TABLE 1

TESTS FOR POLIOMYELITIC VIRUS IN SEWAGE SPECIMENS

Sewage specimen	Original amount	Interim between collection and inoculation	Amount of inoculum		Monkey No.	Result
			Intraperitoneal	Subcutaneous		
(A) Pump	3.5 liters	6 and 7 days	36 cc	22 cc	1229	Abscess of abdominal wall; acute peritonitis. Killed on 4th day.
(B) Har.	0.5 liter	7 days	24 "		1231	Chronic and acute peritonitis. Died on 18th day.
(C) Pump (1)	8 liters	1 and 2 days	120 "		1227	Poliomyelitis. Killed on 10th day. Passage to monkey 1248 successful.
(2)		3 days	125 "		1232	Poliomyelitis. Died on 8th day. Passage not done.
(D) Hosp. (1)	7 "	5 "	75 "	125 "	1239	Infected abdominal wall. Died on 2nd day.
(2)		6 "	25 "	46 "	1240	Abscess of abdominal wall. Killed on 4th day.

monkeys develop such a severe form of the experimental disease when inoculated with material from a human source, and so we have suspected that the amounts of virus in the inocula here were not small.

The results of our experiments to date on this type of material appear in Table I. Here it will also be seen that our other attempts to isolate virus from Charleston sewage were unsuccessful as a result of the premature death of the monkey from bacterial infection. Obviously, methods have not yet been perfected for handling or testing this type of material.

Our criteria for the diagnosis of poliomyelitis in these experiments have been practically the same as those we have used in previous work[2] on the isolation of poliomyelitic virus from extra-neural human sources, namely, that the inoculated animal developed signs and symptoms compatible with those of the experimental disease; that typical lesions were found in the spinal cord, and that the strain was successfully passed to another monkey. All these criteria were met in the case of monkey 1227; one of them is omitted in monkey 1232, for a passage of this strain was not attempted.

It is not evident from this work whether the presence of poliomyelitic virus in sewage is a direct or even an indirect link in the chain which leads this infectious agent from one patient to another in this disease. Our report merely calls attention to the fact that poliomyelitic virus may not only be present in urban sewage but also that it may possibly be present in appreciable quantities.[3]

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

DEMONSTRATION OF THE SHAPE OF CILIA IN NORMAL MOTION

IT is often desirable to demonstrate the shape and motion of cilia without the use of methods which may cause distortion or prevent an estimation of the rate of beating. An easily made stroboscope meets this need.

To make a periodically moving object like a cilium appear stationary, it must be made visible repeatedly at only one point in its path. This can be achieved by a light flashing on the object at exactly the same frequency as its motion. If the synchronization is not quite perfect, the cilium will appear to be moving very slowly because each flash of light will reveal it at a slightly different position. Should the synchronization be much poorer, the entire effect will be lost. These principles constitute the basis of the stroboscope.

An obvious means of producing a flashing light is to have a disk with a number of regularly spaced radial slits rotating in front of a fairly strong light source. Four slits have been found most suitable. In projection, it makes little difference where the disk is located; for convenience it may be placed between the ocular and the screen.

A satisfactory method for the adjustment of the disk's speed is an important element in the success of the stroboscope. A rheostat in series with the motor attached to the disk is adequate; however, finer adjustment is obtainable with two rheostats in series if the resistance per unit length of one is considerably larger than the other's. In the way of a motor, even those obtainable in metal construction kits are usable. A possible arrangement of the apparatus is shown in the diagram.

The rate of ciliary motion can easily be determined from the disk's speed when synchronization, as judged from the cessation of movement, is attained. In general, it will be found a simple matter to count the

[2] Criteria reviewed by A. J. Vignec, J. R. Paul and J. D. Trask, *Yale Jour. Biol. and Med.*, 11: 15–31, 1938.

[3] Aided by grants from the National Foundation for Infantile Paralysis.

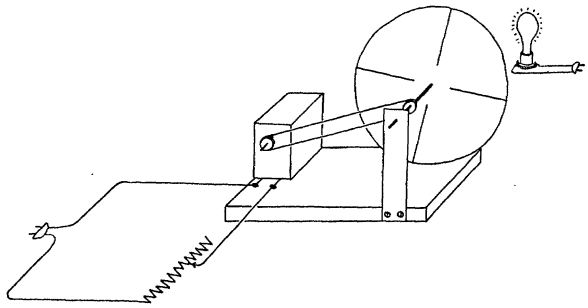

FIG. 1. A simple stroboscope.

number of rotations of the disk in a given period of time, which, when multiplied by the number of slits, will give the corresponding number of ciliary beats.

Organisms which will not move about much or side-view preparations of ciliated epithelium are best suited for demonstration purposes. Rotifers like Philodina and various attached Infusorians are excellent material, although with proper handling even an active form like Paramecium can be used.

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A NEW MATERIAL FOR MOUNTING NERVE TISSUE SECTIONS IN PARAFFIN FOR SILVER STAINING OR RESTAINING

EGG albumin solution used to affix nerve tissue sectioned in paraffin to slides has not proven completely satisfactory. Sections so mounted are easily lost when the slides are passed through the various solutions. The histological picture obtained in those sections successfully carried through is not absolutely clear. While attempting to remove the haziness from such material, it soon became evident that the proteins of the albumin are responsible for the gray background. Some other mounting media were therefore considered. One which would have no reducing properties and which would, at the same time, hold the sections in place was desired. Because of the inertness of starch, a paste of this substance was tried. The following form of paste gave the most satisfactory results: 1 gm starch thoroughly mixed with 10 cc of cold water was added to 20 cc of boiling distilled water and constantly stirred until the suspension, which is opalescent, is uniform and free from lumps. Two drops of hydrochloric acid were then added, and the solution was boiled for an additional three to five minutes. After cooling, a small crystal of thymol was added as a preservative. The result is a clear thin paste which is used very much the same as the albumin fixative. The paraffin sections which are placed on the slide covered with the hydrolyzed starch suspension are allowed to dry for two to three days in an oven at 45° C.

This fixing material was used on sections from various sized blocks of tissue which had been impregnated with silver according to Bielschowsky's or Cajal's method. These blocks were sectioned and mounted serially. Out of 500 sections of one series, only one section was lost in the solutions. In all cases, the resulting pictures were clear. No precipitate was evident.

In another series of tests, fresh formalin fixed tissue was sectioned in paraffin and impregnated after being fixed to the slide. In this way a quite even staining reaction was obtained. The inevitable gradations which occur in mass staining were avoided. In addition, the time necessary for impregnation was reduced to less than half. These sections were mounted and dried, passed through xylol into water, through the graded alcohols, and washed for about fifteen minutes and then allowed to remain in pyridine over night. Next, the tissues were washed in distilled water for about ten minutes and put into a solution of 5 per cent. silver nitrate for three hours in the dark. They were then transferred into a solution of ammoniacal silver nitrate, *i.e.,* 200 cc of 5 per cent. silver nitrate solution plus 5 cc of 10 per cent. sodium hydroxide. Add ammonia, drop by drop, until the precipitate dissolves. The sections are allowed to remain in this solution for half an hour, then rapidly washed in distilled water, placed in a 10 per cent. formalin solution for about 5 minutes. After this, they are washed again and may be toned in gold and left in hypo for two minutes, then dehydrated, cleared and covered in the usual way.

This method stains the cells as well as neurofibrillae. Further work is being done in applying this method to other routine neurological techniques, such as Bodian's, modified Bielschowsky's and Ranson-pyridine methods.

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