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Effect of Drugs on Human Erythrocytes. V.[1] Inhibition of Cellular Metabolism and Adenosine Triphosphatase Activity by Drugs and Relation of the Inhibition to Drug-induced Hemolysis[2]

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In an attempt to clarify the effects of drugs on the metabolism, transport mechanism and life time of human erythrocytes, the effects of chlorpromazine and clemastine on the ATP content, sodium pump and glucose-6-phosphate (G-6-P) dehydrogenase were studied. Chlorpromazine at 2 and 3×10^{-4} M caused only a 5% reduction in the ATP level of erythrocytes and the drug at 8×10^{-4} and 10^{-3} M induced 20 and 22% decreases in the amount of the ghost membrane, respectively. The incubation of erythrocytes in an ATP-generating system had only a slight protective effect against the drug-induced hemolysis. The Na^+, K^+-ATPase activity in the ghost membrane was markedly decreased by chlorpromazine at 6×10^{-4} to 10^{-3} M without inhibition of Mg^{2+}-ATPase activity, and inhibition of the Na^+, K^+-ATPase activity was observed a short time after the drug treatment. There was also a partial inhibition of G-6-P dehydrogenase activity by the drugs. Complete inhibition of Na^+, K^+-ATPase activity with ouabain resulted in no increment of the directly drug-induced hemolysis and no extreme shape changes. Therefore, the directly drug-induced hemolysis is not necessarily an energy-dependent process. However, the cells exposed to chlorpromazine at prelytic concentrations were gradually hemolyzed upon prolonged incubation in isotonic solution at 37°. Consequently, the drug treatment appears to shorten the survival time of the cells, probably due to inhibition of cellular metabolism and to membrane damage.

Keywords——drug effect on metabolism and transport; Na^+, K^+-ATPase inhibition by drugs; G-6-P dehydrogenase inhibition by drugs; ATP level of erythrocytes; hemolysis during prolonged incubation; erythrocytes

Studies on the effects of drugs on erythrocytes have been performed by a number of investigators and many kinds of drugs, such as tranquilizers, antihistaminics and anesthetics, are known to cause hemolysis in varying degrees at high concentrations,[4] while at low concentrations these drugs protect erythrocytes against hypotonic hemolysis.[4a,b,5] Therefore, a

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4) *a*) P. Seeman and J. Weinstein, *Biochem. Pharmacol.*, **15**, 1737 (1966); *b*) P. Seeman, *Biochem. Pharmacol.*, **15**, 1753 (1966); *c*) J. Dausset and L. Contu, *Ann. Rev. Med.*, **18**, 55 (1967); *d*) I.P. Lee, *J. Pharmacol. Exp. Ther.*, **196**, 525 (1976); *e*) T. Ogiso, M. Watanabe, K. Yamauchi, T. Sato, and Y. Kato, *Nippon Yakurigaku Zasshi*, **72**, 145 (1976).
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biphasic effect is observed as a function of drug concentration. Generally, lysis of erythrocytes by drugs is thought to occur by one of two basic mechanisms.[4c,6] The first involves direct association of the drug with the red blood cell membrane, resulting in changes in membrane structure, increased permeability, osmotic swelling and hemolysis. In the second mechanism, the drug or its metabolite first penetrates into the cell interior where it or its oxidation products interfere with cellular metabolism, ultimately resulting in membrane damage and hemolysis. The mechanism of hemolysis of the cells at high drug concentrations has been studied by some investigators: hexachlorophen induces the efflux of Na^+ and K^+ from red blood cells by directly altering the permeability of the cellular membrane, and secondarily induces osmotic swelling and subsequent hemolysis[7]; ellipticine-induced hemolysis is due to disruption of membrane structure as a result of the drug-phospholipid and drug-protein interactions.[4d] We have also proposed that hemolysis induced by chlorpromazine and clemastine is probably due to changes in the arrangement of the phospholipids and to an increase in membrane permeability accompanied by a perturbation of lipid-protein interactions.[8]

