

A group of nurses who were treated with 2,000 skin test doses experienced no inconvenience whatever from the treatment. There were sixteen in this group. In seventeen days only 37.5 per cent were negative. We believe, therefore, that larger doses are indicated for older children and adults. Since large doses cause the patient no inconvenience, we recommend using 4,000 skin doses, as the larger doses apparently give a higher percentage of immunity than the smaller ones.

The soap-toxin should be allowed to stand at room temperature for four hours before using, to allow it to come to equilibrium. If cloudiness develops upon standing, it should not be used. The soap, as well as the soap-toxin mixtures, must be kept in hard glass containers.

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#### The effect of concentration upon the neutralization of toxin by sodium ricinoleate.

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It has previously been shown that sodium ricinoleate possesses the property of detoxifying bacterial toxins.<sup>1, 2</sup> In the present study an attempt has been made to show the effect of the concentration of both the toxin and the soap upon the efficiency of the detoxification.

One L+ diphtheritic toxin contained in a volume of 0.4 cc. was added to 1.0 cc. samples of a series of soap solutions ranging in concentration from 1 per cent to 9 per cent. After 15 minutes standing, these mixtures were injected into guinea pigs. The results of this experiment are given below:

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<sup>1</sup> Larson and Nelson, PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 278.

<sup>2</sup> Larson, Evans and Nelson. *Ibid.*, 1924, xxii, 194.

TABLE 1.  
Effect of Varying the Concentration of the Soap.

| Dose of Toxin | Con. of Soap Used | Total Volume | Result.                  |
|---------------|-------------------|--------------|--------------------------|
| L+            | 1 per cent        | 1.4 cc.      | Died in 3 days           |
| "             | 2 per cent        | "            | Died in 11 days          |
| "             | 3 per cent        | "            | Died in 28 days          |
| "             | 4 per cent        | "            | Paralysis but re-covered |
| "             | 5 per cent        | "            | Alive after 78 days      |
| "             | 6 per cent        | "            | Alive after 78 days      |
| "             | 7 per cent        | "            | Alive after 78 days      |
| "             | 8 per cent        | "            | Alive after 78 days      |
| "             | 9 per cent        | "            | Alive after 78 days      |

In this experiment concentrations below 5 per cent did not protect, whereas concentrations of soap of 5 per cent or above did protect. This experiment has been repeated and confirmed. Studies were made in the following experiment to show the effect of changing the concentration of both the toxin and the soap. In these experiments the effect of aging was also studied. To do this a series of samples was prepared, each sample containing an L+ dose for each 1 cc. of 4 per cent soap solution added. One of these was injected without diluting; to the other varying amounts of water were added. One series of guinea pigs was injected after the mixture had stood 15 minutes. Another series was injected after the mixtures had remained in the ice box for 24 hours. The results of the experiment are given in the tables below. The concentrations of soap and toxin given are

TABLE 2.  
Effect of Varying the Concentration of Both the Soap and Toxin (Injected after 15 Minutes Standing).

| Con. of Toxin<br>L+ per cc. | Con. of Soap in<br>per cent. | cc. Injected | Result          |
|-----------------------------|------------------------------|--------------|-----------------|
| .715                        | 2.86                         | 1.4          | Died in 41 days |
| .417                        | 1.70                         | 2.4          | Lived           |
| .294                        | 1.18                         | 3.4          | Died in 3 days  |
| .227                        | .90                          | 4.4          | Died in 2 days  |

TABLE 3.  
Effect of Varying the Concentration of Both the Soap and Toxin (Injected after 24 Hours' Standing).

| Con. of Toxin<br>L+ per cc. | Con. of Soap in<br>per cent | cc. injected | Result          |
|-----------------------------|-----------------------------|--------------|-----------------|
| .715                        | 2.86                        | 1.4          | Died in 30 days |
| .417                        | 1.70                        | 2.4          | Lived           |
| .294                        | 1.18                        | 3.4          | Died in 2 days  |
| .227                        | .90                         | 4.4          | Died in 2 days  |

the actual concentrations of the final mixture. A sufficient volume was injected in each case to represent one L+ dose of toxin.

This experiment has been confirmed several times.

This experiment clearly demonstrates the fact that the concentration of both the soap and the toxin is of fundamental importance, of more importance than the actual quantity of toxin injected. It also clearly shows that equilibrium must be reached in a relatively short time, since both sets show about the same results.

Other experiments have been performed in which it was shown that the action of the soap upon the toxin is a reversible one in which the toxin is not destroyed. This was done by preparing a mixture of soap and toxin that would protect, so that when the guinea pig was injected it showed no ill effects. After this mixture had stood for 24 hours, long enough to establish an equilibrium, the equilibrium could be disturbed by adding a quantity of salt solution, as evidenced by the fact that it would now no longer protect.

These experiments tend to show that the neutralization of toxin by sodium ricinoleate is an adsorption phenomenon.

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### Antidiphtheritic immunization using sodium ricinoleate as a detoxifying agent.

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The property of sodium ricinoleate to detoxify bacterial toxins has been pointed out in previous papers.<sup>1, 2</sup> We have studied this problem further to determine whether it is possible to immunize children and adults against diphtheria with toxin which has been detoxified in this way.

A quantity of toxin was mixed with a 1 per cent solution of

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<sup>1</sup> PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 278.

<sup>2</sup> *Ibid.*, 1924, xxii, 194.