Hemolysis of Erythrocytes in Various Iso-osmotic Solutions

By E. R. HAMMARLUND† and KAJ PEDERSEN-BJERGAARD‡

A study has been made of the degree of hemolysis of human erythrocytes that occurs when the red blood cells are incubated in many different pharmaceutically employed iso-osmotic solutions. Many distinct differences were noted which further demonstrate the frequent dissimilarities between iso-osmotic and isotonic concentrations. In addition to the hemolytic differences, one of the other main factors in the consideration of the use of isotonic solutions *versus* iso-osmotic solutions is clearly that of the quantities and proportions of the substances used and the resulting rapidity and ease of dilution of the medicinal solution by the body fluids.

IN A PREVIOUS study of iso-osmotic and isotonic solutions Hammarlund and Pedersen-Bjergaard (1) determined the sodium chloride equivalents for 332 pharmaceutical compounds. These were calculated from experimentally obtained measurements of the lowering of the vapor pressure and the depression of the freezing point of their aqueous solutions. It is well known that solutions of some medicinally used substances such as ammonium chloride, boric acid, ethanol, glycerin, and urea fail to prevent the hemolysis of red blood cells in iso-osmotic concentrations. For such substances that pass through or alter the erythrocyte membrane, the iso-osmotic concentration differs markedly from the isotonic concentration. Husa and co-workers (2–9) have investigated the red blood cell hemolytic activity of a number of solutions in reference to a determination of their van't Hoff *i* values or "isotonic coefficients." However, since the hemolytic activities of a large number of pharmaceutical solutions have not been compared previously on an equal osmolar basis, except those examined by Husa (2–9) and by Setnikar and Temelcou (10), this study was undertaken. The purpose was to determine the degree of hemolysis which results when defibrinated blood is added to solutions in iso-osmotic concentration for 161 substances from our previous study. The items selected were those which are completely soluble to the extent of their iso-osmotic concentrations.

METHOD

The method used is essentially the same as that employed by Husa and co-workers (2–9) and is a modification of the method by Hunter (11). The principal deviations from Husa's method were that iso-osmotic concentrations were employed and an aqueous saponin purum,[1] 100 mg./L., was used as the 100% hemolyzing solution for the erythrocytes instead of 0.1% sodium carbonate solution. Finholt (12) and others used a saponin solution for a standard since the alkalinity of the carbonate solution produces a darker red hemolyzed solution which results in a higher colorimetric reading than would be obtained at the body pH of approximately 7.4. The color given by the hemolysis was determined as oxyhemoglobin and this was done as rapidly as possible following the incubation of the defibrinated blood in the solutions being tested.

Fresh venous blood from human volunteers was drawn into a flask containing glass beads. It was immediately defibrinated by rotating the flask with the beads until the fibrin separated. The blood was poured into a small flask and aerated by rotating gently for five minutes. The blood was shaken gently by swirling immediately before each 0.1-ml. sample was withdrawn with a volumetric pipet. The blood was preserved under refrigeration and was not used if it had been drawn more than twenty-four hours earlier.

The solutions were freshly prepared with distilled water; all chemicals used in the study were identical in purity to those reported in the previous study (1).

Quantitative Determination of the Per Cent Hemolysis.—Ten milliliters of each solution studied was added to each of three centrifuge tubes. Three tubes containing 10 ml. of 0.9% sodium chloride served as the colorimetric blanks. Three tubes containing 10 ml. of saponin solution served as the reference color for complete hemolysis; the colorimetric readings of which were made at the beginning, in the middle, and at the end of the experimental determinations. Exactly 0.1 ml. of fresh defibrinated and aerated blood was added to all tubes by means of a volumetric pipet. The tubes were stoppered and inverted several times. They were placed in a water bath at 25 ± 1° for forty-five minutes and were immediately centrifuged for five minutes at approximately 2,000 r. p. m. The supernate was decanted and its absorbance was determined in an EEL photoelectric colorimeter with a standard green filter. The per cent of hemolysis was calculated by dividing the absorbance reading for each unknown solution investigated by the reading obtained from the average complete he-

