Isotonic Solutions XIII

Hemolysis of Red Corpuscles by Various Substances in the Presence of Sodium Chloride

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Various compounds which cause hemolysis in the presence of 0.6% sodium chloride were studied in environments of higher concentrations of sodium chloride. An increase in the concentration of sodium chloride reduced the degree of hemolysis produced by a compound in the presence of 0.6% sodium chloride in some instances, but this effect did not occur as a general rule for all compounds. Since 0.1% sodium carbonate did not cause an appreciable discoloration of oxyhemoglobin, it was retained as a standard for total hemolysis in the hemolytic method employed.

PREVIOUS INVESTIGATORS have emphasized the uncertainty associated with the use of physicochemical data to determine whether a solution is isotonic with the blood since methods utilizing colligative properties of solutions ignore the selective permeability of the corpuscular membrane and other pertinent factors. This variation is vividly shown by the fact that some substances cause hemolysis even in the presence of 0.6% sodium chloride which would prevent hemolysis if no other substance were present (1–3).

Compounds studied in this investigation generally exhibited a common property in their behavior toward red blood corpuscles when examined in the presence of 0.6% sodium chloride, *i.e.*, at a certain point, a relatively small change in concentration of the drug generally caused an abrupt increase in the degree of hemolysis. Exceptions to this were procaine hydrochloride and 2-propoxyprocaine hydrochloride.

Marcus and Husa (2) found that solutions of procaine hydrochloride ranging in concentration from 4.00 to 10.00% caused hemolysis in the presence of 0.6% sodium chloride. Using the hematocrit method, Setnikar and Temelcou (4) found that a solution of 5.05% procaine hydrochloride in 0.6% sodium chloride produced hemolysis of rabbit erythrocytes, but hemolysis was prevented when the sodium chloride concentration was increased to 1.3%.

The purpose of the present investigation was to determine by use of the hemolytic method whether the presence of hyperosmotic concentrations of sodium chloride might have general application in preventing the destruction of red blood cells by substances which cause hemolysis in an environment of 0.6% sodium chloride. In addition, a recent report (5) indicated that the alkalinity of 0.1% sodium carbonate solution caused a discoloration of oxyhemoglobin which would invalidate its use as a standard for 100% hemolysis in the hemolytic method. Five hemolyzing solutions were compared in regard to the degree of hemolysis each produced and to determine whether there was any significant change in the color of oxyhemoglobin upon mixing with each hemolyzing solution.

EXPERIMENTAL

Collection of Blood.—The human blood used in various experiments was obtained primarily from the veins of the arms of a 27-year old white female by means of a hypodermic syringe. Blood was taken occasionally from other white volunteers, and a fresh blood sample was utilized for each experiment. Each fresh blood sample was treated in the manner previously described by Grosicki and Husa (6).

Chemicals.—The medicinal chemicals were contributed by the manufacturers. The other chemicals were of C.P. grade with the exception of saponin which was a purified grade.

Preparation of Solutions.—Solutions were prepared by diluting aliquot portions of appropriate stock solutions with triple distilled water.

Quantitative Determination of Per Cent Hemolysis.—Determination of the degree of hemolysis produced by various solutions under examination was accomplished by use of the hemolytic method described by Grosicki and Husa (6). Fragility tests are used to minimize deviations due to the variability of blood when hemolysis is due entirely to osmotic effects. However, no standard method has been established to compensate for the variability of blood when some factor such as an effect on the corpuscular membrane is operative in the presence of 0.6% sodium chloride which would prevent hemolysis if no other compound were present. Consequently, fragility tests offered no advantage in

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the majority of experiments performed in this investigation and were deemed unnecessary in obtaining information which would reveal whether or not the addition of hyperosmotic sodium chloride concentrations would prevent hemolysis by certain compounds which cause hemolysis in the presence of 0.6% sodium chloride. With the elimination of the fragility tests, solutions of sodium chloride alone, corresponding to the sodium chloride concentrations utilized in a particular experiment, were used as controls.

