IN THE LABORATORY

A Technique for Aeration of Sterile Liquid Culture Medium

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Sterile aeration of liquid culture medium can be accomplished simply by inserting a sterile tube through the cotton plug at the mouth of the tube and introducing sterile air through this tube. This method, however, does not allow for convenient manipulation of the culture flasks, making transfers, or moving the flask from the sterile air connection. In order to provide a more convenient and efficient means of aerating sterile root cultures the flask illustrated in Fig. 1 was constructed.

Sintered glass filters were prepared according to the method

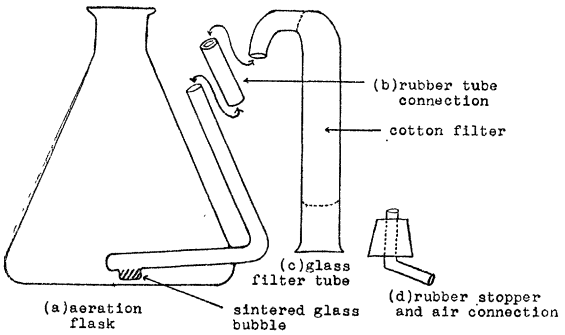

FIG. 1

of Kirk, Craig, and Rosenfels (*1*) and fused to the inside wall of the Erlenmeyer flasks as illustrated (a). An external arm, long enough to allow ample room for the culture medium to flow out without overflowing if the air pressure were cut off, was fused in place at the exit of the filter tube and bent up about parallel to the side of the flask. The sintered glass bubble was placed, open face down, in about the center of the flask bottom. Erlenmeyer flasks (250 ml.) were used.

To provide sterility, a glass tube was made in the shape shown (c), filled with cotton, and fitted with a one-hole rubber stopper and glass tube (d) in the outer end and a rubber connection (b) on the small end to hook to the glass side arm of the flask.

This flask was made and connected in sets of 10 to 14 with a glass manifold, the pressure and air flow in each flask being regulated with a screw clamp on the short rubber tube between the glass tube (d) and the manifold. Some special care had to be taken to attach the cotton filter tube to the side arm of the flask, but, once set up, the flasks were quite substantial. The cotton filter tubes were placed so that they lay against the flask. To avoid the danger of strains at the base, the flasks must be annealed well when the sintered glass filter tubes are inserted. It is also important to test the filters carefully for consistency in bubble size and porosity if the flasks are to be used in sets; otherwise, it is difficult to adjust the air flow evenly.

Further precautions were taken to ensure sterility by passing the laboratory compressed air used through a 2-l. suction flask of sterile water, protected on each side by glass, cotton-filled tubes (15 x 1 inches), before passing it into the glass manifold with the individual take-offs. The water, in saturating the air, prevented the cultures from drying out. Cotton plugs were used at the top of the flasks as usual. The air intakes may be detached and reconnected without contaminating the cultures.

Excised root cultures were kept sterile and aerated in these flasks over a period of three months and could have been kept longer if desired.

Reference

1. KIRK, P. L., CRAIG, R., and ROSENFELS, R. S. *J. ind. eng. Chem.* (Anal. ed.), 1934, **6**, 154–155.

Studies on the Stability of Streptomycin in Solution

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In the process of developing satisfactory assay methods in this laboratory, preliminary studies have been made of the stability of the F.D.A. streptomycin working standard (*2*) when held in solution in different concentrations and at three pH levels. The working standard, which was established on November 20, 1945, has a potency of 400 μg. of activity/mg. when streptomycin base is assigned a value of 1,000 μg./mg. It was established early that streptomycin is considerably more stable in solution than penicillin, and it appeared to be particularly desirable to determine the most efficient concentration and pH level that could be utilized for the establishment of a working standard solution. Accordingly, the working standard was dissolved in sterile 0.05M potassium phosphate buffer at pH levels of 6.0, 7.0, and 8.0 in quantities sufficient to give solutions of 100 μg./ml. and 1,000 μg./ml. at each pH level. The two concentrations at each pH level were divided into 100-ml. amounts and tightly stoppered to minimize evaporation and chances of mold contamination. The solutions were held at 10°C. and assays[1] performed by both the turbidimetric and plate methods (*2*) every week for a period of three months. The data obtained were subjected to statistical

[1] These solutions were assayed against a standard solution prepared monthly to contain 100 μg. of the working standard/ml. in .005M potassium phosphate buffer—pH 6.

analysis, and it was shown that there was no appreciable drop in potency by either assay technique. Certain other variations, however, are worthy of mention.

