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On the Nature of the Toxin-Antitoxin Neutralization Studied on Collodion Particles.

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Diphtheria and tetanus toxins can be adsorbed upon collodion particles and can then be neutralized by the adsorption of their antitoxins.[1, 2] When, however, the particles were treated first with tetanus antitoxin and then with tetanus toxin a phenomenon, at first sight paradoxical, appeared, which may be interpreted as the combination of toxin with antitoxin without neutralization. Further observations are presented here.

Collodion particles prepared according to Loeb[3] and unconcentrated antitoxic horse serums* were used in 3 types of experiment: A—first treatment with the antitoxin, second treatment with the toxin; B—first treatment with normal serum or a non-corresponding antitoxin, second treatment with the toxin; C—treatment with the toxin alone.

Tetanus toxin. With antitoxin dilution 1:1 and toxin 1:1 or 1:10, and with antitoxin 1:10 and toxin 1:1 or 1:10, the mice in experiment B showed no symptoms at all; in experiments A and C they either died or had severe symptoms of tetanus. When antitoxin was used in dilution 1:10 and toxin 1:100, the mice in experiments A and B had no symptoms and in experiment C they showed symptoms of tetanus.

Diphtheria toxin. Collodion particles treated with diphtheria toxin alone produce a nodule in the skin of guinea pigs, and the skin over the nodule becomes red and scaley 2 or 3 days after the injection. Toxin + antitoxin produces a nodule over which the skin shows no signs of inflammation. With toxin + normal serum the reaction is the same as with toxin alone. If, however, the order of treatment is reversed, that is, if the particles are first treated with anitoxin and then with toxin, the skin in experiment A shows severe inflammation, and in experiment B no inflammation. In

[1] Freund, J., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 65.

[2] Freund, J., *J. Immun.*, 1931, in press.

[3] Loeb, J., *J. Gen. Phys.*, 1923, **5**, 109.

* The toxin and antitoxin preparations were obtained through the courtesy of Prof. K. F. Meyer of the George Hooper Foundation, and Dr. John H. Reichel of the H. K. Mulford Company.

repeated experiments the inflammation with particles treated with antitoxin + the corresponding toxin was strikingly more intense than with those treated only with toxin.

The results of the experiments are summarized as follows:

A tetanus antitoxin + tetanus toxin: toxic.

B diphtheria antitoxin + tetanus toxin: not toxic.

A′ diphtheria antitoxin + diphtheria toxin: toxic.

B′ tetanus antitoxin + diphtheria toxin: not toxic.

Two questions naturally arise. (1) Why are the particles that have been treated with a non-corresponding serum + the toxin, not toxic, when those treated with the corresponding antitoxin and toxin, are toxic? The latter observation, but not the former, could be satisfactorily explained as an adsorption of the toxins as well as of the antitoxins upon the collodion particles. In view of the results, however, the question can be answered by assuming that collodion particles treated with the non-specific serums adsorb or retain little if any of the toxin, whereas those treated with antitoxin are capable of combining with the corresponding toxin, as if in a reversed Castellani absorption experiment.

(2) Why are the particles, treated with antitoxin + toxin, toxic when the opposite order of treatment yields non-toxic particles? A tentative answer can be given. A combination between toxin and antitoxin is evidently not sufficient for neutralization. This conclusion is also supported by the fact that under certain conditions toxin can be recovered from toxin-antitoxin mixtures, suggesting that the neutralization following chemically specific combinations is a physical phenomenon. Neutralization takes place only when toxin and antitoxin are combined in a certain way. They are combined whatever the order of treatment of the collodion particles, but it has been seen that the toxin is neutralized only when the antitoxin is in the top layer, i. e. when the antitoxin covers the surface of the toxin molecule. Similarly Jones[4] has shown that collodion particles treated with precipitinogen + precipitin are flocculated, but not with the reverse treatment. Antitoxins adsorbed on charcoal are incapable of neutralizing their toxins.[5] These observations suggest that the toxin-antitoxin reaction is essentially similar to other antigen-antibody reactions, particularly precipitation, agglutination and phagocytosis.[6]

[4] Jones, F. S., *J. Exp. Med.*, 1928, **48**, 183.

[5] Eisler, M., *Biochem. Z.*, 1923, **135**, 416; 1924, **150**, 350.

[6] Mudd, S., Lucké, B., McCutcheon, M., Strumia, M., *J. Exp. Med.*, 1930, **52**, 313.

This conclusion is strengthened by experiments showing that tannin adsorbed on red blood cells mediates agglutination, promotes phagocytosis, and prepares the cells for lysis by complement,[7] and that it combines with, and detoxifies, toxins adsorbed on collodion particles.

I am indebted to Miss Elizabeth S. Stivers for valuable assistance.

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Studies on Mouse Leukemia. IV. Specificity of Susceptibility to Different Lines of Inoculated Leukemia.*

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Susceptibility and resistance to a particular line of inoculable mouse leukemia may, for present purposes, be defined in terms of the presence or absence of conditions necessary for the survival of the active agent. A group of 11 such lines of agent, each of which originated in a different case of spontaneous lymphatic leukemia in a highly inbred strain of mice designated as C58, found the necessary conditions for survival in mice of the same strain.[1] Although different lines of agent can grow in the same pure bred strain of mice, these different lines do not all have the same requirements for survival, as has been shown by hybridization experiments.[2] The present report gives direct evidence of the specificity of requirements of different lines of agent, without the use of hybridization, by means of a new line that originated from a spontaneous case in another highly inbred strain of mice.

The new line of agent is designated line L. The spontaneous case that gave rise to this line was a mouse in strain 89; the line is carried in mice of this same strain. During the course of 13 trans-

[7] Reiner, L., and Fischer, O., *Z. f. Immunitatsf.*, 1929, **61**, 317; Reiner, L., and Kopp, H., *Ibid.*, 1929, **61**, 397; Freund, J., PROC. SOC. EXP. BIOL. AND MED., 1922, **26**, 876; Neufeld, F., and Etinger-Tulczynska, R., *Zentralbl. f. Bakt.*, 1929, **114**, 252.

* Aided by a grant from the Carnegie Corporation and an appropriation for technical assistance from the Research Fund of Columbia University.

[1] Richter and MacDowell, *J. Exp. Med.*, 1930, **51**, 659.

[2] MacDowell and Richter, *J. Cancer Res.*, 1930, **14**, 434.