Comparison of Hemolyzing Solutions Producing Approximately Complete Hemolysis.—The hemolyzing solutions tested exhibited no appreciable degree of absorbance *per se*. The hemolytic method was employed to compare the degree of hemolysis elicited by triple distilled water, 0.1% sodium chloride, 0.1% sodium carbonate, 0.01% saponin, and a solution containing 0.1% sodium carbonate and 0.01% saponin. The greatest difference in the average colorimeter readings was 1.3% when per cent hemolysis was calculated on the basis of triple distilled water producing complete hemolysis; this difference was considered to be within experimental error. In other tests, colorimeter readings were determined immediately after mixing each of the hemolyzing solutions with oxyhemoglobin solution prepared by laking red blood cells in triple distilled water. Colorimeter readings were also taken after storage in a refrigerator for 4- and 24-hour periods. Results indicated that no appreciable discoloration of oxyhemoglobin occurred within the time interval required for hemolytic determinations. Consequently, 0.1% sodium carbonate solution was retained as a standard for complete hemolysis in the hemolytic method employed in this investigation.

Reproducibility of Results.—Parallel results and duplicate colorimeter readings were generally obtained when the degree of hemolysis approximated 0 or 100%. However, experimental data associated with concentrations of some compounds producing an intermediate degree of hemolysis exhibited excessive variations, which might be attributed to factors the hemolytic method employed, is incapable of controlling. However, ascertaining the exact percentage of hemolysis produced by the compounds was not essential in determining whether or not the addition of hyperosmotic quantities of sodium chloride would prevent hemolysis produced by the compounds in the presence of 0.6% sodium chloride.

Experiments were conducted to determine whether an uncontrollable factor might be introduced in hemolytic determinations during the time lapse between the addition of blood to the solution under study and the subsequent inversion of the colorimeter tubes to insure thorough mixing. Experiments were performed in which the solutions under study, as well as the 0.85% sodium chloride blank and 0.1% sodium carbonate standards for 100% hemolysis, were treated in the same manner with the exception that one portion of the tubes was shaken immediately after the addition of blood, whereas their counterparts were treated in the usual way. Results indicated that the time lapse between the addition of blood and shaking of the mixture did not account for the variations found in duplicate tubes.

TABLE I.—DEGREE OF HEMOLYSIS PRODUCED BY VARIOUS CONCENTRATIONS OF COMPOUNDS IN VARIOUS CONCENTRATIONS OF SODIUM CHLORIDE[a]

Compound	Per Cent Compound	Degree of Hemolysis[b] NaCl, % 0.6	0.9	1.2	1.5
Procaine HCl	4.00	L	–	–[c]	–
	6.00	M	M	–[c]	–
	10.00	M	M	–[c]	–
2-Propoxy-procaine HCl	1.00	–	–	–	–
	2.00	M	–	–	–
	7.00	–	–	–	–
NH_4 Salicylate	1.00	–	–	–	–
	2.20	M	–	–	–
	2.60	H	M	M	L
NH_4 Benzoate[d]	2.40	–	–	–	–
	3.20	M	L	L	L
	4.00	H	H	H	H
Pramoxine HCl[d]	0.10	–	–	–	–
	0.12	M	L,M	L,M	M
	0.15	H	H	H	H
Benoxinate HCl[d]	0.10	–	–	–	–
	0.30	L,M	L	L	–
	0.90	H	H	H	H
Hexylcaine HCl[d]	0.70	L,M	–,L	–,L	–
	0.90	M	M	L,M	M
	2.00	H	H	H	H
Butacaine SO_4[d]	0.30	–,L	–,L	–,L	–,L
	0.35	L,M	L,M	L,M	L,M
	0.50	H	H	. . .[e]	. . .[e]
Phenmetrazine HCl[d]	3.00	L,M	L,M	L,M	M
	4.20	M	M	M,H	H
	5.40	H	H	H	H

[a] Results of two blood samples unless otherwise indicated. [b] –, no hemolysis; L, <15%; M, 15–85%; H, >85%. [c] Determined in 1.3% sodium chloride. [d] Results of four blood samples. [e] Precipitation before addition of blood.

DISCUSSION

Higher concentrations of sodium chloride generally caused a significant reduction in the degree of hemolysis produced by procaine and 2-propoxyprocaine hydrochlorides, ammonium salicylate, and ammonium benzoate in the presence of 0.6% sodium chloride. In most cases, hemolysis was eliminated. However, a smaller inhibitory action was exhibited by solutions of ammonium benzoate. Furthermore, a notable exception was apparent in 4.00% solutions of ammonium benzoate which caused approximately total hemolysis in environments of 0.6, 0.9, 1.2, and 1.5% sodium chloride as indicated in Table I. Absence of hemolysis in procaine hydrochloride solutions ranging in concentration from 4.00 to 10.00% in the presence of 1.3% sodium chloride indicates that the results obtained by Setnikar and Temelcou (4) for rabbit blood also hold true for human blood.