However, neither the role of the cellular metabolism nor the participation of sodium pump activity in drug-induced hemolysis is understood, although the inhibition of tissue Na^+, K^+-ATPase by phenothiazines,[9] anesthetics[5m] and ethacrinic acid,[10] and ATP depletion of chicken erythrocytes by anesthetics and tranquilizers[11] were found by some investigators. Ben-Bassat *et al.*[12] found that erythrocyte membrane invagination or endocytosis induced by drugs, including chlorpromazine, was an energy-dependent process. In an attempt to clarify the effect of drugs on the metabolism and the transport mechanism and life time of erythrocytes, and to determine whether or not drug-induced hemolysis is an energy-dependent process, we carried out various experiments. This report deals with the effects of chlorpromazine and clemastine on the ATP content, sodium pump, and glucose-6-phosphate (G-6-P) dehydrogenase activity, and with the involvement of their inhibition in drug-induced hemolysis, in addition to the effect of prolonged incubation on drug-treated cells.

Experimental

Materials——Chlorpromazine hydrochloride (Nihon Shinyaku) and clemastine fumarate (Sankyo Co.) were used throughout these experiments. ATP disodium salt and ouabain were obtained from Sigma Chemical Co. and E. Merck A. G., respectively. Hexokinase from yeast was obtained from Miles-Seravac. Inosine and adenine were obtained from Kohjin Co., Ltd. Nicotinamide adenine dinucleotide phosphate (NADP), G-6-P dehydrogenase and G-6-P were purchased from Oriental Yeast Co., Ltd.

Preparation of Erythrocyte Suspension——Human erythrocyte suspension was prepared by the method described in a previous paper.[13] The hematocrit value was normally $40\pm1\%$ and in some experiments $50\pm1\%$.

Preparation of Hemoglobin-free Erythrocyte Ghosts——Hemoglobin-free erythrocyte ghosts were prepared according to the method of Dodge *et al.*[14] The ghosts obtained were immediately resealed by the procedure of Mueller and Morrison.[15]

Drug-induced Hemolysis and Exposure of Ghosts to Drugs——Drug-induced hemolysis of erythrocytes was measured as described in a previous paper.[16] In the experiment using the ghosts, a mixture consisting of 1.5 ml of erythrocyte ghost suspension (6 mg protein/ml) and 15.0 ml of drug dissolved in 0.9% NaCl–40 mM

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Tris-HCl buffer, pH 7.4, was incubated at 37° for 1 hr, followed by centrifugation at 20000×*g* for 5 min. The ghost pellet was washed once with the same buffer and centrifuged. The ghosts collected were frozen and thawed twice and 0.5 ml aliquots of the suspension were used for assay of Na^+, K^+- and Mg^{2+}-ATPase activities.

Determination of ATP——A mixture of 1 ml of erythrocyte suspension (hematocrit value, 40±1%) or ghost suspension and 10 ml of the drug (1.1×10^{-5}—3.3×10^{-4} M) dissolved in an isotonic NaCl solution (NaCl 9.0 g, $NaH_2PO_4\cdot2H_2O$ 0.343 g and $Na_2HPO_4\cdot12H_2O$ 3.443 g/l, pH 7.4) was incubated at 37° for 1 hr and then centrifuged. ATP in erythrocytes or ghosts was extracted with 0.96 M $HClO_4$ and after centrifugation the extract was neutralized with 10 N KOH.[17] ATP in the supernatant fraction was measured enzymatically by coupling the hexokinase and G-6-P dehydrogenase reactions as described by Lamprecht and Trautschold.[18]

Assay of Na^+, K^+-ATPase and Mg^{2+}-ATPase Activities——ATPase activity was assayed by measuring the inorganic phosphate released from ATP during incubation at 37° according to the method of Kramer *et al.*,[19] except that the activity was assayed at pH 7.7. Mg^{2+}-ATPase activity was measured in the presence of 10^{-3} M ouabain.[19] The Na^+, K^+-dependent portion of the activity was calculated by subtracting the Mg^{2+}-dependent activity from the total activity. The activity was expressed as μg Pi/mg membrane protein/hr.