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† Recipient of 1958–1959 Gustavus A. Pfeiffer Memorial Research Fellowship. Present address: College of Pharmacy, University of Washington, Seattle.
‡ Apoteker, Ph.D., Valby Apotek, Copenhagen, Denmark.
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[1] Merck and Co., Rahway, N. J.

molysis times 100%. The instrument was previously standardized to read zero absorbance for the 0.9% sodium chloride blank solutions. The test was repeated on another day using different blood samples and new solutions for each of those substances which gave hemolysis. These two sets of values were then averaged and are reported in Table I.

The pH of each experimental solution was determined prior to the addition of the blood and this value to the nearest 0.1 is included in the results in Table I. After the 1% blood sample was added, the pH changed slightly in the direction of 7.4. The amount of change usually was small and depended upon the buffer capacity of the solution.

If an experimental solution was colored before the addition of blood, then this same colored solution was employed as the blank. A few solutions were too intensely colored for an accurate colorimetric determination of hemolysis and for these the method was modified as follows: after centrifuging down the erythrocytes and ghosts, the colored supernate was decanted; the remaining erythrocytes were then resuspended in normal saline and centrifuged again. The resulting erythrocyte volume was compared to the volume in the standard 0.9% sodium chloride solution for an approximate percentage of cells hemolyzed. In some instances it is possible that the color of the solution and the color of oxyhemoglobin are not exactly additive. However, only three solutions in the study (cupric sulfate, oxophenarsine hydrochloride, and sodium ascorbate) were colored appreciably, and no tests were made to determine if these colors were additive.

The absorbance readings from some solutions resulted in a greater than 100% hemolysis due to such factors as the darkening of the red color in alkaline solution and oxidation or reduction reactions with the blood. These solutions are reported as 100% if there were no unhemolyzed erythrocytes in the sediment.

After this study was completed Ansel and Husa (13) showed that zinc ions precipitate oxyhemoglobin; therefore, data for the zinc compounds are not included in the study. Furthermore, it is possible that other compounds similarly are incompatible with oxyhemoglobin since the compatibility of the compounds in Table I with oxyhemoglobin was not determined. But, any apparent change in the physical appearance of any of the solutions or of the unhemolyzed erythrocytes following incubation is reported in the footnotes for Table I.

RESULTS AND DISCUSSION

Hemolytic Values.—The percentage of hemolysis found for the 161 compounds studied are listed in Table I including the iso-osmotic concentration (1) used for each and the pH of each iso-osmotic solution.

Solutions of 90 substances prevented hemolysis in iso-osmotic concentration and 71 substances showed varying degrees from slight to complete hemolysis. Solutions of the inorganic salts of moderate pH prevented hemolysis of erythrocytes. The carbohydrates and most alkali salts of organic acids also prevented hemolysis. Most inorganic and organic acids and alkalies with more extreme pH values or with marked oxidation or reduction properties usually penetrated or altered the erythrocyte sufficiently to give some degree of hemolysis.

Many amine salts usually of a monovalent anion failed to prevent hemolysis. This is in agreement with the results of the permeability of erythrocytes to weak electrolytes obtained by Jacobs and Stewart (14), Jacobs, Glassman, and Parpart (15), and Davson (16), which is summarized well by Thomasson and Husa (7) and Marcus and Husa (9).

From an examination of the hemolytic data from the many compounds studied in equal osmolar concentrations, it is evident that there is no distinct pattern indicating the predominance of any single main mechanism of penetration of the erythrocyte by the solute and solvent which results in hemolysis of the erythrocyte. It appears to be a combination of factors such as pH, lipoid solubility, the molecular and ionic size of the particles, and the inhibition of cholinesterase in the cell membrane (9), to name a few. There are also other complex forces which may also play a role because of their possible denaturing actions on the plasma membrane proteins: substances such as alcohols and urea are known to rupture hydrogen bonding; low surface tension forces may denature proteins; and acids, alkalies, and oxidizing agents often change the structure of proteins owing, in part, to their effects on benzene ring structures, such as those present in the amino acids tyrosine and histidine. The picture is still incomplete.

