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Effects of Triethanolamine on Hemolysis.*

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The author's previous work[1] on the surface tension lowering substance, sodium taurocholate, and its effects on red blood cells as demonstrated by hemolysis suggested like observations on triethanolamine, N $(C_2H_4OH)_3$,[2] a new surface tension lowering compound now used commercially as an emulsifying agent and superior to similar agents previously used because of its low alkalinity, ability to cause finer and more stable dispersions, its slightly bactericidal powers, etc.

Approximately 25 human bloods were examined, a drop of blood drawn from the patient by syringe being added directly to each tube containing 2 cc. of the solution to be observed. Varying dilutions of sodium chloride, potassium chloride, calcium chloride, sodium hydroxide, hydrochloric acid, acetone, saponin, sodium taurocholate, venom from the bothrops atrox and sulphanilic acid were observed for hemolysis as such and containing 5% triethanolamine and the results compared. The influence of triethanolamine on the pH of the solutions and the time of hemolysis were also noted. The first readings registered were taken 2 hours after the addition of the blood. Normal sodium chloride was used as a vehicle with the exception of the sodium, potassium and calcium chloride dilutions and where otherwise noted. The pH of the distilled water and normal sodium chloride ranged from 5.6-5.8.

In the case of the sodium chloride dilutions, hemolysis began at the .40% concentration of the salt without triethanolamine and at the .30% concentration with the triethanolamine, the triethanolamine in each case definitely increasing the alkalinity of the solutions. Similar results were obtained with the potassium and calcium chlorides. Hemolysis began at .65% in the case of the potassium salt without triethanolamine and at .45% with triethanolamine, and the alkalinity of the dilutions was increased markedly by the tri-

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I wish to thank the Touro Infirmary for the blood specimens which made this work possible.

[1] Williams, J. W., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 913.

[2] Wilson, A. L., *Ind. Eng. Chem.*, 1930, **22**, 143.

ethanolamine. Hemolysis began in the case of calcium chloride at .90% without and .60% with triethanolamine, and the alkalinity of the solutions was increased definitely by the triethanolamine.

In the case of sodium hydroxide a brown sediment appeared in concentrations of 1:128 and greater, both with and without the triethanolamine, and the pH was little affected. Likewise in the case of acetone a brown sediment appeared in concentrations of 1:4 and greater, both with and without the triethanolamine added. The pH of pure acetone changed from 5.4 to 6.8 on the addition of triethanolamine and the pH of the 1:2 dilution changed from 6.8 to 8.4 on the addition of the same substance.

In the case of hydrochloric acid hemolysis occurred in concentrations of 1:3360 and greater without triethanolamine, and in concentrations of 1:40 and greater with triethanolamine, the pH changing to 7.2 in the 1:20 dilution and to 8.4 in the 1:320 dilution on the addition of triethanolamine. Sulphanilic acid acted similar to hydrochloric. Hemolysis occurred in concentrations of 1:3200 and greater without triethanolamine, and in concentrations of 1:50 and greater with triethanolamine. The pH of the sulphanilic acid in the 1:50 dilution increased to 8.4 on addition of triethanolamine.

In the case of sodium taurocholate hemolysis began in concentrations of 1:100 without and 1:1600 with triethanolamine, and the triethanolamine in each case increased the alkalinity of the solutions. Saponin reacted in a somewhat similar but less pronounced manner, hemolysis beginning in the 1:6400 dilution without and the 1:12800 dilution with triethanolamine, and likewise the alkalinity of the solutions was increased by the triethanolamine.

In the case of venom, hemolysis did not occur either with or without triethanolamine in concentrations ranging up to 1:25 the latter of which was very opalescent. The alkalinity of the solutions, however, was increased.

Triethanolamine dilutions of 1:6389760 in distilled began to influence the pH toward the alkaline and at the dilution 1:24960 the pH was 8.4. Dilutions of triethanolamine in normal saline of 1:4 and greater do not as a rule show immediate hemolytic effects, nor do they show effects in 2 hours. However, there is a delayed hemolytic effect which occurs in 6 hours. A like delayed hemolytic effect was observed in all 5% triethanolamine dilutions of sodium chloride, potassium chloride, calcium chloride, sodium hydroxide, acetone, saponin, sodium taurocholate, venom with the exception of the 1:25 dilution which showed no hemolysis, sulphanilic acid with the exception of the unhemolysed dilutions of concentrations

greater than 1:800 which took 32 hours, and hydrochloric acid with the exception of unhemolysed concentrations greater than 1:640 which took 18 hours.

