that an agent isolated by one of their group could be passed directly to the cotton rat, but not after filtration through a Berkefeld N filter. The relation of this virus to pneumonitis has not yet been established.

Following an outburst in 1942 of continued and sporadic cases of primary atypical pneumonias, occurring in male personnel, several hundred mice, 12 ferrets and 8 hamsters were given intranasal inoculations of pooled and unpooled throat washings and sputa from acute x-ray positive cases. The results obtained were insignificant. Mice would die with some regularity if unfiltered washings were used. Where Berkefeld V filtration of throat washings and ground sputum was used intranasally, occasional mice of a series would die in 7 to 12 days, or longer, but successive serial transfers of the sterile lung suspensions were negative.

In 1942, sputum and nasal washings were collected from twelve patients (subsequently Lygranum negative) exhibiting typical symptoms by roentgenogram of severe primary atypical pneumonia. Their sputum and nasal washings were suspended in veal infusion broth emulsified by grinding and shaking and filtered through Berkefeld V candles. Previous experiments with N filtrates had already been found to be negative for mice and 2 human volunteers.

The veal infusion filtrates were given intranasally to 5 human volunteers in amounts from 20 to 40 cc each. The volunteers remained healthy and did not subsequently exhibit signs of illness. Control mice also remained healthy.

The use of human volunteers is reported. The negative results obtained are consistent with the apparent relatively low degree of infectivity.

The twelve patients mentioned were Naval personnel. The five human volunteers were non-military personnel.

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# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## STERILITY TEST FOR PENICILLIN

WITH the introduction of the use of penicillin in the successful treatment of systemic and wound infections caused by many gram positive and some gram negative bacteria,[1, 2, 3, 4, 5, 6] the necessity of providing a suitable method for neutralizing the antibacterial effects of this highly active substance prior to sterility test becomes apparent. The approach used in developing a satisfactory method for testing penicillin for sterility involved a consideration of some chemical or physical agent which would inactivate the substance completely and yet in itself have no antibacterial effects on possible contaminating organisms in the product.

Among the various agents tested, which appeared to meet the necessary requirements given, were two enzyme preparations, Taka-diastase and particularly Clarase,[7] a more active diastatic enzyme system. These enzymes were found to inactivate the antibacterial effects of penicillin in two hours or less when incubated in the presence of the agent in a water bath at 40° C and tested by the Oxford cup-plate method. Taka-diastase was effective under these conditions at pH 6.0 and 8.0 but not in buffer solution of pH 4.0. Clarase, on the other hand, neutralized penicillin activity completely at all the pH ranges given. Additional studies on the relative inactivating actions of the two preparations indicated that while Taka-diastase was effective in this test in dilutions of 1:200, a similar action was had with Clarase in a final dilution of 1:4,000.[8]

Since it was found that relatively high dilutions of Clarase would neutralize the antibacterial effects of penicillin when tested by the Oxford cup-plate procedure, it appeared worth while to study the effects of the enzyme system against the agent in the presence of a fluid medium. This study was carried out as follows: A 1 per cent. stock solution of Clarase was prepared in buffer solution, of pH 7.0 and sterilized by Berkefeld filtration. Samples of sodium penicillin powder,[9] as received in the sterility control laboratory, were inoculated with dry cultures of *Clostridium tetani, Clostridium septique, Bacillus subtilis, Eber-*

[1] E. P. Abraham, E. Chain, C. M. Fletcher, A. D. Gardner, N. G. Heatley, M. A. Jennings and H. W. Florey, *Lancet*, 2: 177, August 16, 1941.
[2] J. E. Bordley, S. J. Crowe, D. A. Dolowitz and K. L. Pickrell, *Ann. Otol., Rhin. and Laryngol.*, 51: 891, 1942.
[3] M. E. Florey and H. W. Florey, *Lancet*, 1: 387, March 27, 1943.
[4] W. E. Herrell, *Staff Meet. Mayo Clin.*, 18: 65, 1943.
[5] W. E. Herrell, E. N. Cook and L. Thompson, *Jour. Am. Med. Asn.*, 122: 289, 1943.
[6] C. H. Rammelkamp and C. S. Keefer, *Am. Jour. Med. Science*, 205: 342, 1943.
[7] "Standardized" Clarase from the Takamine Laboratories, Clifton, N. J.
[8] Details of this and additional studies on enzymes *vs.* penicillin to be published elsewhere.
[9] 10 mg containing approximately 240 units per mg.