Ammonium Compounds.—It is well known that the ammonium salts are an exception to the generally accepted view that iso-osmotic solutions of inorganic salts of monovalent anions are osmotically indifferent to red blood cells.

The results of the several ammonium compounds in this study are presented in Table II for comparative study. The results of ammonium compounds from Cadwallader and Husa (6) are also included.

The erythrocyte membrane is permeable to the anions in the first column (about 100%) and impermeable to those in the second column (0%). Since ammonium sulfate and phosphate protected erythrocytes from hemolysis, Jacobs and Stewart's (14) statement that ammonium salts of weak acids and strong acids will cause hemolysis should be amended to include only strong acids of monovalent anions. It is only necessary for a membrane to be impermeable to a single species of ion, i. e., anion or cation, for it to be impermeable to the salt of which this ion is a part (16).

Ammonium Chloride.—Since ammonium chloride solution is occasionally employed as an i. m. or i. v. injection or infusion for the prevention of alkalosis, special mention is made of its hemolytic activity. Figure 1 shows a graph of the degree of hemolysis of erythrocytes in various concentrations of ammonium chloride solutions. Instead of obtaining the characteristic (17) S-shaped curve (sigmoid) as in the case of sodium chloride solutions, one obtains a radically different curve, Fig. 1. As the concentration of ammonium chloride increases, the degree of hemolysis decreases from 93% until it reaches a minimum point between 4 and 5% ammonium chloride concentration. Four per cent ammonium chloride solution was found to require

