acute influenza became resistant to intranasal inoculation of the experimental infectious agent and "natural" influenza virus. Hence, the use of both streptococcic and viral vaccines in prophylaxis should afford protection.

The possibility that the infectious agent obtained in these experiments might represent pickups of latent or spontaneous pneumotropic virus, described by others,[3-7] in mouse stocks was considered and a report has been withheld until the evidence against such possibility seemed conclusive. Since the different strains of the infectious agent obtained by me from pneumotropic streptococci and "natural" influenza virus isolated by others are alike, the possibility of pickups of the latent virus in mice applies equally to my experiments and to the isolation and propagation in mice of "natural" influenza virus by others. The controls in both instances suffice, it would seem, to eliminate this possibility. Control inoculations made in 712 mice during the course of these experiments with (1) emulsions or filtrates of emulsions of lungs of normal mice and of the few uninoculated mice in which lesions of lungs had been found, which, however, clearly were different from those in test mice; (2) filtrates of dextrose-brain broth cultures of the streptococcus, and (3) filtrates of dextrose-brain broth and chick-embryo medium, and inoculations made in 1,142 mice with non-pneumotropic streptococci from sources remote from influenza, did not yield the infectious agent.

The data obtained indicate that the pneumotropic, filtrable, transmissible infectious agent obtained from pneumotropic streptococci appears to be true influenza virus, as now understood, and that pneumotropic streptococci in influenza and related respiratory infections, such as primary atypical pneumonia and influenzal bronchopneumonia, may be an important source of what is now considered virus.

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# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## SCREW-CAPPED BACTERIOLOGICAL CULTURE TUBES

A NEW screw-capped bacteriological test-tube has been placed on the market by the Will Corporation.[1] This test-tube has been tried in our laboratory and has been found to have certain advantages over the conventional cotton-plugged culture tube. For example, media that need to be made up only occasionally can be kept in the screw-capped tubes fresh and moist at room temperature for many months. The experience with this new tube has shown, however, that it still could be improved considerably by extending the length of the screw cap and the neck of the tube from the present length of about 10 mm to the least 25 mm without changing the thread.

Fig. 1 shows the suggested shape and length of the neck with the screw thread and the cap. Three important improvements could be achieved by such modification of design. (1) Tubes with long caps and necks can be incubated with the caps slightly unscrewed to permit the same free exchange of air as through the usual cotton plugs, without any increased danger of contamination, whereas with the short caps contamination occurs readily if the caps are not closed tightly. (2) Since the present short caps must be closed tightly immediately after the removal of the test-tubes from the sterilizer, because otherwise they do not protect the medium sufficiently from contamination during the process of cooling, a negative pressure develops in the cooled-off tubes. When such

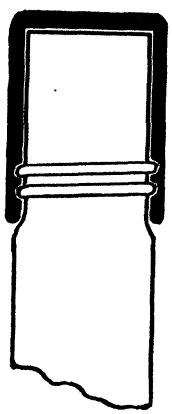

FIG. 1

partly evacuated tubes are opened for inoculation, the air current entering the tubes may sometimes introduce contaminating microorganisms. Also, the short cap will stay in place only when screwed on so far that the vinylite lining of the cap sticks to the end of the tube, whereupon inrushing air forces the lining suddenly down into the tube. By having the necks and the screw caps much longer than the present ones,

[3] A. R. Dochez, K. C. Mills and B. Mulliken, *Proc. Soc. Exp. Biol. and Med.*, 36: 683, 1937.
[4] F. B. Gordon, Gustave Freeman and J. Marion Clampit, *Proc. Soc. Exp. Biol. and Med.*, 39: 450, 1938.
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[6] Helen V. Karr, *Jour. Infect. Dis.*, 72: 108, 1943.
[7] Clara Nigg, SCIENCE, n.s., 95: 49, 1942.
[1] Catalog Supplement Number Three, p. 21, No. 16535.

the test-tubes could be cooled off slowly with the caps entirely unscrewed but still fitting tightly enough over the long necks of the test-tube to prevent the contamination, thereby permitting the equalization of air pressure on the inside and the outside of the test-tubes without contamination. (3) Longer caps can be held more securely between the fingers when the cultures are being transferred.

The test-tubes with properly constructed screw caps can prove to be more versatile, and in certain types of bacteriological work can be superior, to conventional cotton-plugged tubes.

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## PREPARATION AND STORAGE OF AUTOPSY NERVE GRAFTS

THE use of prepared nerve grafts in bridging nonsuturable peripheral nerve gaps is a recognized neurosurgical procedure of great value.[1-5] As a consequence, means have been devised of preparing and storing graft material which facilitate its use at operation. Applicable primarily to experimental work are those measures utilizing direct aseptic transfer.[6-8] In addition efforts at relatively permanent storage utilizing freezing-drying, dehydration and cold storage have been introduced by Weiss and Taylor.[9] The disadvantages in elaborate preparation and storage are at once apparent. The following procedure is offered as a simple flexible method of preparing and storing autopsy nerve grafts which is adaptable to any size or complex of nerves, and simplifies pre-operative sterilization through the expedient of alcohol immersion of the containing vial similar to sterilization of suture material.

The nerve or nerve complex is dissected as cleanly as possible at autopsy and placed in tap water. Using clean instruments the material is freed of all excess fibrous tissue. The stripping of straight nerves such as antibrachial cutaneous, ulnar, median, musculocutaneous, radial, femoral, obturator and sciatic imposes no difficulty. Cleaning of more elaborate complexes such as the brachial plexus requires repeated moistening and dissection of the unwanted tissue. The material is next sized. For most purposes lengths of 7 centimeters suffice. For nerves of smaller diameter this length is rarely required. Appropriate lengths are cut and the pieces of larger girth (median, radial etc.) tied under moderate tension to segments of glass tubing with the string passed through the cylinder of glass. The smaller pieces (antibrachial cutaneous, obturator, etc.) are fixed to ordinary mimeograph paper strips by inserting the ends under slit loops.

Fixation is accomplished by immersion in 10 per cent. formalin for three days. The material is then subjected to running tap water wash over night and dehydrated to 70 per cent. ethyl alcohol. Three changes of ethyl alcohol are employed over a period of 3 days to assist in sterilization. The tissue is then hydrated aseptically by repeated washes of distilled water, then placed in sterile saline overnight. The nerves are then transferred to saline in sterile vials of soft glass and sealed. Sterility is checked by aerobic and anaerobic cultures of the first saline wash.

The larger nerves retain their position in fixation and are freed from the supporting glass for introduction into the vials. The paper mounts of the smaller nerves may be rolled and introduced into the vial with the nerves attached to prevent distortion of the smaller structures freed of their support. Bulky preparations (brachial plexus) do not lend themselves to the storage technique described above. On the few occasions these have been required the material has been spread out and sutured to thick white cardboard and passed through the formalin into 70 per cent. ethyl alcohol in which they have been stored until just prior to operation.

Material prepared as described above has been retained at room temperature for periods up to 5 months with no sign of deterioration. Experiments employing these grafts have given excellent results and the procedure is now standard in the division of neurosurgery.

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