hurried, for the successful transfer depends on the maintenance of an alcohol concentration of about 95 per cent. in and around the bag. Once inside, the top of the bag is drawn shut and the free ends of the thread are cut off near the knot. For greater security the overhand knot may be continued into a square knot before cutting off the ends. There is little danger of tearing the bag upon pulling it shut.

The entire bag plus contents is now to be dehydrated, cleared, infiltrated, imbedded and sectioned, though it is advisable to stain it in alcoholic eosin to facilitate orientation. Since the thread can not be sectioned, the section plane must be at right angles to the longitudinal axis of the bag. Begin cutting tangentially to the deepest part of the bag and proceed toward the thread.

If it is desirable to keep the protozoa concentrated in the bottom of the bag, a metal insect pin may be thrust through the free edges of the bag above the thread. With suitable wire supports at the ends of the pin, it may be kept in a horizontal position with the bag hanging downward from it throughout the entire procedure, including imbedding.

C. D. BEERS

UNIVERSITY OF NORTH CAROLINA

THE PRESERVATION OF TETANUS TOXIN BY THE LYOPHILE PROCESS[1]

TETANUS toxin (tetanospasmin), obtained by drying the ammonium sulfate precipitate from a filtered broth culture, must be preserved under rigorous precautions. Aqueous solutions and stock bottles of powder which are repeatedly opened deteriorate rapidly. An additional difficulty is created by the actively hygroscopic behavior of the dried powder which causes errors in the weighing. MacConkey[2] recommended the use of a stable solution prepared by dissolving toxin in equal parts of pure neutral glycerine and distilled water. Most investigators consider it essential to perform guinea pig titrations of their stock toxin with each successive experiment. At the National Institute of Health in Washington, D. C., purified toxin, used as the basis for standardizing therapeutic antiserums, is preserved within small ampoules in vacuo under the influence of pentaphosphoric acid in a cold dark place.[3] The minimum lethal dose of this purified powdered material for 350 gram guinea pigs remains constantly at 0.006 mgm.

In an attempt to avoid these difficulties, the Flosdorf-Mudd lyophile technique[4] has been applied to the problem of tetanus toxin preservation. A known quantity of standard toxin, obtained from the National Institute of Health, was dissolved in distilled water and distributed in aliquot portions into a large number of small rubber-stoppered glass ampoules. The solutions were immediately frozen in a bath of dry ice and methyl cellosolve at −78° C., and then dried by high vacuum distillation from the frozen state. The containers were sealed individually under vacuum by heat fusion of the pyrex glass exhaust tubes, and stored away under ice refrigeration (8–10° C.). The powdery residue dissolved instantly when distilled water was reintroduced by syringe and needle through the rubber stopper, in contrast to the slow solubility of the original material.

For example, on one occasion a solution of standard government toxin, which had been obtained from the National Institute of Health, was distributed into 25 lyophile ampoules in 10 cc quantities. The original solution had been prepared in such fashion that, after processing, 0.8 mgm was left in each ampoule as a dry residue. On repeated titrations the MLD of this material was regularly found to be 0.008 mgm.

Five separate solutions of tetanus toxin have been processed by this technique. In each instance a slight inconstant diminution of the initial potency was observed, although within each batch of ampoules there was a consistent uniformity of titer as measured by the MLD test on guinea pigs. These solutions were of various strengths depending on the nature of the experiments in which they were to be used. This lyophile toxin has proved itself most dependable in the course of several series of experiments on tetanus intoxication and treatment carried on over a three year period. No single batch was used for longer than a one year period, however, since beginning deterioration was detected after that time.

In summary, therefore, the lyophile method of preserving tetanus toxin has been found a valuable and time-saving adjunct to experimental investigation.

IRVING J. WOLMAN

CHILDREN'S HOSPITAL OF PHILADELPHIA
AND
DEPARTMENT OF PEDIATRICS,
UNIVERSITY OF PENNSYLVANIA

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[2] A. T. MacConkey, *Jour. Hyg.*, 22: 473–476, 1923–24.

[3] M. J. Rosenau and T. F. Anderson, *Hygienic Lab. Bull.* No. 43, March, 1908.

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