TABLE I.—PERCENTAGE HEMOLYSIS OF ERYTHROCYTES IN ISO-OSMOTIC SOLUTIONS

Substance	Iso-osmotic Concentration, %	Hemolysis, %	Approx. pH
Acetazoleamide sodium	3.85	0	9.2
Alcohol U. S. P.	1.39	100	6.0
Alcohol, dehydrated N. F.	1.28	100	6.1
Alum (potassium) N. F.	6.35	24[a]	3.4
Amiodoxyl benzoate	4.42	2	4.4
Ammonium carbonate U. S. P.	1.29	97	7.7
Ammonium chloride U. S. P.	0.80	93	5.0
Ammonium lactate	2.76	98	5.9
Ammonium nitrate	1.30	91	5.3
Ammonium phosphate, dibasic	1.76	0	7.9
Ammonium sulfate	1.68	0	5.3
Amobarbital sodium U. S. P.	3.60	0	9.3
d-Amphetamine HCl	2.64	98	5.7
Amphetamine phosphate N. F.	3.47	0	4.5
Amphetamine sulfate U. S. P.	4.23	0	5.9
Amydricaine HCl	5.74	84	6.8
Amylcaine HCl	4.98	100	5.6
Antipyrine N. F.	6.81	100	6.1
2-Methylamino-6-hydroxy-6-methylheptane (Aranthol)	3.96	100[b]	12.0
Arecoline HBr N. F.	3.88	41	4.4
Ascorbic acid U. S. P.	5.04	100[c]	2.2
Atropine sulfate U. S. P.	8.85	0	5.0
Barbital sodium	3.12	0	9.8
Boric acid U. S. P.	1.90	100	4.6
Butethamine formate	4.56	93	6.3
Caffeine and sodium benzoate U. S. P.	3.92	0	7.0
Caffeine and sodium salicylate U. S. P.	5.77	0	6.8
Calcium chloride U. S. P.	1.70	0	5.6
Calcium chloride ($6H_2O$)	2.50	0	5.7
Calcium chloride, anhydrous	1.30	0	5.6
Calcium lactate N. F.	4.50	0	6.7
Calcium pantothenate U. S. P.	5.50	0	7.4
Chloramine-T N. F.	4.10	100[c]	9.1
Citric acid U. S. P.	5.52	100[c]	1.8
Cocaine HCl U. S. P.	6.33	47	4.4
Codeine phosphate U. S. P.	7.29	0	4.4
p-Propylaminobenzoic acid-γ-dimethylamino-β-oxypropyl ester HCl (Cornecaine)	7.30	100	6.0
Cupric sulfate N. F.	6.85	trace[d]	3.9
Cupric sulfate, anhydrous	4.09	trace[d]	4.0
Cyclopentamine HCl	2.68	100	5.7
Decamethonium bromide	5.00	0	5.7
Dextroamphetamine phosphate N. F.	3.60	0	4.7
Dextroamphetamine sulfate U. S. P.	4.20	0	5.9
Dextrose U. S. P.	5.51	0	5.9
Dextrose, anhydrous	5.05	0	6.0
Diethylcarbamazine citrate	6.29	100[c]	3.7
Dihydrostreptomycin sulfate U. S. P.	19.40	0	6.1
Dipyrone	4.65	0	7.3
Edrophonium chloride	3.36	0	4.5
Ephedrine HCl N. F.	3.20	96	5.9
Ephedrine sulfate U. S. P.	4.54	0	5.7
Epinephrine bitartrate U. S. P.	5.70	100[c]	3.4
Ethylmorphine HCl U. S. P.	6.18	38	4.7
Ethylnorepinephrine HCl	3.32	5	4.2
Fluorescein sodium U. S. P.	3.34	0	8.7
d-Fructose	5.05	0[e]	5.9
Galactose	4.92	0	5.9
Glycerin U. S. P.	2.60	100	5.9
Glyphylline	10.87	95	5.9
Guanidine HCl	1.47	0	4.7
Hexamethonium bromide	4.99	0	6.0
Hexamethonium chloride	3.30	0	4.7
Histalog	1.91	100[c]	1.6
Histamine di-HCl	2.24	79[f]	3.7
Homatropine HBr U. S. P.	5.67	92	5.0
Hydroxyamphetamine HBr U. S. P.	3.71	92	5.0
Intracaine HCl	4.97	85	5.0
Iodophthalein sodium U. S. P.	9.58	100	9.4
Isoniazid U. S. P.	4.35	100	7.1
Lactic acid U. S. P.	2.30	100[c]	2.1
Lactose U. S. P.	9.75	0[e]	5.8
Magnesium chloride	2.02	0	6.3
Magnesium sulfate	6.30	0	6.2
Mannitol N. F.	5.07	0[g]	6.2
Menadione sodium bisulfite U. S. P.	5.07	0	5.3
Meperidine HCl U. S. P.	4.80	98	5.0
Mephentermine sulfate U. S. P.	4.74	0	4.5
Methacholine bromide	3.77	0	5.0
Methacholine chloride U. S. P.	3.21	0	4.5
Methadone HCl U. S. P.	8.59	100[c]	5.0
Methamphetamine HCl U. S. P.	2.75	97	5.9
Methenamine U. S. P.	3.68	100	8.4
Methoxamine HCl U. S. P.	3.82	88	5.2
Methylatropine bromide	7.03	0	5.7
Monoethanolamine N. F.	1.76	100	11.4
Naphazoline HCl N. F.	3.99	100	5.3
Neoarsphenamine	2.32	17	7.8
Nicotinamide U. S. P.	4.49	100	7.0
Nikethamide U. S. P.	5.94	100	6.9
Oxophenarsine HCl U. S. P.	3.67	trace[a]	2.3
Penicillin G, potassium U. S. P.	5.48	0	6.2
Pentylenetetrazole U. S. P.	4.91	100	6.7
Phenobarbital sodium U. S. P.	3.95	0	9.2
Phenol U. S. P.	2.80	0[h]	5.6
Phenylephrine HCl U. S. P.	3.00	0	4.5
Phenylpropanolamine HCl	2.60	95	5.3
Phenylpropylmethylamine HCl	2.70	95	5.4
Pilocarpine HCl U. S. P.	4.08	89	4.0
Potassium chlorate N. F.	1.88	0	6.9
Potassium chloride U. S. P.	1.19	0	5.9
Potassium iodide U. S. P.	2.59	0	7.0
Potassium nitrate N. F.	1.62	0	5.9
Potassium phosphate N. F.	2.08	0	8.4
Potassium phosphate, monobasic	2.18	0	4.4
Potassium sulfate	2.11	0	6.6
Probarbital sodium N. F	3.10	0	10.0
Procaine HCl U. S. P.	5.05	91	5.6

