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Hemolytic Actions of Short-Chain Alkylamine Hydrochlorides

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Abstract □ The hemolytic actions of a number of short-chain alkylamine hydrochlorides were studied. Primary salts, produced complete hemolysis but secondary, tertiary, and quaternary salts produced incomplete hemolysis. This was attributed to the increased bulkiness and lipophilicity of the successively alkyl-substituted amine cations. In each group of the salts, the hemolytic activity increased with increasing alkyl chain length. The importance of the interaction of the alkylamine cations with phospholipid in the erythrocyte membrane was emphasized on the basis of the amount of lipids released by the cations from erythrocytes.

Keyphrases □ Alkylamine hydrochlorides—*in vitro* hemolytic action □ Hemolytic effect—alkyl chain length □ Erythrocyte aggregation—alkylamine hydrochlorides

A previous paper (1) has reported a remarkable difference in hemolytic activity between short-chain anionic and cationic surface-active electrolytes. Thus, sodium alkyl sulfates and sodium carboxylates with shorter hydrophobic chain than hexyl radical are hemolytically inactive whereas the corresponding members of alkylamine hydrochlorides and alkyl pyridinium iodides are capable of causing hemolysis. Furthermore, the release of a considerable amount of phospholipids by the surface-active cations were detected by means of TLC prior to lysis (1, 2).

These findings have prompted the study of the hemolytic actions of a number of short-chain alkylamine hydrochlorides in relation to their molecular structure.

EXPERIMENTAL

Preparation of Short-Chain Alkylamine Hydrochlorides—The amine salts were prepared by passing dry hydrogen chloride through benzene solutions of the amines. The precipitated salts were collected and purified by recrystallization from ethanol.

Hemolysis of Dog Erythrocytes—The method of preparing the erythrocyte suspension from dog blood was the same as that used in the previous work (1) except that aqueous 0.9% NaCl solution was employed as the washing liquid and the suspending medium of erythrocytes instead of the isotonic phosphate buffer solution.

The percent hemolysis was estimated, as in the previous work (1), by determining spectrophotometrically the amount of released hemoglobin in the supernatant liquid by the amine hydrochlorides after centrifuging the unhemolyzed cells. The concentration of erythrocytes was 2.5% v/v. Some of the amine salts used in this work caused the aggregation of erythrocytes at low concentrations, and the aggregates formed were observed under a microscope and photographed.

Determination of the Amount of Lipids Released—The procedures of determining the amount of lipids released from the erythrocytes were identical with those adopted in the previous work (2).

RESULTS AND DISCUSSION

With the secondary, tertiary, and quaternary salts, complete hemolysis was not observed presumably due to the limited solubility of these salts and a strong interaction with the released hemoglobin. Figure 1 shows a typical hemolysis curve for mono-, di-, and triethylamine hydrochlorides.

In Table I are listed the hemolytic concentrations of the amine salts, determined from the hemolysis curves, at three different degrees of hemolysis.

The hemolytic activity increases with increasing alkyl chain length in each group of the primary, secondary, and tertiary salts. The same is true of the quaternary salts though the results are not given in the table. Thus, the hemolytic concentrations required of tetramethyl, tetraethyl, and tetrabutyl ammonium chlorides to produce 10% lysis were 1.5, 1.0, and 0.5 moles/l., respectively. These results would come from the increasing surface activity of the salts with alkyl chain length.

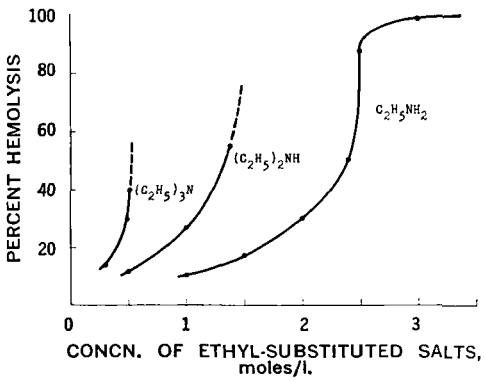

Figure 1—*Hemolysis curves for mono-, di-, and triethylamine hydrochlorides.*

Table I—Hemolytic Concentrations in Moles Per Liter of Alkylamine Hydrochlorides[a]

