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Deterioration of Staphylococcal Toxin in Saline.

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There is some evidence of union between certain bacterial toxins and susceptible tissues. The harmful effects of tetanal toxin are neutralized when it is mixed *in vitro* with brain and cord tissues of rabbits and guinea pigs as shown by Wassermann and Takaki.[1] Metchnikoff observed that brain-tissue from animals entirely insusceptible to tetanal toxin possesses absolutely no neutralizing properties.[2]

Doerr[3] found that rabbits' spinal cord macerated in saline had little effect on the lethal action of tetanal toxin.

The action of some toxins on certain tissues *in vivo* endangers the life of the animal. The injury produced in connective tissue by diphtheric toxin, ricin, and abrin can only be explained by anchoring of the toxin on the cells, according to Dean.[4]

Staphylococcal toxin when injected intravenously in the rabbit may produce immediate death.[5] If the animal survives the acute reaction, necrosis may occur in the majority of the organs.[6] When the toxin is injected locally necrosis occurs in the immediate area.[7]

The present study was made to determine the *in vitro* effect of rabbit brain, liver, kidney, and muscle on a staphylococcal toxin. The tissues were macerated and mixed immediately with the toxin and kept at 5-15°C for 24 hours. The toxin-tissue mixtures were centrifuged and the supernatant liquid was titrated for hemotoxin with 1 cc of a 2% suspension of rabbits' red blood cells. Lysis was recorded after the tubes had been in a waterbath at 37°C for 2 hours and in the icebox for 18 hours. The degrees of hemolysis in the different tubes were compared with that produced by a known amount of toxin diluted with saline.

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1 Wassermann, A., and Takaki, T., *Berl. Klin. Woch.*, 1898, **35**, 5.

2 Metchnikoff, E., *Ann. de l'Inst. Past.*, 1898, **12**, 81.

3 Doerr, R., *Hyg. und Infektionshkr.*, 1936, **118**, 212.

4 Nuttall, C. H. F., and Graham-Smith, G. S., *The Bacteriology of Diphtheria*, Cambridge University Press, 1913, p. 546.

5 Kraus, R., and Pribram, E., *Wien. Klin. Wchnschr.*, 1906, **19**, 493.

6 Rigdon, R. H., *Arch. Path.*, 1935, **20**, 201.

7 Rigdon, R. H., *Arch. Path.*, 1937, **23**, 634.

Table I shows variation in the effect of staphylococcal toxin of different tissues from the same rabbit and from a different rabbit. The supernatant liquids from the tissues of rabbit A have more toxin than the control diluted with saline. The amount of hemotoxin recovered from the liver and brain of rabbit B is more than that in the saline control. However, the supernatant fluid from the muscle and kidney contains less than the saline control.

TABLE I.
Effect Produced on Hemolysin by Mixing Staphylococcal Toxin with Rabbit Tissues Macerated in Saline.

	Rabbit A			Rabbit B		
Tissue	Hemolysin in mm³ necessary to produce same degree of lysis		Avg amt of hemolysin	Hemolysin in mm³ necessary to produce same degree of lysis		Avg amt of hemolysin
Kidney	.3 .4	.5 .6	.45	.8	.1	.9
Liver	.2 .3	.3 .4	.3	.3	.4	.35
Brain	.23 .25	.35 .35	.295	.3	.4	.35
Muscle	.32	.55	.435	.6	1.1	.85
Saline Control	.5	.8	.65			

The experiment was repeated. Infusion-broth, pH 7.4, was substituted for the saline. The results were quite similar.

The 2 experiments show that more hemotoxin is present in the supernatant fluid from the livers and brains of the 4 rabbits than in the control mixtures of toxin with broth and with saline. There is less hemotoxin from the muscle in 3 of the 4 animals and less from the kidney in 2 of the 4 rabbits. These results suggest that the toxin may deteriorate either when diluted with saline or in the presence of macerated muscle and kidney tissues. Other tissues appear to enhance the hemolytic activity of the toxin toward rabbits' red blood cells.