TABLE I (*continued*)

Substance	Iso-osmotic Concentration, %	Hemolysis, %	Approx. pH
Propylene glycol U. S. P.	2.00	100	5.5
Quinine and urea HCl N. F.	4.50	64[f]	2.9
Racephedrine HCl N. F.	3.07	94	5.7
Resorcinol U. S. P.	3.30	96	5.0
Scopolamine HBr U. S. P.	7.85	8	4.8
Silver nitrate U. S. P.	2.74	0[d]	5.0
Silver protein, mild N. F.	5.51	0	9.0
Sodium acetate, anhydrous	1.18	0	8.1
Sodium aminosalicylate U. S. P.	3.27	0	7.3
Sodium arsenate, dibasic	3.83	0	8.8
Sodium ascorbate	3.00	0	6.9
Sodium benzoate U. S. P.	2.25	0	7.5
Sodium bicarbonate	1.39	0	8.3
Sodium biphosphate, anhydrous	2.10	0	4.1
Sodium biphosphate U. S. P.	2.45	0	4.1
Sodium biphosphate ($2H_2O$)	2.77	0	4.0
Sodium bisulfite U. S. P.	1.50	0[i]	3.0
Sodium borate U. S. P.	2.60	0	9.2
Sodium cacodylate N. F.	3.30	0	8.0
Sodium carbonate, anhydrous	1.32	100	11.1
Sodium carbonate, mono hydrated U. S. P.	1.56	100	11.1
Sodium chloride U. S. P.	0.90	0	6.7
Sodium citrate U. S. P.	3.02	0	7.8
Sodium iodide U. S. P.	2.37	0	6.9
Sodium lactate	1.72	0	6.5
Sodium metabisulfite	1.38	5[j]	4.5
Sodium nitrate	1.36	0	6.0
Sodium nitrite U. S. P.	1.08	0[a]	8.5
Sodium phosphate, exsiccated, N. F.	1.75	0	9.1
Sodium phosphate N. F.	3.33	0	9.2
Sodium phosphate, dibasic ($2H_2O$)	2.23	0	9.2
Sodium phosphate, dibasic ($12H_2O$)	4.45	0	9.2
Sodium propionate N. F.	1.47	0	7.8
Sodium salicylate U. S. P.	2.53	0	6.7
Sodium sulfate N. F.	3.95	0	6.1
Sodium sulfate, anhydrous	1.61	0	6.2
Sodium thiosulfate N. F.	2.98	0	7.4
Sorbitol ($^1/_2 H_2O$)	5.48	0	5.9
Succinylcholine chloride U. S. P.	4.48	85[a]	3.5
Sucrose U. S. P	9.25	0	6.4
Sulfacetamide sodium U. S. P.	3.85	0	8.7
Sulfadiazine sodium U. S. P.	4.24	0	9.5
Sulfathiazole sodium N. F.	4.82	0	9.9
Tartaric acid N. F.	3.90	75[f]	1.7
Tetraethylammonium bromide	3.17	0	5.7
Tetraethylammonium chloride	2.67	0	4.7
Thiamine HCl U. S. P.	4.24	87[f]	3.0
Tolazoline HCl U. S. P.	3.05	93	4.8
Trimethadione U. S. P.	4.22	100	6.0
Tropacocaine HCl	4.92	75	5 7
Tuaminoheptane sulfate N. F.	3.40	0	5.9
Urea U. S. P.	1.63	100	6.6
Urethan U. S. P.	2.93	100	6.3
Vinbarbital sodium	3.55	0	9.7