Assay of G-6-P Dehydrogenase Activity——An aliquot of 0.3 ml of erythrocyte suspension (hematocrit value, 40±1%) was treated with the drug solution (10^{-5}—3×10^{-4} M in the incubation mixture) at 37° for 30 min and washed once with 0.9% NaCl·40 mM tris-HCl buffer, pH 7.4, followed by centrifugation at 1500×*g* for 3 min. Isotonic buffer (0.5 ml) was added to the cell pellet and the suspension was frozen and thawed twice. A portion (0.1 ml) of the hemolysate was subjected to assay for G-6-P dehydrogenase activity according to the method of Marks.[20]

Protein Determination——Protein concentration was determined by the procedure described by Lowry *et al.*[21] with bovine serum albumin, fraction V, as a standard. The protein content (including hemoglobin) of intact erythrocytes was determined following hemolysis in 10 volumes of water.

Scanning Electron Microscopy——Erythrocytes, after treatment with the drug and centrifugation, were fixed with 1.5% glutaraldehyde in an isotonic phosphate buffer, pH 7.2. After 1 hr, the cells were washed thoroughly with the isotonic buffer and then dehydrated 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.

Results

Effect of Chlorpromazine on the ATP Content of Erythrocytes and Ghosts

It is tempting to speculate that the drug-induced hemolysis may be due to depletion of ATP, since the treatment of chicken erythrocytes with chlorpromazine resulted in a drastic decline in the ATP level of the cells, a 78% depletion by 2.4×10^{-4} M drug and a 90% depletion by 4.8×10^{-4}M drug.[11] Thus, the ATP content of human erythrocytes exposed to 10^{-5} to 3×10^{-4} M chlorpromazine for 1 hr was measured. The drug at 2 and 3×10^{-4} M caused only a small reduction (about 5%: controls, 5.72±0.48 $n=4$; 3×10^{-4} M drug-treated cells, 5.41±0.41 nmol/mg protein $n=4$) in the ATP level, while the drug at concentrations below 10^{-4} M had no effect on the level.

Table I also shows the ATP level of the ghosts treated with chlorpromazine. The ATP level in the ghosts treated with the drug was slightly decreased. Chlorpromazine at 8×10^{-4} and 10^{-3} M, at which concentrations complete hemolysis was produced, induced 20 and 22% decreases of the ATP level as compared with the control, respectively. These results indicate that the drug does not have a major effect on the ATP level of human erythrocytes and the membrane.

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Table I. ATP Content of Ghost Membranes treated with Chlorpromazine

Concentration of drug ($\times 10^{-4}$ M)	ATP (nmol/mg protein)	%
None	30.3±1.2	100
1	29.7±2.1	98.2
2	31.0±3.0	102.4
4	27.5±1.6	90.8
5	26.9±0.5[a)]	88.8
6	26.2±2.5[a)]	86.7
8	24.1±3.0[a)]	79.7
10	23.6±1.0[b)]	78.0

A mixture consisting of 1.5 ml of the ghost membrane suspension (6 mg protein/ml) and 10 ml of the drug dissolved in isotonic NaCl solution was incubated at 37° for 1 hr and then centrifuged. The pellet obtained was washed once with the same isotonic solution, followed by centrifugation, and the ghost pellet was subjected to ATP and protein determinations. Values represent the means±S.D. of 4 experiments.
a) $p<0.025$, *b)* $p<0.01$.

Effect of ATP Regeneration on Drug-induced Hemolysis

It was observed by Nakao *et al.*[22] that the incubation of erythrocytes with glucose, inosine and adenine sulfate changed the cells, which were initially to smooth spheres, to a biconcave disk or a shallow cup form. Thus, the effect of ATP regeneration by incubation of these agents with erythrocytes on drug-induced hemolysis was examined in an attempt to clarify the involvement of ATP in this lysis. It was found that incubation of the cells in the ATP-regenerating system, which resulted in about a 10% increase in ATP level, produced only a slightly protective effect (5—7% decrease in hemolysis) against the hemolysis induced by the drug at 4×10^{-4} to 6×10^{-4} M. Consequently, the generation of ATP under the conditions tested appears not to have a significant protecting effect on the drug-induced hemolysis.