In summary we will attempt to explain some of the differences which are apparent when triethanolamine is added. The temporary increase in resistance of red blood cells to hemolysis when triethanolamine is added to the sodium, potassium and calcium chloride dilutions might possibly be explained by the ability of triethanolamine to cause absorption of the salt on the surface of the red blood cells, or rather on the surface of the triethanolamine film surrounding the red cells, so that as a result the media adjacent to the red cells is less hypotonic than the figures would indicate, and equilibrium is maintained until the physico-chemical changes in the red cells destroy this equilibrium and hemolysis is effected. There is no or very little difference in the dilutions in which hemolysis takes place in the case of acetone and sodium hydroxide with and without triethanolamine, but it is to be expected that a substance of low alkalinity such as triethanolamine would have little effect on a strong alkali or a substance of the reactive properties of acetone. When triethanolamine is added to saponin and sodium taurocholate we in all probability have an addition of forces militating toward hemolysis, since we have substances toxic to red cells with abilities to lower surface tension and emulsify combined in one solution. In the case of hydrochloric and sulphanilic acids, triethanolamine probably acts as a protective of the red cell by forming a surrounding envelope and thus acting to maintain equilibrium between physico-chemical forces outside and those within the red cell. In addition, triethanolamine may interfere with the dissociation of these acids and thus reduce their activities. It should be noted here that the forces tending to maintain equilibrium are such that hemolysis is staved off longer in certain of the dilutions. In the case of venom very little effect is shown (unfortunately it was only possible to obtain a small quantity from the Antivenom Institute of America) and the probable reason why hemolysis did not take place in the 1:25 dilution when triethanolamine was added was that the phase was a triethanolamine and red cell in venom solution resulting in no triethanolamine being directly in contact with the red cell. Undoubtedly in the above case reaction plays a part, especially in the case of acids where dissociation is interfered with and the acid changed toward the alkaline. The maintenance of equilibrium where mentioned is an important factor in the delayed type of

hemolysis. This delayed hemolysis to the author's knowledge has not been described for any substance or substances. This delayed hemolytic effect probably results from the gradual softening of the red cell resultant from the emulsifying action of triethanolamine on certain of its constituents with eventual complete dissolution of its structure.

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Oxygen Capacity and Hemoglobin Content of Normal Blood of Men.

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(Introduced by Henry Laurens.)

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It is almost universally recognized that Haldane's figure for the oxygen capacity of human blood (18.5 vol. p. c.) is too low. In spite of this the old standard is still used. A recent text book[1] states that "the standard (referring to Haldane's) is regarded by American workers as too low," and Drinker,[2] in describing a hemoglobin standard, writes that "although the blood of normal men in this country . . . . contains on the average 16.6 gm. of hemoglobin which would correspond to an oxygen capacity of about 22 p. c., it was thought best to keep Haldane's standard for the present." It appears unreasonable to accept as a standard a scale on which every normal blood is well over 100 p.c. Haldane's figure was based on an original series of 12 men[3] and substantiated later by a second series of equal size.[4] Although Haldane's method of analysis has long since outlived its usefulness, and we now have an accurate and simple method for determining blood oxygen, no extensive observations on normal subjects have been reported.

We have determined, during the winter months, the oxygen capacity of the blood of 115 healthy male students ranging in age from 18 to 30, most of whom were born and have always lived in the South. Specimens were taken during the morning, oxalated (20 mg. per 10 cc.), and determinations made the same afternoon. The

[1] Wright, Samson, *Applied Physiology*, 1929, 3rd Ed., 205.
[2] Drinker, C. K., *Oxford Medicine*, **2**, 538.
[3] Haldane, J., and Smith, L., *J. Physiol.*, 1900, **25**, 331.
[4] Haldane, J., *J. Physiol.*, 1900, **26**, 497.