

sodium ricinoleate, so that each cc. of the mixture contained 0.125 L+ toxin. Immediately after mixing, 1 cc. of the mixture was injected subcutaneously into the arms of a group of laboratory workers and volunteer medical students. In all the cases there developed a local redness with some induration, which persisted for five or six days. There were no general reactions observed. Another group was injected with like amounts of soap toxin which had stood at room temperature for six hours to allow the soap-toxin to come to equilibrium. In this group there were either no local reactions or mild reddening at the point of inoculation. No general reactions were observed. In another group, injected with soap-toxin which had stood at room temperature for twelve hours, there were no local reactions.

One hundred forty-nine cases of children and adults with positive Shick tests have been treated with one dose of soap-toxin. Sixty-nine of these have been retested within six weeks after treatment. Of this group 50.2 per cent gave negative skin reactions. We have frequently found the Shick test to be negative as early as four weeks after inoculation.

We wish to emphasize the importance of using only toxin-soap solutions which are perfectly clear. Injections of cloudy solutions are invariably followed by severe local reactions. This point has been emphasized in an earlier publication in which animal experiments were reported.² It is imperative that the soap solution as well as the soap-toxin mixtures be kept in hard glass containers.

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The preparation of pure sodium ricinoleate.

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The sodium ricinoleate used by Larson in his work on toxin neutralization has been prepared from commercial castor oil.

Castor oil is a triglyceride of ricinoleic acid. It contains small amounts of stearic, hydroxystearic, and oleic acids. The object

of the purification process is to remove practically all traces of these impurities.

In saponifying the castor oil to obtain the crude ricinoleic acid any standard method may be used. The fatty acid obtained is a brownish, viscous liquid that forms considerable sediment upon standing. After about two weeks' standing the semi-clear oil is decanted from the sediment. This is converted into the sodium soap in an aqueous solution. This solution is made up to about 20 per cent, and to it is added a 10 per cent solution of barium chloride until no more precipitate is formed. The barium ricinoleate is removed by filtration, and dissolved in 95 per cent alcohol. The barium soap recovered from 50 grams of fatty acid will require about one liter of alcohol. The barium ricinoleate is soluble in hot alcohol, whereas the barium stearates, hydroxystearates, and oleates are much less soluble. The solution is cooled to about 50° C. and filtered. Activated charcoal is added to the resulting filtrate, and after heating for about ten minutes it is again filtered. The portion that does not dissolve in the alcohol should be discarded. When this alcoholic solution is cooled to 5° C., the barium ricinoleate crystallizes out almost quantitatively. The solution should be kept at that temperature for a day to allow the crystallization to come to completion. The soap is separated from the alcohol by filtration. The process should be repeated, using 800 cc. of 95 per cent alcohol, redissolving, treating with more charcoal, filtering and allowing to recrystallize.

The pure barium soap is converted into the fatty acid by treating it with a 10 per cent HCl solution. After thorough shaking and six hours' standing, the HCl solution is removed in a separatory funnel. The oil is then treated with a 10 per cent H_2SO_4 solution in order to remove the last traces of barium that may be present. Too strong acids must not be used in this step because they have a tendency to break down the fatty acid. By using HCl the greater part of the barium can be recovered in the form of chloride, which can be used again without purification.

After the sulphuric acid treatment, the fatty acid is clarified by centrifuging to remove the barium sulphate suspended in the oil. The resulting clear oil is then separated from the sediment, weighed and dissolved in enough 95 per cent alcohol to make the solution about 10 per cent. To this is added, with constant stirring, the theoretical equivalent of pure NaOH dissolved in the

minimum amount of water. This solution is filtered until it becomes perfectly clear, and then evaporated down to one-fourth its original volume. This solution is allowed to jell and harden, and the resulting cake is cut into fine chips and dried at 35° C.

Soap made in this way consistently resulted in a pure white product that shows within experimental error 100 per cent of the theoretical iodine number. This product will make a water clear solution which shows no turbidity when kept at 5° C. for long periods of time, even though the concentration is as high as 10 per cent. Soaps that will not stand this test do not detoxify efficiently.¹

The sediment that forms in solutions of impure soaps may be either stearates, hydroxystearates, oleates, or polymerization products of the ricinoleate. Due to the tendency of this chemical to polymerize, the pure fatty acid must never be kept in the form of fatty acid longer than is absolutely necessary.

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A note on the photoactivity of cod liver oil.

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As a preliminary step in the investigation of the relationship between cod liver oil and ultraviolet radiation as antirachitic factors, the experiments of Kugelmass and McQuarrie¹ on the photoactivity of cod liver oil were repeated. In every case negative results were obtained.

An apparatus similar to that described by these investigators was employed. Eastman's Speedway plates, possessing properties similar to Seed's Graflex 60, were used. Since these plates proved to be sensitive to the red light in the room, the whole experiment was carried out in total darkness. The cod liver oil was a sample of Mead and Johnson's, guaranteed as to vitamin activ-

¹ Larson, Evans and Nelson, *Proc. Soc. Exp. Biol. and Med.*, 1924, xxii, 194.

¹ Kugelmass, F. N., and McQuarrie, I., *Science*, 1924, lx, 272.