Effects of Chlorpromazine on Na^+, K^+-ATPase and Mg^{2+}-ATPase Activities

The effects of chlorpromazine on the Na^+, K^+-ATPase and Mg^{2+}-ATPase activities of the membrane preparations are shown in Fig. 1, with the drug-induced hemolysis. This drug decreased the levels of the Na^+, K^+-ATPase activity, and especially at higher concentrations, 6×10^{-4} to 10^{-3} M, the activity was significantly decreased (controls, 6.08±0.42; 6×10^{-4} M drug-treated ghosts, 0.53±0.36 μg Pi/mg protein/hr). It is interesting that even at lower drug concentrations of 5×10^{-5} and 10^{-4} M the activity was slightly inhibited. The Mg^{2+}-ATPase activity was not affected even at a higher concentration with chlorpromazine (controls, 6.22±0.86; 10^{-3} M drug-treated ghosts, 5.94±0.77 μg Pi/mg protein/hr). The time course of the inhibition of Na^+, K^+-ATPase activity by 6×10^{-4} M chlorpromazine is shown in Fig. 2. At a short time after the addition of the drug Na^+, K^+-ATPase activity was significantly inhibited; 5 min after the addition there were a 40% inhibition and 10 min after a 59% inhibition as compared with the control, while no inhibition of Mg^{2+}-ATPase activity was found in any of the cases tested.

Effects of Chlorpromazine and Clemastine on the G-6-P Dehydrogenase Activity of Erythrocytes and Hemolysates

The G-6-P dehydrogenase activity of erythrocytes treated with the drugs at prelytic concentrations was measured after freezing and thawing. As shown in Table II, the drugs at relatively low concentrations partially inhibited the activity; chlorpromazine and clemastine at 3×10^{-4} M inhibited the activity by 24 and 32% as compared with the control, respectively. The G-6-P dehydrogenase activity in the hemolysates, prepared by freezing

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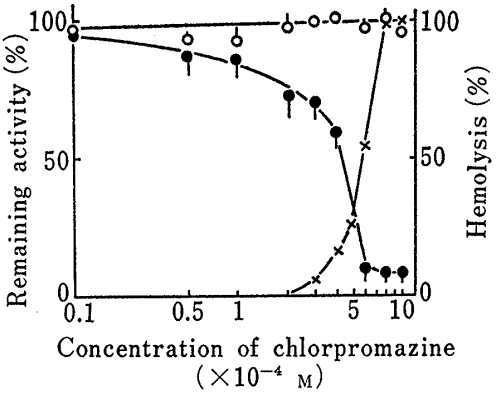

Fig. 1. Effects of Chlorpromazine on Na^+, K^+–ATPase and Mg^{2+}–ATPase Activities in Ghost Membranes

Ghost membranes (6 mg protein/ml) were treated with the drug and the activities were assayed by the method described in the text. Points represent the means ± S.D. of 5 experiments. (●) Na^+, K^+–ATPase activity; (○) Mg^{2+}–ATPase activity; (×) hemolysis.

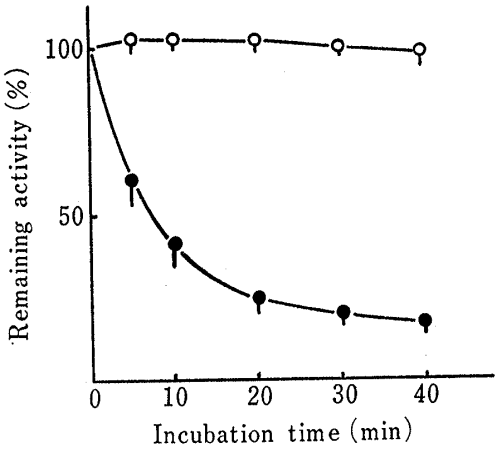

Fig. 2. Time Courses of Inhibition of Na^+, K^+–ATPase Activity in Ghost Membranes by Chlorpromazine

Ghost membranes (6 mg protein/ml) were incubated with the drug for the indicated time at 37° and immediately after washing the membranes once with 0.9% NaCl in 40 mM tris-HCl buffer, pH 7.4, the activity was assayed as described in the text. Points represent the means ± S.D. of 3 experiments. (●) Na^+, K^+–ATPase activity; (○) Mg^{2+}–ATPase activity.

and thawing of intact erythrocytes, was also assayed in the presence of the drugs at concentrations ranging from 10^{-5} to 8×10^{-4} M. As shown in Table II, the activity was increasingly inhibited as the concentrations of the drugs increased. Thus, these drugs have a weakly inhibitory effect on G-6-P dehydrogenase in the cells.

