When thio-acetyl choline is placed in freshly drawn blood and allowed to stand at room temperature for 30 minutes to one hour, its action is greatly reduced and in some cases reversed.

The results suggest that thio-acetyl choline chloride and thio-acetyl-gamma-homo choline chloride have a nicotine-like action; however, when respiratory failure occurs, stimulation of the phrenic nerve still causes tetanic contraction of the diaphragm. Thio-acetyl-beta-methyl choline chloride has a muscarine-like action; however, its action is not augmented by physostigmine.

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Attenuation of Toxins by Interfacial Adsorption.*

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The writer has previously demonstrated that crystalline insulin[1] can be attenuated by interfacial adsorption. In the same communication reference was made to the fact that bacterial toxins can also be attenuated by a similar procedure. It has been found by additional experimentation that ricin, tetanus toxin, staphylococcus toxin and snake venom may be completely detoxified by this method and still retain antigenic properties adequate for purposes of immunization, as demonstrated with rabbits and mice. The following data are representative of the results which have been obtained.

"C.P. ricin" which will produce a pronounced erythema, induration and necrosis when 0.2 cc of a solution of 1 part of ricin in 25,000,000 parts of saline are injected intradermally into rabbits will produce only a faint erythema when equal volumes of the same ricin in dilutions of 1 part of ricin in 1,000 parts of saline are injected after 2 treatments of 48 hours each with chloroform. When triple injections of untreated ricin in dilutions of 1 part ricin in 1,000,000 parts of saline were injected intradermally into rabbits which had been immunized with completely detoxified ricin, only a faint erythema was produced at the site of each injection. When

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1 Johlin, J. M., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 523.

the serum of each immunized rabbit was mixed with an equal volume of a solution of ricin containing 1 part of ricin in 200,000 parts of saline, and the mixture (0.2 cc) injected intradermally into normal rabbits, no symptoms of any kind were produced.

Tetanus toxin† containing 70,000 (guinea pig) M.L.D.'s per cc was completely detoxified when treated twice for 48 hours by interfacial adsorption. Five cc of the detoxified toxin produced no symptoms of any kind when injected subcutaneously into guinea pigs. Groups of mice (50 each) treated with the detoxified toxin all survived this treatment and smaller groups of these subsequently survived injections of 1 and 2 M.L.D.'s of untreated toxin without symptoms of tetany. A smaller proportion of other groups survived injections of 3 to 15 M.L.D.'s of untreated toxin with symptoms of tetany.

Solutions of staphylococcus toxin† "containing 8,000 dermonecrotic units and 700 hemolytic units per cc" was completely detoxified by 2 treatments of 48 hours each. Some of the solution, in which all hemolytic, lethal and skin necrotizing action had thus been eliminated by adsorption, was injected into rabbits and increased the protection of these animals towards staphylococcus toxin about 20 times.‡

The toxic and dermonecrotic action of 0.2% solutions of moccasin venom was entirely eliminated after treatment for a 36-hour period. In groups of mice immunized with the treated toxin, as many as 13 out of 15 survived subcutaneous injections of 0.1 cc of a 1% solution of the untreated toxin, while 2 out of 3 of each of 3 groups of controls died when injected with 1/4, 1/2, and 1/1 times this amount.

Since toxins, on standing for longer periods of time, are spontaneously converted into toxoids, the effect produced by surface adsorption is undoubtedly that of the catalytic action of surface concentration and orientation. Further tests are being made with virus suspensions. The results indicate that this method of attenuation might be generally applied to biological products of a protein-like nature.

† Obtained through the courtesy of the Lederle Laboratories.

‡ Experiments with staphylococcus toxin were performed conjointly with Dr. R. H. Rigdon.