In the specifications drawn up by the F.D.A. the final assay solution for the turbidimetric assay contains 100 μg./ml. of streptomycin. The 100-μg./ml. solution of standard streptomycin was therefore assayed undiluted, and the 1,000-μg./ml. solution was diluted 1:10 with distilled water to bring it to the proper

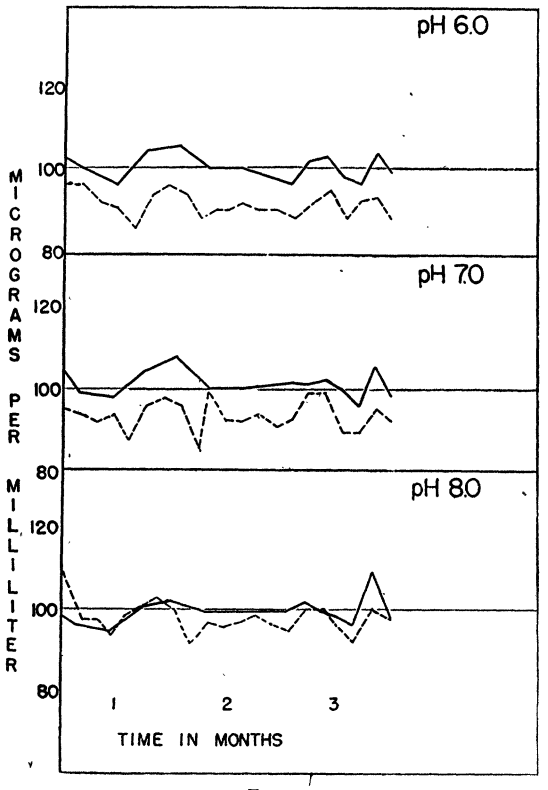

FIG. 1

assay dilution. This gave a 100-μg./ml. solution in 0.05M phosphate buffer and a 100-μg./ml. solution in 0.005M phosphate buffer at each pH level. The assay values obtained were as follows: At pH 6.0 the mean average of the 0.05M buffer concentration was 91.5 per cent activity and that of the 0.005M concentration, 100 per cent. At pH 7.0 the mean average of the 0.05M buffer concentration was 93.7 per cent activity and that of the 0.005M, 100 per cent. At pH 8.0 the 0.05M concentration showed 97.9 per cent activity, while the 0.005M buffer concentration showed 99.9 per cent activity. In other words, when the concentration of phosphate salts was increased, the activity of the streptomycin decreased, at least when that activity was measured by a turbidimetric method. The lowering of activity, however, was not the same at the three pH levels. At pH 6.0 the 100-μg. standard solution was 8.5 per cent lower than the 1,000-μg. standard; at pH 7.0, 6.3 per cent lower; and at pH 8.0, 2.0 per cent lower. The assay values are given in Fig. 1. The solid line represents the standard in .005M buffer (original, 1,000 μg./ml.), and the broken line represents the standard in .05M buffer (original, 100 μg./ml.). In three additional assays run turbidimetrically on these same solutions the 1,000-μg./ml. standard solution was diluted with 0.05M buffer instead of water. This had the expected effect of *lowering* the average assay figure for this solution closer to that of the 100-μg./ml. standard solution at all three pH levels. Apparently, a combined depressor effect is exerted on the activity of streptomycin by the increased concentration of buffer salts and the lowering of pH. Waksman (*1*) has demonstrated a similar restriction of the activity of streptothricin by buffer salts.

Summary. Streptomycin held in solution in concentrations of 100 and 1,000 μg./ml. at 10°C. is stable at pH 6.0, 7.0, and 8.0 for a period of three months. The depressor effect observed in the turbidimetric assay appears to be the result of two factors, buffer salt concentration and pH, and is most marked at pH 6.0. Almost normal activity reappears at pH 8.0 even in the presence of the greater concentration of phosphates.

References

1. WAKSMAN, S. A., *et al. Proc. staff Meet., Mayo Clin.*, 1944, **19**, 537–548.
2. ————. *Minimum specifications for streptomycin, standards of identity, strength, quality, and purity.* Washington, D. C.: Food and Drug Administration, 1945.

A New Type of Electroencephalographic Electrode Coordinator With Semipermanent Electrodes

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Gibbs (*1*) has made the statement that ideal electrodes for electroencephalographic purposes should not produce artifacts; should be easy to apply, keep on, and get off; and should be cheap and painless. Keeping these requirements in mind, most electroencephalographic laboratories employ enamel-insulated copper wires (usually 29- or 30-gauge), with the common solder-type pellets attached as electrodes. Unfortunately, this type of electrode can be used adequately only for 5–10 tracings and must then be replaced. In one way or another, a good deal of time on the part of the electroencephalographic laboratory personnel is therefore lost in the purely technical process of making and remaking electrodes. Furthermore, these commonly used electrodes frequently become tangled and bent because of their inherent mechanical characteristics, subsequently producing artifacts. Another point of difficulty is tracing the electrodes to their respective outlets at the terminal board.

Some workers (Ogilvie, *2*) have advised the use of "tinsel wire." In our experience, however, this carries too heavy a weight for the attachment on the scalp, and additional artifacts are produced by insufficient contact between the end of the "tinsel wire" and the silvered union which is commonly used.

It should be mentioned that a device for reducing the time to apply electrodes is the "headband electrode holder." This device, described by Ulett and Claussen (*3*), also avoids the use of collodion, depending entirely on pressure. However, an increased number of artifacts are unavoidable with this and