*thella typhi, Escherichia coli* and *Staphylococcus aureus*. To the dry contaminated powders was added 2 cc of the prepared Clarase solution. The enzyme penicillin solutions or suspensions were transferred immediately to several tubes of Brewers' fluid thioglycollate medium (approved medium of the National Institute of Health). The contents of the tubes were mixed thoroughly by swirling and placed at 37° C to incubate. A luxuriant growth of the inoculated test organisms was had in all tubes at the end of 48 hours. Control tubes containing gram-positive organisms and penicillin, but without enzyme, failed to show evidence of visible growth at the end of 7 days incubation. However, many of the tubes containing gram-negative organisms and penicillin without enzyme showed some growth after several days. These results could be expected on the basis of known resistance of most gram negative bacteria to the antibacterial agent.

*Method:* With the information obtained in this and additional studies, the following method for routine sterility tests on penicillin powder is proposed. A stock of 1 per cent. sterile solution of Clarase in phosphate buffer, pH 7.0, is distributed in 2 cc amounts in sterile cotton plugged test-tubes or in ampules which may be sealed. The sterile enzyme solution, stored in a refrigerator, will retain its anti-penicillin activity for a period of at least two months. The contents of an ampule of penicillin are dissolved or suspended in 2 cc[10] of prepared Clarase solution and transferred immediately to tubes of Brewers' fluid thioglycollate medium. The inoculated tubes are placed at 37° C. and examined for possible bacterial contamination throughout a period of 7 days. An additional 7 days incubation should be allowed for detection of possible mold contamination.

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## QUIETING PARAMECIUM FOR THE ELEMENTARY STUDENT

THIS new mechanical method of "hog-tying" Paramecium for classroom study has proved to be completely reliable and "foolproof" in the hands of our large student body at Washington Square College, New York University. It is somewhat simpler and more stable than the method reported recently in SCIENCE,[1] and much more dependable than any of the older methods.

The method depends upon the high viscosity, the low tonicity and the non-toxic properties of a methyl cellulose solution; and upon the fact that this solution displays very little change of viscosity as it is warmed (as by the lamp of the microscope). Best results are obtained by using a ten per cent. solution of methyl cellulose (Dow Chemical Company; viscosity type, xx low). Place one large drop of this solution on a slide in contact with an equal-sized drop of the Paramecium culture, stir the two drops together with a toothpick, put on a cover slip (no bristle or other support is necessary), and the preparation is ready for immediate study.

The slowing effect of this solution is remarkable. Now it takes the specimen about ten minutes to cross the high power (4 mm) field. Nevertheless, the form remains altogether normal, and even the rotational character of the locomotion is retained. The ciliary beat is so slow that it is quite easy to differentiate the "active" and "recovery" phases of the strokes. The progressive passage of the individual food vacuoles throughout the body can be followed (especially if a little carmine suspension is added to the culture just before mixing with the cellulose solution), and the process of defecation can be observed, as the specimen leaves a trail of fecal granules along the slow path of its progress. The untrained student also finds little difficulty in getting a good look at the other structures of the specimen, including the pulsating contractile vacuole, the gullet and undulating membrane, the trichocysts and in some cases even the macronucleus.

The further advantages of this method are inherent in the stability of the methyl cellulose solution and of the resulting "wet mount." Even without sterilization and at room temperature, the stock cellulose solution will keep for months. The "wet mount," due to the very slow evaporation of the solution, far outlasts any ordinary preparation. Without any attention at all, it does not deteriorate appreciably in two to three hours.

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[10] This quantity of Clarase solution will inactivate as much as 50 mg of penicillin containing a total of potency of 10,000 or more Oxford units.

[1] John B. Buck, SCIENCE, 97: 2526, 494, May 28, 1943.

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