The effect of washing on adherence was tested using organ bath II. The preparation was washed with 5 ml of water immediately after administration of the drug. The results are shown in Table III. After washing, the force needed for detachment was only about 30% of that without washing.

Doxycycline has caused most of the reported drug-induced injuries to the esophagus (3, 11). The adherence properties of all doxycycline products at present being marketed in Finland were therfore studied. In addition, two experimental formulations of doxycycline were included in the study. As can be seen from Table IV, there were significant differences between the products. Hard gelatin capsules required the most force to dislodge. In addition, the forces for detachment of experimental formulations G and H were significantly lower than those for the best commercially available products.

In the present study about 60 esophageal preparations from 30 different pigs were used, but no marked interindividual variation was observed. The same preparation could be used for 20–30 consecutive measurements without excessive variation in results. If the mucosa was washed, 50–60 measurements with the same preparation were possible. In the experiments, esophagi stored at 4° in Tyrode solution for 24 hr as well as fresh esophagi were used, but no significant differences between the two were noted. The effect of possible spontaneous esophageal contractions on the detaching force was eliminated by using a large number of n-values (usually $n = 20$).

As can be seen from Figs. 3–4 and Table IV there were significant differences in the tendency for adherence, as between pharmaceutical formulations. The adherent tendency of uncoated potassium chloride tablets and sugar-coated tablets was only about 15–20% that of gelatin capsules. Thus, it is reasonable to try to develop drug products with less tendency to adhere. Large sizes should be avoided, especially in the case of drugs that are known to cause esophageal stricture or ulceration.

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Erythrocyte Changes in Aqueous Polyethylene Glycol Solutions Containing Sodium Chloride

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Abstract □ The behavior of rabbit erythrocytes in aqueous solutions of polyethylene glycol 300 (I) and polyethylene glycol 400 (II) containing sodium chloride was investigated during 2–120 min incubation at 37°. No hemolysis was found in I (0–10.1%) and II (0–12.9%) solutions in the presence of sodium chloride (0.45–1.35%), but prelytic potassium ion loss and changes in the appearance of the erythrocytes proceeded with the passage of time. The potassium ion loss increased with increasing concentration of polyethylene glycol and/or sodium chloride. The mean cellular volume of erythrocytes decreased temporarily (during the first 2 min) in both I (6.7%) and II (8.6%) solutions containing sodium chloride (0.68–1.35%), and then increased progressively to the same value as that determined by solution of sodium chloride at the same concentration but without polyethylene glycol (~30 and 120 min in I and II solutions, respectively). Both I (10.1%) and II (12.9%) induced a stomatocytic transformation of erythrocytes, but at the higher concentrations (0.9–1.35%) of sodium chloride, II accelerated the progress of spontaneous transformation to echinocytes. The results indicate that these solutions were not isotonic with rabbit erythrocytes.

Keyphrases □ Erythrocytes—changes in aqueous polyethylene glycol solutions, sodium chloride □ Sodium chloride—erythrocyte changes in aqueous polyethylene glycol solutions □ Polyethylene glycol—aqueous solutions containing sodium chloride, erythrocyte changes

The hemolysis of rabbit and human erythrocytes occurs in polyethylene glycol even at iso-osmotic concentrations, while the hemolysis is almost completely inhibited in the presence of a suitable amount of sodium chloride (1, 2). However, little is known about the retention of the normal characteristics of erythrocytes in polyethylene glycol solutions containing sodium chloride.

The experiments described deal with the quantitative variations of hemolysis, potassium ion loss, mean cellular volume, and shape of rabbit erythrocytes produced in aqueous polyethylene glycol 300 (I) and polyethylene glycol 400 (II) solutions with reduced sodium chloride content.

EXPERIMENTAL

Materials—Polyethylene glycol 300[1] and 400[1] in reagent grade were used without further purification. All other reagents and chemicals used were reagent grade or high purity.