Two trends appeared evident upon examination of the experimental data obtained for pramoxine, benoxinate, and hexylcaine hydrochlorides, and butacaine sulfate. Results generally indicated that additional quantities of sodium chloride inhibited hemolysis somewhat when concentrations of the drug were used such that the degree of hemolysis produced in the presence of 0.6% sodium chloride was low. However, in concentrations of the compound causing degrees of hemolysis ranging from intermediate to essentially complete in 0.6% sodium chloride, the addition of hyperosmotic quantities of sodium chloride did not appear to inhibit hemolysis.

A crystalline precipitate appeared in solutions of several concentrations of butacaine sulfate in 1.2 and 1.5% sodium chloride before the addition of blood. In contrast to the effect produced on butacaine sulfate solutions by increased proportions of sodium chloride, several solutions of pramoxine hydrochloride in some concentrations of sodium chloride exhibited evidence of precipitation after incubation and centrifugation of the blood-salt mixtures. Higher concentrations of sodium chloride appeared to decrease the precipitation until the effect was eliminated as far as visual observation could detect.

Experimental data indicated that phenmetrazine hydrochloride did not behave in a manner similar to any of the other compounds studied in this investigation. As the concentration of sodium chloride was increased, a corresponding augmentation in the degree of hemolysis, compared to that produced in the presence of 0.6% sodium chloride, was effected. The only exception to this trend was exhibited by 5.40% phenmetrazine hydrochloride solutions in environments of 0.6, 0.9, 1.2, and 1.5% sodium chloride; essentially complete hemolysis was elicited in the presence of 0.6% sodium chloride, and increasing the concentration of sodium chloride afforded no apparent change in the degree of hemolysis.

SUMMARY

1. An increase in the concentration of sodium chloride reduced the degree of hemolysis produced by procaine hydrochloride, 2-propoxyprocaine hydrochloride, and ammonium salicylate solutions in an environment of 0.6% sodium chloride. A similar effect was noted in most of the concentrations of ammonium benzoate studied.

2. Increasing the concentration of sodium chloride in solutions of pramoxine, benoxinate, and hexylcaine hydrochlorides, and butacaine sulfate afforded some reduction in the degree of hemolysis produced by the compound in 0.6% sodium chloride in cases where the degree of hemolysis was slight. No reduction in the degree of hemolysis was observed as a result of augmenting the sodium chloride concentration when the compound under examination caused degrees of hemolysis ranging from intermediate to essentially complete in the presence of 0.6% sodium chloride.

3. Higher concentrations of sodium chloride generally increased the degree of hemolysis produced by phenmetrazine hydrochloride solutions in the presence of 0.6% sodium chloride. In the case of 5.40% solutions of phenmetrazine hydrochloride, hemolysis was essentially complete in presence of 0.6, 0.9, 1.2, and 1.5% sodium chloride.

4. No appreciable discoloration of oxyhemoglobin resulted from the presence of 0.1% sodium carbonate. Triple distilled water, 0.1% sodium chloride, 0.1% sodium carbonate, 0.01% saponin, and a solution containing 0.1% sodium carbonate and 0.01% saponin caused the same degree of hemolysis within experimental error.

5. The slight time lapse between the addition of blood to the solution under study and the subsequent shaking of the mixture seemed to produce no appreciable variation in experimental data.

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Modification of Physical Properties of Certain Antitussive and Antihistaminic Agents by Formation of N-Cyclohexylsulfamate Salts

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The N-cyclohexylsulfamic acid salts of four well known therapeutic agents were prepared. Salts of two of the compounds, dextromethorphan and chlorpheniramine, were found to have greatly improved taste and increased solubility. Accelerated aging studies indicate good stability.

PROMINENT among the factors which must be considered in the development of oral dosage forms are those of solubility and taste acceptability. These factors present no great problem provided the drug is to be administered in such dosage forms as capsules or tablets which are designed to be swallowed as a unit. However, in those cases where it is desirable to administer a drug in liquid form, chewable tablet, lozenge, or other such forms, solubility and taste acceptability become factors of prime consideration.

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