[a] R. B. cells turned black color. [b] Solution turned olive-green color. [c] Solution turned brown-black color. [d] R. B. cells shrunk in size and turned black color. [e] R. B. cells clumped. [f] Solution and R. B. cells darkened. [g] Solution became slightly turbid. [h] R. B. cells turned brown color and solution became milky. [i] R. B. cells turned violet color. [j] Solution and R. B. cells turned violet color.

the presence of 2.5% dextrose to prevent hemolysis. The degree of hemolysis is then greater in the ammonium chloride solutions from this minimum point up to 10%.

Various proportions of sodium chloride were added to three different concentrations of ammonium chloride solution: 0.8, 0.6, and 0.4%. It was found that an approximately identical proportion of sodium chloride 0.65%, was required to prevent hemolysis of erythrocytes by each of the ammonium chloride solutions, Table III.

The commonly accepted iso-osmotic composition of 0.8% ammonium chloride gives 93% hemolysis. To prevent hemolysis of erythrocytes, it was found under the conditions of the procedure mentioned previously that 0.65% sodium chloride or 4% dextrose must be added to the 0.8% ammonium chloride solution. This will give an isotonic but hyperosmotic solution.

Amphetamine Compounds.—The hemolysis obtained in solutions of the amphetamine salts of monovalent and polyvalent anions provides an interesting comparison with the ammonium salts. Iso-osmotic solutions of amphetamine hydrochloride, hydrobromide, and methamphetamine hydrochloride each produced about 100% hemolysis while the solutions of amphetamine phosphate and sulfate prevented hemolysis. The erythrocytes are more permeable to the monovalent anions of amphetamine than to the polyvalent sulfate and phosphate anions. This is in agreement with Davson (16) and Thomasson and Husa (7) who found that the sulfate and other polyvalent anions penetrate the erythrocyte very slowly in comparison with univalent anions.

Ephedrine Compounds.—Thomasson and Husa (7) mentioned the difference in the hemolytic activity between ephedrine hydrochloride and sulfate solutions. In the present study racephedrine hydrochloride was also found to allow nearly the same degree of hemolysis as ephedrine hyprochloride 94 and 100%, whereas ephedrine sulfate prevented hemolysis. This is, one would assume, because the membrane is more permeable to the chloride than to the sulfate ion.

Because of the great difference in their hemolytic effects, solutions of ephedrine hydrochloride and sulfate were investigated further in order to find out how much sodium chloride and sodium sulfate must be added to prevent the hemolysis of their iso-

TABLE II.—ERYTHROCYTE HEMOLYSIS IN ISO-OSMOTIC SOLUTIONS OF VARIOUS AMMONIUM SALTS

Substance	Hemolysis, %	Substance	Hemolysis, %
Ammonium acetate[a]	100	Ammonium citrate[a]	0
Ammonium benzoate[a]	100	Ammonium phosphate, dibasic	0
Ammonium carbonate	98	Ammonium sulfate	0
Ammonium chloride	93	Ammonium tartrate[a]	0
Ammonium lactate	98	Tetraethyl-ammonium bromide	0
Ammonium nitrate	92	Tetraethyl-ammonium chloride	0
Ammonium salicylate[a]	100		

[a] Results from Cadwallader and Husa (6).