Preparation of Solutions—The polyethylene glycol and sodium chloride solutions were weight-in-volume percentage preparations, and were adjusted to pH 7.4 by addition of 3 N HCl. Iso-osmotic concentration of polyethylene glycol was estimated by the freezing point depression data.

Preparation of Erythrocyte Suspension—Fresh rabbit (albino) erythrocytes, using heparin (100 U/ml of blood) as an anticoagulant re-

[1] Wako Pure Chemical Industries, Ltd., Osaka, Japan.

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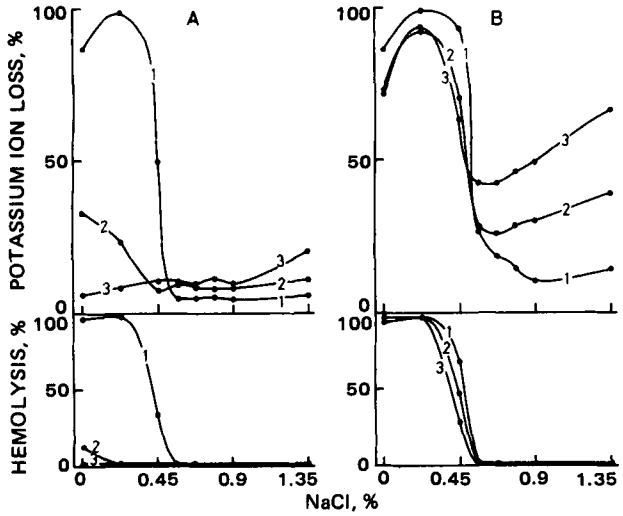

Figure 1—*The percent release of potassium ion (A, B upper) and hemoglobin (A, B lower) from rabbit erythrocytes in I–sodium chloride solutions in various proportions after incubation for 2 min (A) and 120 min (B) at 37°. Curves 1–3 represent the percent release in I (0, 6.7, and 10.1%, respectively) solutions. Each point is the mean value of three observations.*

agent, were washed three times with a chilled 0.9% NaCl solution to remove plasma and the buffy coat and resuspended at 80% hematocrit in the same solution.

Erythrocyte Incubation—To a mixture of 1 ml of polyethylene glycol solution (0–10.1% for I and 0–12.9% for II in the final medium) and 1 ml of NaCl solution (0–1.35% in the final medium), 0.5 ml of erythrocyte suspension (hematocrit value 80%) was added and mixed immediately. The mixture was incubated for a certain period (up to 120 min) at 37°, and an aliquot was withdrawn from the suspension and analyzed for the mean cellular volume and morphology of erythrocytes. The remaining suspension was centrifuged at 2000×*g* for 3 min, and both hemoglobin and potassium ion in the supernate were quantitatively determined. The degree of hemoglobin loss (hemolysis) and potassium ion loss were expressed as a percentage of their total losses induced by an aqueous saponin solution (100 mg/liter).

Estimation of Mean Cellular Volume—The mean volume of erythrocytes was calculated from the total cell volume, which was determined by the microhematocrit method and from the number of erythrocytes determined with a micro cell counter[2].

