Although there is a high degree of variability in virus assay methods, it appears that the infectivity of the trypsin purified protein is as great or greater than that of the controls. The virus protein after treatment with trypsin had been precipitated by ammonium sulfate at pH 4.5–5.0, filtered on Celite, and recrystallized three times by Stanley's procedure,[6] using CaO for elution, and acetic acid in ammonium sulfate for crystallization. It is probable that this subsequent treatment freed the preparation of trypsin, which either has only a temporary inactivating effect,[5] or acts on cells of inoculated plants so as to prevent entry of virus into living cells, when it is still present in the inoculum.[7]

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CATALYTIC REDUCTION OF THE METHYL ESTER OF 2:3:4-TRIACETYL α-METHYL-GALACTURONIDE TO METHYL-GALACTOSIDE

IN a recent note[1] there was described the reduction of methyl ester of 2:3:4-trimethyl α-methyl-d-galacturonide to 2:3:4-trimethyl α-methyl-d-galactoside. We now wish to report on the reduction of the methyl ester of 2:3:4-triacetyl α-methyl-galacturonide to methyl-d-galactoside. Thus, in one step, reduction of the $-COOCH_3$ and deacetylation are accomplished.

The composition of the methylgalactoside was as follows: C 43.51, H 7.4, OCH_3 16.91 (for $C_7H_{14}O_6$ Calc. C 43.30, H 7.3, OCH_3 15.98).

The application of this method to the study of aldobionic acids and other uronic acid derivatives is in progress.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A METHOD FOR THE SECTIONING OF PROTOZOA EN MASSE

OF the methods commonly used for sectioning protozoa *en masse,* those of Sharp[1] (imbedding in a hard paraffin mold), Calkins[2] (entanglement in zooglea) and Belar[3] (glass tubing closed at one end by bolting cloth) are no doubt the most practicable. In an attempt to reduce the loss of specimens, the present method of imbedding in a bag made of mammalian mesentery was devised. I have used the method successfully in sectioning several lots of large endocommensal ciliates, and an account of the procedure may be useful to others.

To make a form over which the bag may be shaped, roll a bit of warmed hard paraffin of such size that a sphere 2 mm in diameter results; while still warm impale it on the end of a round wooden toothpick or on a wooden pin, pointed at both ends, about 5 cm long and 0.6 mm in diameter near the ends.

Excise a transparent piece of fresh mesentery (cat or rabbit) 1.5 cm in diameter, transfer it to a dish filled with warm salt solution to a depth of 7 cm and, after rinsing, dip it out with the paraffin head of the pin so that it hangs symmetrically over the head. Stick the pin head-upright into a piece of sheet cork and tie with fine, black, cotton thread (No. 80) an overhand knot securely around both mesentery and pin just below the head. Handling the pin by its cork base, plunge it head first into a deep dish of Bouin's fluid and shake it about head-downward in the fixative; the free edge of the bag can thus be made to stand out nearly at right angles to the pin and to harden in this position. Leave it immersed head-downward for 24 hours, allowing the cork to serve as a float.

Remove the pin from the cork, wash in 80 per cent. alcohol as usual, dehydrate and transfer to xylene for four hours to dissolve out the paraffin interior of the bag. Reimmerse in absolute alcohol, transfer to 80 per cent. for a day and with forceps push the bag off the pin. There results a tough, fairly rigid, thin-walled bag for receiving the protozoa; it has an opening equal to the diameter of the pin and has a draw string in place; it may be stored in alcohol until needed.

To get the fixed protozoa into the bag, transfer them to a watch glass of 80 per cent. alcohol. Make a small basket or low cylinder of fine-mesh wire gauze just large enough to receive the bag, immerse the basket completely in a watch glass of 95 per cent. alcohol and set the bag into the basket with its opening uppermost. With a pipette having a straight, slender tip about 2 cm long, transfer the protozoa to the bag under the dissecting binocular. They need merely to be released from the pipette directly above and near to the opening in the bag; they will drop or stream into it because of the greater specific gravity of the 80 per cent. alcohol. The process should not be

[6] W. M. Stanley, *Jour. Biol. Chem.*, 115: 673, 1936.
[7] W. M. Stanley, *Phytopath.*, 24: 1055, 1934.
[1] R. G. Sharp, *Univ. Calif. Publ. Zool.*, 13: 43, 1914.
[2] G. N. Calkins, *Jour. Exp. Zool.*, 27: 293, 1919.
[3] K. Belar, *Methodik der Wiss. Biol.*, 1: 735, 1928.
[1] SCIENCE, 86: 2232, 332, October 8, 1937.

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.

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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.

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[1] Aided by a grant from the Eli Lilly Fellowship Fund.

[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.

[4] E. W. Flosdorf and S. Mudd, *Jour. Immunol.*, 389–425, November, 1935.

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