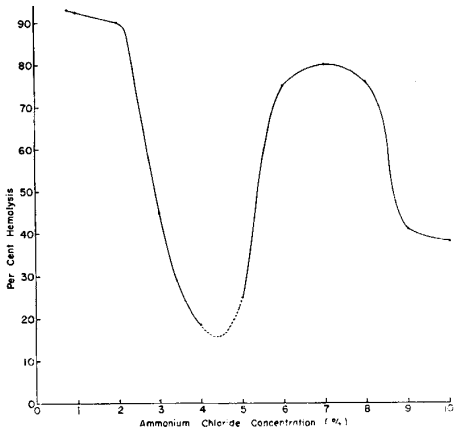

Fig. 1.—Erythrocyte hemolysis in ammonium chloride solutions of varying concentrations.

osmotic solutions and of various dilutions of these. Various proportions of sodium chloride were added to iso-osmotic solutions of ephedrine hydrochloride and the degree of hemolysis was determined, Table IV.

When 0.6% sodium chloride is added, the hemolysis is prevented. This is slightly more than the required amount of sodium chloride which alone would retard hemolysis. Thus the ephedrine hydrochloride is similar to ammonium chloride and other substances, as reported by Thomasson and Husa (7), which act in a negative manner in preventing hemolysis in the presence of sodium chloride. In other words, their iso-osmotic solutions cause an increased fragility of erythrocytes.

The per cent of hemolysis given by various proportions of the iso-osmotic concentration of ephedrine hydrochloride solutions containing the necessary amounts of sodium chloride or of sodium sulfate to maintain iso-osmoticity was determined

TABLE III.—ERYTHROCYTE HEMOLYSIS IN AMMONIUM CHLORIDE SOLUTIONS CONTAINING VARYING AMOUNTS OF SODIUM CHLORIDE

NaCl Added, %	Hemolysis, % Ammonium Chloride Concentration 0.8%	0.6%	0.4%
0.50	82	80	76
0.55	45	43	39
0.60	3	6	2
0.65	0	0	0
0.70	0	0	0
0.80	0	0	0

TABLE IV.—ERYTHROCYTE HEMOLYSIS IN ISO-OSMOTIC EPHEDRINE HYDROCHLORIDE SOLUTIONS (3.2%) CONTAINING VARYING PROPORTIONS OF SODIUM CHLORIDE

NaCl Added, %	Hemolysis, %
0.3	94
0.4	91
0.5	6
0.6	0
0.7	0

TABLE V.—ERYTHROCYTE HEMOLYSIS IN VARIOUS EPHEDRINE HYDROCHLORIDE SOLUTIONS MADE ISO-OSMOTIC WITH SODIUM CHLORIDE

Ephedrine HCl Iso-osmotic Multiple	Concn., %	NaCl Added Iso-osmotic Multiple	Concn., %	Hemolysis, %
1 1/4	4.0	0	0	99
1	3.2	0	0	96
3/4	2.4	1/4	0.23	96
1/2	1.6	1/2	0.45	94
3/8	1.2	5/8	0.56	1
1/4	0.8	3/4	0.68	0

TABLE VI.—ERYTHROCYTE HEMOLYSIS IN VARIOUS EPHEDRINE HYDROCHLORIDE SOLUTIONS MADE ISO-OSMOTIC WITH SODIUM SULFATE

Ephedrine HCl Iso-osmotic Multiple	Concn., %	Na_2SO_4 Added Iso-osmotic Multiple	Concn., %	Hemolysis, %
1	3.2	0	0	96
3/4	2.4	1/4	0.38	16
1/2	1.6	1/2	0.76	0
1/4	0.8	3/4	1.14	0

and the results are presented in Table V for the addition of sodium chloride and Table VI for the addition of sodium sulfate.

Ephedrine hydrochloride solutions have strong hemolytic activity until their concentrations are reduced to less than one-half iso-osmotic value with sodium chloride.

The presence of sodium sulfate depresses much more strongly the hemolytic activity of ephedrine hydrochloride than does an equivalent quantity of sodium chloride.

The per cent of hemolysis given by various proportions of the iso-osmotic concentration of ephedrine sulfate solutions adjusted with sodium chlo-

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