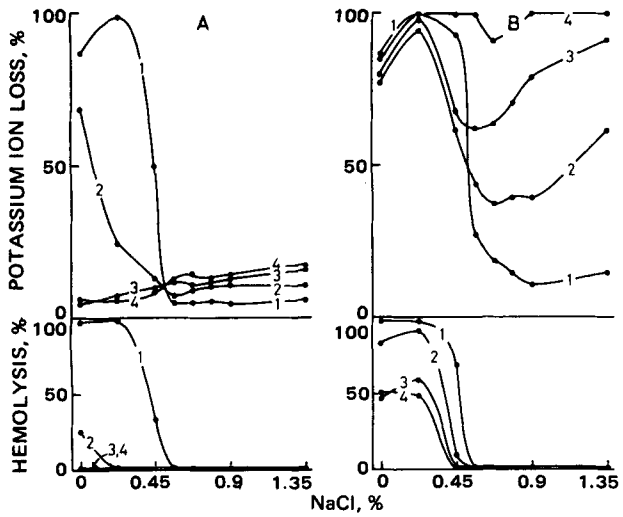

Figure 2—*The release percentage of potassium ion (A, B upper) and hemoglobin (A, B lower) from rabbit erythrocytes in II–sodium chloride solutions in various proportions after incubation for 2 min (A) and 120 min (B) at 37°. Curves 1–4 represent the percent release in II (0, 4.3, 8.6, and 12.9%, respectively) solutions. Each point is the mean value of three observations.*

[2] Model CC-107, Toa Medical Electronics Co., Ltd., Kobe, Japan.

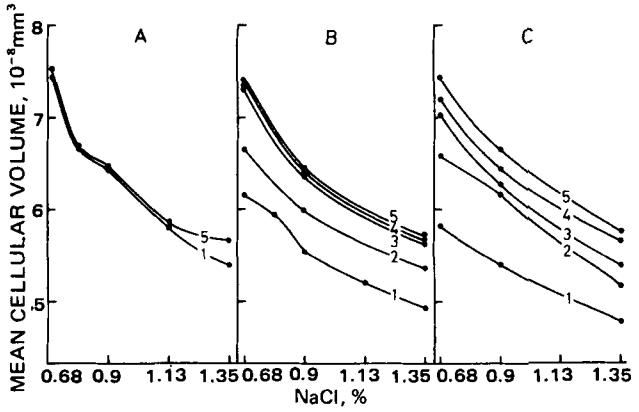

Figure 3—*The mean cellular volume of rabbit erythrocytes in the aqueous solutions of sodium chloride (A), 6.7% I–sodium chloride (B), and 8.6% II–sodium chloride (C) at 37°. Curves 1–5 represent the mean cellular volume after incubation for 2, 10, 30, 60, and 120 min, respectively. Each point is the mean value of three observations.*

Morphological Study of Erythrocytes with Scanning Electron Microscopy—Erythrocytes were fixed in 2.5% glutaraldehyde by standing overnight, followed by treatment in 2% osmium tetroxide for 2 hr in an isotonic phosphate-buffered saline solution (pH 7.4). The erythrocytes were dehydrated in a graded series of ethanol and air dried, followed by coating with gold–palladium alloy with an ion coater[3]. The erythrocytes were observed and photographed with a scanning electron microscope[4]. Morphology was defined according to Bessis' classification (3).

Determination of Hemoglobin (4)—The hemolyzates were added to ferricyanide–cyanide reagent, and the pigments were converted to cyanomethemoglobin. Their optical densities were analyzed with a spectrophotometer[5] at 540 nm.

Determination of Potassium Ion—Before measurements, proteins present in the hemolyzates were precipitated by boiling for 10 min and centrifuged. Potassium ion in the supernate was determined with an atomic absorption spectrophotometer[6] (air acetylene flame).

Measurement of Freezing Point Depression—The freezing point depression measurement was made on aqueous solutions of I and II by means of an osmometer[7].

RESULTS

Hemolysis and Potassium Ion Loss—Figures 1 and 2 show the release percentage of hemoglobin (hemolysis) and potassium ion from rabbit erythrocytes in various I and II solutions, respectively, with reduced content of sodium chloride at 37°.

After 2 min of incubation in I (Fig. 1A) and II (Fig. 2A) solutions with 0–0.45% NaCl, the percentage of hemolysis and potassium ion loss decreased with increased concentration of polyethylene glycol, and no loss occurred at iso- (I: 6.7%; II: 8.6%) or hyperosmotic concentration of polyethylene glycol. This finding was similar to that obtained for sodium chloride. Sodium chloride (>0.45%) prevented both the hemolysis and the potassium ion loss, regardless of the concentration of polyethylene glycol.