Effect of Ouabain Pretreatment on Drug-induced Hemolysis

Considering that the inhibition of Na^+, K^+-ATPase activity might cause the hemolysis, we measured the extent of the drug-induced hemolysis of erythrocytes pretreated with ouabain. The results are shown in Table III. The inhibition (the activity was completely inhibited

Table II. Effects of Drugs on Glucose 6-Phosphate Dehydrogenase Activity in Erythrocytes and Hemolysate

Concentration of drug (×10⁻⁴ M)	Erythrocytes[a]		Hemolysate[b]	
	Chlorpromazine	Clemastine	Chlorpromazine	Clemastine
None	5.54±0.36	5.64±0.15	788±19	788±19
0.1	5.71±0.46	5.80±0.75	789±35	743±48
0.5	5.58±0.40	5.43±0.33	745±26	709±47
1	5.31±0.25	5.37±0.43	671±27[c]	689±31[c]
2	4.64±0.48	4.59±0.48[c]	609±38[c]	707±56[c]
3	4.23±0.51[d]	3.83±0.58[d]	—	—
4	—	—	544±37[c]	563±39[c]
6	—	—	373±41[d]	522±37[c]
8	—	—	359±30[d]	489±35[c]

The erythrocytes were treated with drugs for 1 hr at 37°, washed once with 0.9% NaCl in 40 mM Tris-HCl buffer, and centrifuged. The cells were frozen and thawed twice, then diluted 5 times with water (*a*). The washed cell suspension (hematocrit value, 40±1%) was frozen and thawed twice. A mixture consisting of 0.3 ml of the hemolysate and 3 ml of the drug solution at the indicated concentration was incubated for 30 min at 37° (*b*) and the enzyme activity in the mixture was immediately assayed. Values represent the means± S.D. of 3—4 experiments. The activity is expressed as *a*) NADPH nmol/mg protein/min or *b*) NADPH nmol/ml/min. *c*) $p<0.05$. *d*) $p<0.01$.

Table III. Effect of Ouabain Pretreatment on Drug-induced Hemolysis

Concentration of chlorpromazine ($\times 10^{-4}$ M)	Control	Hemolysis (%) ouabain	
		5×10^{-5} M	1.5×10^{-4} M
None	0.2	0.3	0.3
2	1.3±0.6	0.8±0.3	0.8±0.1
3	5.2±0.5	4.3±0.2	5.3±0.1
4	14.4±0.9	13.9±1.5	11.8±0.9
5	26.1±2.0	27.5±2.3	22.7±1.8
6	54.0±2.5	49.9±5.9	40.4±5.8
8	101.2±3.5	99.6±2.3	101.7±2.4
10	100.6±1.0	100.7±1.2	101.0±0.5

A mixture consisting of 4 ml of erythrocyte suspension (hematocrit value, 50±1%) and 1 ml of ouabain (2.5×10^{-4} or 7.5×10^{-4} M) dissolved in isotonic NaCl solution was incubated at 20° for 40 min, and then 0.3 ml of the suspension was exposed to the drug at 37° for 1 hr. Values are the means±S.D. of 3 experiments.