Material	Hemolysis, % 100	50	30
CH_3NH_2HCl	4.5	2.5	2.0
$C_2H_5NH_2HCl$	3.4	2.4	2.0
$C_3H_7NH_2HCl$	2.9	2.2	1.9
$C_4H_9NH_2HCl$	1.9	1.6	1.5
$(C_2H_5)_2NHHCl$	—[b]	1.3	1.1
$(C_4H_9)_2NHHCl$	—[b]	1.0	0.95
$(CH_3)_3NHCl$	—[b]	2.5	2.2
$(C_2H_5)_3NHCl$	—[b]	0.52[c]	0.48
$(C_4H_9)_3NHCl$	—[b]	0.47[c]	0.46

[a] The results on the quaternary salts used in this work were not listed here since they did not cause appreciable hemolysis. [b] The complete lysis was not observed within the limit of solubility. [c] Extrapolated values.

The number of alkyl chains in a molecule has a similar effect on the hemolytic activity of the salts with the same chain length. Thus, the hemolytic activity increases in the order: tertiary > secondary > primary. This may also be related to the same increasing order of surface activity of these salts reported in the literature (3).

Since the erythrocyte surface is negatively charged due to the presence of the dissociated carboxyl residues of mucosaccharide (4, 5), the alkylamine cations will be taken up by the negatively charged sites. The adsorption of the cations will neutralize the negative charges of erythrocyte surface and make it lipophilic, thereby permitting the aggregation of erythrocytes prior to lysis. In fact, the amine salts produced the aggregates with the exception of the primary ones. Figure 2 shows an example of the aggregates formed.

The fact that the primary amine salts can hardly cause the aggregation of erythrocytes prior to lysis although they are strongly hemolytically active indicates the necessity of taking another factor into account. This would be the penetrability of the alkyl amine cations into the interior of the erythrocyte membrane because the incorporated cations only can interact with various components in the membrane strongly enough to cause complete hemolysis. The extent to which the penetration can occur will depend on the shape and size of the cations. Thus, the successive substitution of an alkyl radical for a hydrogen atom attached to the nitrogen atom of a primary amine cation makes the cation more lipophilic and bulkier, and the penetration would consequently be harder than before. This may be a reason for the incomplete hemolysis of the successively alkyl-substituted amine salts, though the hemolytic activity of these compounds increases with the number of alkyl chains in a molecule at the initial stage of hemolysis.

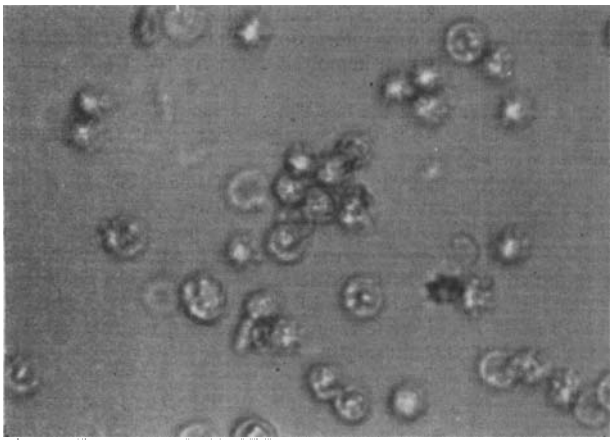

Figure 2—*Photomicrograph of the aggregates formed in the presence of* $(C_2H_5)_2NHHCl$.

Table II—Relative Amount Released of Cholesterol and Phospholipids from Erythrocytes by 0.001 m*M* Ethyl-Substituted Alkylamines[a]

Amine	Cholesterol	Cephalin	Lecithin
None (saline alone)	1.0	1.0	1.0
$C_2H_5NH_2HCl$	1.0	2.1	6.8
$(C_2H_5)_2NHHCl$	1.1	3.7	7.3
$(C_2H_5)_3NHCl$	1.2	4.3	7.5
$(C_2H_5)_4NCl$	1.5	5.5	8.6

[a] Values are the averages of duplicate experiments.

In connection with this, the amount released of cholesterol and phospholipids by 0.001 m*M* solutions of ethyl-substituted salts from erythrocytes is given in Table II.

The amount of cholesterol released is not appreciably affected by the presence of the amine salts, while cephalin and lecithin are considerably released. It is likely, therefore, that hemolysis would be brought about by the interaction of the amine cations with phospholipids in the erythrocyte membrane. Several workers (6, 7) have recently suggested, independently of the present authors, the importance of phospholipid portion of erythrocyte membrane in hemolysis by cationic surface-active agents. This suggestion appears to be consistent with the experimental results reported here.

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