THE FLOCCULATION OF TETANUS TOXIN AND TOXOID. I.

by

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For the determination of the antitoxin-binding power of diphtheria toxin and toxoid the flocculation method described by Ramon and Descombes (1926) has been used for some decades now. This method is based on the fact that, when mixtures of a constant quantity of toxin (or toxoid) and increasing quantities of antitoxin (serum) are prepared in a number of tubes, although flocculation eventually occurs in all the tubes, it sets in first in the tube in which the components just neutralize one another. This so-called initial flocculation, therefore, indicates the point of equivalence. The titre of the toxin or toxoid, expressed in flocculation units per ml (Lf/ml), being known, one can therefore calculate the titre of the antitoxic serum which is used, expressed in Antitoxin Units per ml (A.U./ml), and vice versa. This method gives practically no trouble.

When, however, one tries to determine the antitoxin-binding power of tetanus toxin or toxoid in this way, things are quite different. True also in this case a flocculation is seen to set in at the point of equivalence, as has already been pointed out by Ramon and Descombes (1926), but if the series of toxin-antitoxin mixtures is only made long enough, one nearly always finds several flocculation zones, so that the results are no longer unambiguous.
The flocculation picture which we get depends on the origin both of the antitoxic serum and of the toxin or toxoid. In 1937 Ramon reported that he had at his disposal a special serum, which caused only one flocculation, namely at the point of equivalence. This serum he had obtained by immunizing horses in a very special way. Several other publications on this problem have come out. On the whole, serums purified by pepsin digestion proved to be more suitable than crude serums, but even with the former one often gets several flocculations, as was pointed out by Moloney and Hennessy (1944) and others. These workers also developed some methods to tell the "true" flocculation from "false" ones.

The origin of the toxins or toxoids is also important. The experiments described below were made with toxins and toxoids that have been obtained from the medium used at the National Institute for Public Health for routine production after 10-days' incubation. This is a modified Tuzzii broth, which is still prepared in about the same manner as described by Pondman (1939). This broth is of the following composition: 500 g veal, 10 g peptone Witte, 5 g NaCl, 1000 ml water, 1.2% glucose. Final pH 5.8—6. The meat is added in the form of cubes of about 1 cm³. Nowadays, however, the flasks are simply plugged with cotton-wool without using paraffin or other precautions to ensure an anaerobic condition. The broth with the meat is sterilized in portions of 700 ml in litre roundbottles and then inoculated with 1 ml of an inoculated and incubated seeding-culture, which has been produced in a broth prepared in the same way.

Toxins prepared from different batches of this broth all behaved in essentially the same way with regard to flocculation etc, though, naturally, they proved to differ in potency. The same applies to the toxoids prepared from these toxins. However, it would be wrong to take it for granted that conclusions based on these data are also applicable to toxins and toxoids of different origin. We hope to refer to this later.

The flocculations were all carried out with purified Wellcome tetanus serum (pepsin method), batch R.D. 948 A. A serum from the Statens Serum Institut at Copenhagen, purified by the Hansen method (1948), proved to behave in exactly the same way, as regards flocculations.