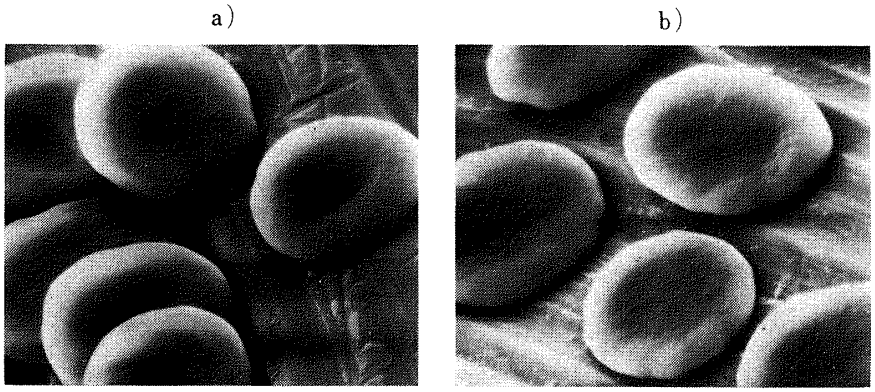

Fig. 3. Scanning Electron Micrographs of Erythrocytes treated with Ouabain

0.3 ml of the erythrocyte suspension (hematocrit value, 40 ± 1%) was incubated with 3 ml of 1.1 mM ouabain dissolved in isotonic NaCl–Tris-HCl buffer, pH 7.4, or with the buffer alone for 1 hr at 37°.
a) normal cells.
b) cells treated with ouabain. Magnification ×7500.

under these conditions, at 20° for 40 min) of Na^+, K^+-ATPase activity with 1.5×10^{-4} M ouabain resulted in no additional increment of the hemolysis induced by chlorpromazine, and there was rather a slight decrease of hemolysis, although no effect on the hemolysis was seen with 5×10^{-5} M ouabain. This result indicates that the inhibition of Na^+, K^+-ATPase by the drug is not involved in the mechanism of the directly drug-induced hemolysis. Scanning electron microscopy showed that the erythrocytes in which Na^+, K^+-ATPase activity had been completely inhibited by incubation with 10^{-3} M ouabain showed no significant shape changes, although minor shrinkage of the cells was observed, as shown in Fig. 3.

Time Course of Hemolysis of Erythrocytes pretreated with Chlorpromazine

When the cells were treated with chlorpromazine at a semilytic or prelytic concentration for 10 min at 37°, the cells treated with the drug above 8.8×10^{-5} M were gradually hemolyzed upon prolonged incubation in isotonic NaCl solution, as shown in Fig. 4. The drug-treated cells were similarly incubated in sterilized Lock's solution containing 1% glucose, but the hemolytic behavior was almost the same as that of the treated cells incubated in isotonic NaCl solution, indicating that the survival time of the drug-treated cells is not prolonged by glucose.

These results suggest that the survival time of the treated cells is much shorter than that of the normal cells.

Discussion

Drug-induced hemolysis may reflect membrane damage in various tissues by the drugs. It is known that some drugs at high concentrations have a lytic action on lysosomes.[23] In an attempt to clarify the effects of drugs on both cellular metabolism and transport systems in human erythrocytes and on the cell life time, we carried out various experiments. Some investigators demonstrated that restoration of ATP to ATP-depleted erythrocytes endowed them with resistance to the attack of phospholipase C,[24] and a major role of ATP in the maintenance of red cell viability, related to the preservation of cell deformability, has been suggested.[25] It was reported that cell membrane invagination or endocytosis induced by chlorpromazine was an energy-dependent process.[12]

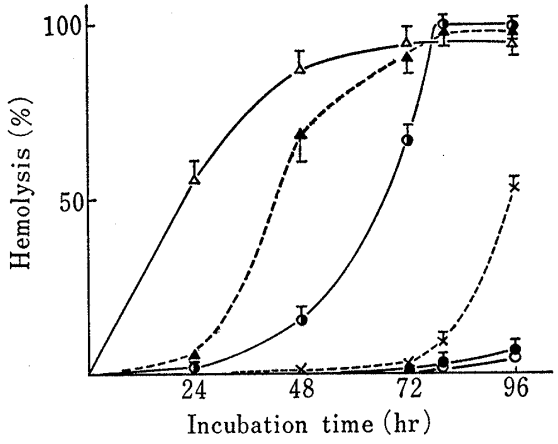

Fig. 4. Time Courses of Hemolysis of Erythrocytes pretreated with Chlorpromazine

The cells were pretreated with the drug for 10 min at 37° and washed once with isotonic NaCl solution. A 0.3 ml aliquot of the cell suspension (hematocrit value, 40%) was added to 3 ml of isotonic NaCl solution and the whole was incubated for the indicated time at 37°. Normal cells (○); drug (●) 4.4×10^{-5} M; (×) 8.8×10^{-5} M; (◑) 1.8×10^{-4} M; (▲) 2.6×10^{-4} M; (△) 4.4×10^{-4} M. Points represent the means ±S.D. of 3—4 experiments.

