4873

Lack of Antigenic Power of a Highly Purified Diphtheria Toxin and Detoxification by Ultraviolet Light.

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The literature concerning the effect of ultraviolet radiation of toxins has been summarized by Norton[1] with the statement that "In the medium in which bacterial toxins are found or are produced they appear to be relatively stable toward ultraviolet light." The work of Lowenstein,[2] and of Hortock, Schurman and Stiner,[3] which led to this conclusion, was done with the usual broth filtrates of organisms and in the case of diphtheria toxin from 6 hours to 20 days radiation was necessary to produce marked lessening of the toxicity of the filtrates. The former investigator was unable to produce an active immunity with radiated toxin. Although a previous investigation[4] had demonstrated the marked absorption of ultraviolet rays between 2000 and 3100 A° by proteins in solution or in suspension, it was thought advisable to determine whether the longer rays from "C" carbons (National Carbon Co.) would penetrate sufficiently to detoxify a broth filtrate of the diphtheria bacillus. Using the apparatus described in a previous article,[5] various dilutions of this toxic broth were radiated at varying distances and for varying periods of time, it was found that detoxification could be brought about but the long period of exposure necessary made it of little practical significance.

A water-clear, colorless diphtheria toxin prepared by the method of Gross[6] and with his aid, was found to be extremely toxic. Toxin prepared in this way contains but little protein. Since it had been found that the period of radiation necessary to insure detoxification varied directly with the potency of the toxin, a dilution of this toxin in physiological salt solution was selected for radiation, which gave a strong intracutaneous reaction in the guinea pigs. This purified

[1] Norton, J. F., "The Newer Knowledge of Bacteriology and Immunology." Univ. of Chicago Press, 1928, 371.

[2] Lowenstein, E., *Z. Exp. Path. u. Therap.*, 1914, xv, 279.

[3] Hortock, O., Schurman, W., and Stiner, O., *Z. Imm. u. Exp. Therap.*, 1914, xxi, 643.

[4] Welch, H., and Perkins, R. G., *J. Prev. Med.*, 1930, iv, 15.

[5] Perkins, R. G., and Welch, H., *J. Prev. Med.*, 1929, iii, 363.

[6] Gross, P., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 696.

toxin was found to be destroyed by the action of "C" carbons in 2 minutes, at a distance of 25 cm. When 3% glycerine, having the same pH (7.8) as the purified toxin, was added it was found that detoxification was brought about in 4 minutes.

To test the antigenic power of Gross' toxin and of the detoxified glycerinated and unglycerinated preparations of this toxin, 6 guinea pigs were given 10 injections at intervals of 5 days with each of these 3 products. Twelve days after the last injection half of each group of animals was given intracutaneous injections of 1/40 and 1/50 M.L.D. of ordinary diphtheria toxin. All showed positive reactions, and when these animals were given 1 M.L.D. of toxin subcutaneously, 2 days later, all died. The remaining animals were tested likewise 30 days after the last injection and were also found to be non-immune.

Diphtheria toxin prepared by the method of Gross is easily destroyed by ultraviolet light. Since, however, this purified toxin itself is *not antigenic,* a radiated non-toxic preparation could not be expected to be antigenic. It is apparent that in the preparation of this toxin the antigenic fraction is lost while the toxin remains. Evaluation of this point will necessitate further work, which, we understand, is to be carried out by the same group which first prepared the toxin.

Since investigation of ricin (to be published at an early date) has indicated that it is possible to detoxify this product with ultraviolet light and still retain its antigenicity, the results noted above do not obviate the same possibility with diphtheria toxin and other toxins under the proper conditions.

4874

Histology of the Anterior Pituitary of the Foetal Pig with Reference to Growth and Maturity.

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It is well known that the pars anterior of the hypophysis cerebri is made up of a variety of cell types, *viz.,* eosinophils, basophils, and chromophobes (Flesch,[1] Kraus[2]). The presence of at least 2 func-

[1] Flesch, M., *Tagebl. d. 57 Vers deutsch Naturf. u. Arzte. Magdeburg,* 1884, 195.

[2] Kraus, E. J., *Zeigler's Beitrage,* 1914, lviii, 159.