ON THE NATURE OF DIPHTHERIA TOXIN.

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The chemical nature and the formation of bacterial toxins has been widely discussed. Diphtheria toxin may be regarded as a classical subject. Most of the earlier authors regarded it as a protein or a product derived therefrom. Recently some American authors, Warden, Connell, and Holly, have emphatically declared the toxin to be a "colloidal" mixture of fats and protein. As to the formation of the toxin, it has commonly been regarded as a secretion of the living bacilli, but some authors claim it to be an autolytic postmortem product. One author, S. Martin, has advanced the theory that the toxin is not secreted from the bacilli directly, but is formed from the proteins of the infected tissue, by means of some agent produced by the microorganism. Several authors, Ehrlich, Morgenroth, and others have assumed the existence of "protoxins" and "toxoids," which in some way or other can be activated into toxins.

The reaction of the medium has been found to play a great rôle in the toxin production. It has been found that toxin is formed when the reaction of the broth is alkaline, but if it is acid, toxin does not appear. Recently Bunker has studied this question with modern methods and declared that the toxin should be harvested when the broth has reached a reaction of pH about 8. A higher alkalinity destroys the toxin. His views have, however, been criticised by Davis, Abt and Loiseaux, and others, who do not believe the pH stands in any constant relation to toxin production at all. Hartley has also studied the influence of pH upon the toxin production and obtained results similar to those of Bunker.

In a series of papers I have studied the production of diphtheria toxin and I shall give a short review of the most important facts recorded.

1. The changes in pH in broth cultures depend upon whether the
broth contains sugar or not. Fig. 2 represents graphically these changes under various conditions.

Fig. 1. Growth of diphtheria bacilli in broth culture at different pH values.

2. In a broth culture of pH = 7.2 initially, the maximum strength of toxin is reached after 6 to 10 days. The pH has then increased to about 8. After the maximum is reached the toxicity decreases rapidly. The increase in the pH is not itself the cause of the toxin destruction, but is a consequence of the proteolytic action of the dead bacilli. Fig. 3 represents these facts.

Consequently the toxin destruction might be an action of the proteolytic enzymes of the bacillus.

3. The idea has been confirmed that proteolytic enzymes, as trypsin and proteases from yeast, *Bacillus pyocyaneus*, *Bacillus proteus*, and others, destroy the toxin very rapidly. The proteolytic enzymes from the diphtheria bacillus itself also destroy the toxin.

4. The diphtheria bacillus contains proteolytic enzymes of the trypsin type, which, however, are rather weak in action.

5. There is no strict parallel between growth and toxin production. Fig. 4 gives a striking example of the necessity of unsplit albumoses or peptones for producing a potent toxin. For good growth a great amount of protein split products seems to be necessary. It is therefore possible that the unsplit albumoses could be the source of the toxic substances.

6. The stability of the toxin at different pH values has been studied. It is rapidly destroyed at reactions lower than pH = 6.0, but this destruction is at any rate partly reversible. In alkaline solutions the
toxin is comparatively stable but is destroyed more rapidly, the higher the pH value. This destruction is irreversible. The reversible inactivation in acid reaction is interesting, because it might be regarded as a dissociation reaction. V. Groer has recently published a paper on this question. We have perhaps a parallel in the behaviour of quinine alkaloids at different pH values (Michaelis and Dernby).

These alkaloids are only active in the undissociated state; as positive ions they are almost inactive.

7. Walbum has shown that the toxicity of toxin broths or emulsions of dead bacilli may be augmented by addition of Witte peptone. I have verified his experiments. Fig. 6 represents such an experiment. These experiments favour the hypothesis that the toxins may be produced from the albumoses present in the medium.

8. The American authors Warden, Connell, and Holly have most emphatically claimed that the toxic agent is a fatty substance, and that real diphtheria toxin can be produced by "colloidal" emulsions of ordinary fatty acids and broth, "if the proper degree of dispersity is
reached.” My experiments have fully shown that the fatty substances of the bacilli or of the toxin broth are not at all toxic. The statements of the authors quoted cannot be confirmed.

The facts referred to form the most important part of my investigation. But it is tempting to try to evolve an hypothesis regarding the formation of diphtheria toxin. The following theory is suggested.

Diphtheria toxin is to its greatest part neither secreted from the living cells, nor is it extracted from the dead bacilli. The bacilli grow, die, and autolyze. Simultaneously proteolytic enzymes of a specific character are liberated. These enzymes attack the albumoses and peptones of the autolyzed bacilli as well as of the broth, and some of the primary split products are the toxic substances. If the proteolysis proceeds further, the toxin will be disintegrated also. Briefly, the theory may be represented thus:

Proteolytic enzymes:

1. Albumoses (non-toxic) $\rightarrow$ Intermediate products (toxic).
2. Intermediate products (toxic) $\rightarrow$ End-products (amino acids) (non-toxic).

This is of course only an hypothesis, but it explains most of the facts regarding the formation of diphtheria toxin in vitro. I would point out that this theory is in good accord with that advanced by Martin.
It is possible that the theory could be applied to other bacterial toxins also.

It would be interesting to see if it also could be applied to conditions in vivo. In fact I have made some experiments with guinea pigs and like previous investigators found that the actual toxin is destroyed very rapidly in the organism, but that lesions in the peritoneum and the suprarenal glands may be caused not by the actual toxin but by the "protoxins" or "toxinases," possibly the proteolytic enzymes of the bacilli.

To carry out similar experiments with a diphtheria patient is of course very difficult, if not impossible. At all events, specialists in this disease with whom I have conferred have told me that theoretically my theory might be applied to human cases. The toxic substances would then to a certain extent be produced from the proteins of the infected tissue by means of the proteolytic enzymes of the autolyzed bacilli.

This theory should only be regarded as a working one, but perhaps it might help to throw some light on the problem of infection in general.

BIBLIOGRAPHY.

The literature given below contains the original papers, relating to my studies of diphtheria toxin. In these, all the other papers referred to can be found.

Dernby, K. G., and Davide, H., J. Path. and Bact., 1921, xxiv, 150.
Dernby, K. G., and Allander, B., Biochem. Z., 1921, cxxiii, 245.