The present study indicates that chlorpromazine at higher concentrations, 4×10^{-4} to 10^{-3} M, decreases the ATP level in the ghost membrane by 10—22% and at 3×10^{-4} M decreases the amount in the cells by about 5% as compared with the control (Table I). The decreases are not as drastic as those observed in chicken erythrocytes.[11] These differences in the decreases of ATP levels of human and chicken cells may be due to differences in their membrane structures or in the "leakiness" of the membranes. The slight decrease in ATP level observed indicates that the directly drug-induced hemolysis is not a metabolic process dependent on energy. This view is also supported by the present data that the cells incubated in the ATP-generating system were not protected against the drug-induced hemolysis.

Chlorpromazine above 6×10^{-4} M strongly inhibited the Na^+, K^+-ATPase activity of the membrane preparations, while the drug had little or no effect on the Mg^{2+}-ATPase activity. The present data are reasonably consistent with those on Na^+, K^+-ATPase activity in rat brain[26] and other membranes[9,10] inhibited by chlorpromazine. It has been unclear whether or not inhibition of Na^+, K^+-ATPase activity by drugs is involved in the directly drug-induced hemolysis. We found that 1) a 100% inhibition of Na^+, K^+-ATPase activity in the cells with ouabain produced no additional increment of the directly drug-induced hemolysis (Table III) and 2) the rates of hemolysis and K^+ efflux induced by the drugs were much greater than would be expected even if these drugs were acting simply as metabolic inhibitors and/or inhibitors of Na^+, K^+-ATPase,[8] and these results indicate that inhibition of Na^+, K^+-ATPase activity by the drugs has little effect on the directly drug-induced hemolysis. The significant inhibition of the Na^+, K^+-ATPase activity produced with 6×10^{-4} to 10^{-3} M chlorpromazine is probably due to an extreme disturbance of the arrangement of phospholipids and of the hydrophobic interactions between lipids and proteins, as reported previously by us.[8] Chlorpromazine

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probably disturbs the phosphatidylserine-protein interactions, since the drug penetrates into the membrane, where it binds to phospholipids bearing a negative charge.[27] The partial inhibition of the Na^+, K^+-ATPase activity by chlorpromazine below 2×10^{-4} M (Fig. 1) suggests that some disturbances of the membrane structure are also produced by the drug at prelytic concentrations. The relative differences in inhibitory tendency towards Na^+, K^+-ATPase and Mg^{2+}-ATPase activities (Fig. 1) cannot be accounted for.

It was reported that a hemolytic anemia is associated with G-6-P dehydrogenase deficiency in human erythrocytes, and that hemolysis occurs in such patients when they are exposed to a number of oxidant drugs such as antihypertensives, sulfonamide and primaquine.[28] As a result of this experiment on G-6-P dehydrogenase, chlorpromazine and clemastine were found to partially inhibit the dehydrogenase activity in human erythrocytes when the drugs were incubated with the cells. Such inhibition of the activity may affect the survival time of the cells, although this inhibition is not involved in the directly drug-induced hemolysis.

The cells pretreated with chlorpromazine above 8.8×10^{-5} M were gradually hemolyzed upon prolonged incubation. This indicates that the survival time of the cells is shortened by the drug treatment. Thus, it is possible that the shortened cell life could be attributed in part to the inhibition of cellular metabolism and transport systems and to direct damage to membrane, as mentioned above.

In conclusion, therefore, the present results lead us to consider that chlorpromazine at high concentrations induces a slight decrease of the ATP content and strongly inhibits Na^+, K^+-ATPase and G-6-P dehydrogenase activities without inhibition of Mg^{2+}-ATPase activity. However, the inhibition of Na^+, K^+-ATPase by ouabain has no additional effect on the directly drug-induced hemolysis, and the inhibition of cellular metabolism and these enzyme activities by the drugs is either not or only slightly involved in the directly drug-induced hemolysis. These results demonstrate that the directly drug-induced hemolysis is not an energy-dependent process. However, the cells treated with chlorpromazine at prelytic concentrations were gradually hemolyzed upon prolonged incubation. Thus, treatment of the cells with drugs at prelytic concentrations may shorten the survival time of the cells, due to inhibition of the cellular metabolism and to direct damage to the membrane.

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