The effect of extracts of rabbit tissues on staphylococcal toxin was studied to obtain additional information. The same types of tissues as previously used were extracted in saline and also in infusion-broth. The toxin was added and the preparations were kept at 5-15°C for 18 hours. The toxin-extract mixtures were then titrated for hemotoxin. The results show that approximately the same amount of hemotoxin is recovered from the extracts of kidney,

liver, and brain as from the controls diluted with an equal quantity of broth. There is, however, in the extracts of kidney, liver, and brain more hemotoxin than in the controls diluted with saline. The amount of toxin recovered from the extract of muscle is less than that recovered from the other tissue-extracts and also less than that in both of the controls. The results of all the experiments suggest that the variation in the amount of hemotoxin recovered from either macerated tissue or tissue-extracts is the result of the effect of the diluent on the toxin rather than an increase in the activity of the toxin or any adsorption of the toxin by the tissues. The toxin deteriorates more when diluted with saline than with infusion-broth. Halter's work[8] suggests that oxidation may be the explanation for the deterioration of tetanal toxin in sodium-chloride solution. There is nothing, however, in these experiments to indicate the process of deterioration of staphylococcal toxin.

The amount of necrosis produced in the rabbit's skin by staphylococcal toxin* diluted with physiological saline and diluted with toxin heated at 100°C for 2 hours was studied. This amount of heat is sufficient to destroy the necrotizing properties in this toxin. The following dilutions of toxin in both diluents were injected endermally in 2 animals: 10.0, 6.66, 5.0, 3.33, 2.5, 2.0, and 1.4%. The areas of necrosis were measured 4 days after the toxin was injected. Fig. 1 shows the size of the different necrotic areas. It is evident from this study that staphylococcal toxin deteriorates faster in saline dilution than when diluted with heated toxin. A 3.33% solution of toxin failed to produce necrosis, while a 1.4% solution of the same toxin diluted with heated toxin produced necrosis in the same rabbit. This experiment also shows that the areas of necrosis are not proportional to the amount of toxin injected. The size of the areas of

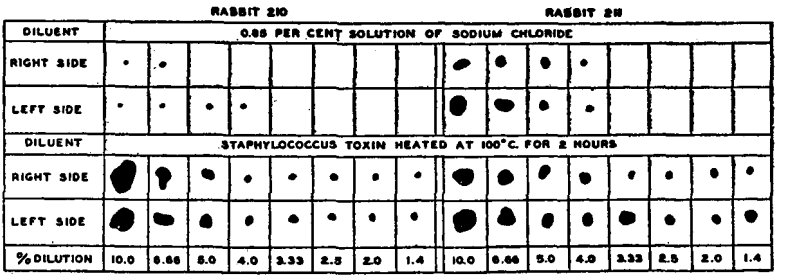

FIG. 1.

Areas of cutaneous necrosis 4 days after the endermal injection of staphylococcal toxin diluted with saline, and heated staphylococcal toxin.

8 Halter, K., *Hyg. Infektionskr.*, 1936, **118**, 246.

* Supplied through the courtesy of Lederle Laboratories.

necrosis apparently is influenced by the extent of diffusion of the toxin.

This study of the effect of rabbit tissues on staphylococcal toxin shows that the toxicity decreases in the presence of some of these tissues. The apparent tissue-adsorption may be due largely to the degree of attenuation attained in the presence of different diluents. These *in vitro* observations with staphylococcal toxin are interesting in view of the interpretation of similar experiments of others with tetanus toxin.

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Studies on the Chemistry and Immunology of Ragweed Pollen Proteins.

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The opinion has been held for some time in our laboratories that many allergens responsible for atopic symptoms have present two types of protein molecules. An extended study of these allergens has led us to the conclusion that there are usually present representatives of the larger variety, loosely typified by crystallizable egg albumin and also representatives of a type of protein molecule much smaller, approaching the size of the peptone group. In the work of the staff of the Allergy Clinic on the preparation of purified proteins from the various foods and environmental materials, it was found that the more these proteins were purified by repeated precipitation and subsequent dialysis, the less skin reactive they became. Some of them lost practically all of this property. Other investigators have recorded this observation. The usual explanation that has been offered is that the protein has been denatured in the process of purification. This explanation is not valid in the light of experimental evidence in connection with crystallizable egg albumin secured by Cole and Aaronson[1] in this laboratory. They found that with each successive crystallization the skin reaction diminished in certain types of egg sensitive cases until with the sixth crystallization this reaction was quite feeble. The precipitinogenic power of the albumin remained unaltered throughout all the crystallization processes.

[1] Cole, A. G., and Aaronson, A. L., personal communication.