During a period from 2 to 120 min (I: Fig. 1B; II: Fig. 2B) at 0–0.45% NaCl, both hemolysis and potassium ion loss proceeded even at iso- or hyperosmotic concentration of polyethylene glycol (more rapidly in I than II solution) in contrast to the degree of hemolysis remaining unchanged for sodium chloride. At 0.45–1.35% NaCl, no hemolysis occurred, but potassium ion loss occurred depending upon the concentration of polyethylene glycol (remarkably in II solution). Furthermore the loss increased with increase of the concentration of sodium chloride. A minimal loss occurred at 0.6% NaCl.

Mean Cellular Volume of Erythrocytes—Figure 3 shows the mean cellular volumes of erythrocytes in solutions of I (6.7%) and II (8.6%) containing various concentrations of sodium chloride at 37°. Erythrocyte occupied a mean cellular volume of 6.4×10^{-8} mm^3 in 0.9% NaCl solution.

[3] Model IB-3, Eiko Engineering Co., Ltd., Ibaragi, Japan.
[4] Model Hitachi-Akashi MSM-4, Akashi Seisakusho Ltd., Tokyo, Japan.
[5] Model UV-VIS 193, Hitachi Ltd., Tokyo, Japan.
[6] Model 503, Perkin-Elmer Ltd., Norwalk, Conn.
[7] Model Osmette 2007, Precision Systems Inc., Sudbury, Mass.

Table I—The Distribution of Rabbit Erythrocyte Shapes[a] in Polyethylene Glycol–Sodium Chloride Solutions

Medium Composition, %	After 2-min Incubation, %			After 120-min Incubation, %		
	Stomatocyte	Discocyte	Echinocyte	Stomatocyte	Discocyte	Echinocyte
0.6 NaCl	3.3	82.6	14.1	2.0	64.8	33.2
0.9 NaCl	2.3	75.8	21.9	1.0	60.9	38.0
1.35 NaCl	8.5	79.2	12.4	0.5	37.6	62.0
10.1 I + 0.6 NaCl	4.4	91.2	4.4	6.4	86.6	7.0
10.1 I + 0.9 NaCl	7.6	90.7	1.7	8.6	70.4	21.0
10.1 I + 1.35 NaCl	24.8	65.2	9.9	11.0	38.2	50.9
12.9 II + 0.6 NaCl	39.5	60.1	0.4	5.4	89.8	4.8
12.9 II + 0.9 NaCl	29.1	69.6	1.4	11.7	44.5	43.8
12.9 II + 1.35 NaCl	31.2	62.8	6.0	3.5	21.5	75.0

[a] The percentage of each cell type (stomatocyte, discocyte, and echinocyte) was estimated in three fields of scanning electron micrograph each containing between 100 and 200 cells/field.

The value increased to 7.53×10^{-8} mm^3 in 0.68% NaCl solution or decreased to 5.39×10^{-8} mm^3 in 1.35% NaCl solution within 2 min. After that, each value remained unchanged. In polyethylene glycol–sodium chloride solutions, the mean cellular volume decreased temporarily and then increased progressively to the same value as that determined by solution of sodium chloride at the same concentration but without polyethylene glycol. It took ~30 and 120 min in I and II, respectively, to reach this stage.

Shape of Erythrocytes—Table I shows the distribution of erythrocyte shapes after a certain period of incubation at 37° in I (10.1%)–, or II (12.9%)–sodium chloride solutions. Both I and II slightly induced cup-formed erythrocytes (stomatocytic transformation) on 2-min incubation. On prolonged incubation the progressive echinocytic transformation was observed with all the solutions examined. After 120 min of incubation, the presence of I retarded the echinocytic transformation regardless of sodium chloride content. II also retarded the transformation at a low concentration (0.6%) of sodium chloride, whereas it accelerated it at a high concentration (0.9–1.35%) of sodium chloride.

DISCUSSION

A previous study (1) reported that sodium chloride prevented hemolysis in polyethylene glycol 200 (≤25%) or I (≤40%) solutions. The present investigation also indicates that ≥0.45% concentration of sodium chloride prevented almost completely the hemolysis of rabbit erythrocyte in I or II solutions, but did not prevent prelytic potassium ion loss, mean cellular volume change, and shape change. In addition, the present experimental data showed that the time course of these erythrocyte changes could be divided into the first phase which occurred during the first 2 min, and the second phase which occurred during further incubation. For example, as shown in Fig. 1 (Curves 2 and 3) and Fig. 2 (Curves 2–4), hemolysis and potassium ion loss in the first phase were prevented in the polyethylene glycol solutions with a small amount of sodium chloride (0–0.45%), while in the second phase it proceeded gradually. Similarly, it was reported (5, 6) that red cells were hemolyzed in hypotonic electrolyte solutions in two phases: an early fast phase due to rapid water entry followed by a slow phase. The time course of hemolysis in the polyethylene glycol solutions was approximately the same as that for the hypotonic electrolyte solutions except that the reaction (for the polyethylene glycol solutions) proceeded more rapidly in the second phase. The process of cellular volume change was also divided into two phases in the polyethylene glycol solutions containing sodium chloride (Fig. 3). In the first phase the mean cellular volume decreased temporarily, as if erythrocytes were placed in the solution in which polyethylene glycol exerted osmotic pressure as well as sodium chloride (Curve 1, Fig. 3). In the second phase the mean cellular volume increased progressively to the same value as that determined by solution of sodium chloride, at the same concentration but without polyethylene glycol (Curves 2–5, Fig. 3). This may be explained from the viewpoint of osmotic support. In the first phase polyethylene glycol acts as an osmotic particle, since most polyethylene glycol molecules remain outside the erythrocyte membrane, while in the second phase the osmotic pressure decreases gradually as they penetrate the membrane.

The potassium ion loss occurred at both hypo- and hypertonic concentration of polyethylene glycol and sodium chloride, and increased with increase in the concentration of polyethylene glycol and/or sodium chloride. This result suggests that polyethylene glycol disturbs the erythrocyte membranes and produces the prelytic potassium ion loss. Discoloration of rabbit erythrocytes has previously occurred at high concentration (>15%) of polyethylene glycol (1). The discoloration also indicates the damage of the cell membranes by polyethylene glycol. Both polyethylene glycol and sodium chloride exert osmotic pressure in the first phase, and such a high osmotic pressure may accelerate the potassium ion loss.

Polyethylene glycol exerted an influence upon the spontaneous transformation of erythrocyte shape to echinocyte, which was said to result from ATP depletion of erythrocytes by incubation (7). This mechanism, however, is yet unclear.

Observation of the behavior of erthrocytes that are suspended in a solution is the direct procedure for determining whether the solution is isotonic, hypotonic, or hypertonic. If the cells retain their normal characteristics, the solution is isotonic (8). The present investigation evaluates the tonicity of polyethylene glycol–sodium chloride solutions. The I and II solutions containing 0.6% NaCl are the most isotonic, because both the prelytic potassium ion loss and the transformation of erythrocyte shape (discocyte: 86.6% in I; 89.9% in II) were relatively inhibited by addition of 0.6% NaCl. Whereas, on the basis of mean cellular volume data (9), the I or II solution containing 0.9% NaCl is the most isotonic, because the mean cellular volume in this solution was the same as in 0.9% NaCl (after incubation for 120 min). An isotonic solution consisting of polyethylene glycol and sodium chloride was not obtained at any concentration of sodium chloride.

Because rabbit erythrocytes were employed in the present investigation, a quantitative transfer of the experimental data to a human erythrocyte system is not possible, however it may be expected that the data apply qualitatively.

A conclusion drawn from this experiment for rabbit erythrocytes was that hemolysis did not occur in I and II solutions with ≥0.45% NaCl, but prelytic potassium ion loss, mean cellular volume change, and shape transformation were observed. Therefore, these solutions were not isotonic.

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