

breaking its surface. We find that with the brand of agar recommended² a concentration of 0.3 per cent. gives satisfactory results. Another brand tried required higher concentration, resulting in diminished diffusion of the active substance. The addition of glucose for staphylococci and of sodium nitrate for *B. coli* insures good growth in the depth of the medium. The medium is sterilized in bulk at 115° C (10 lbs. pressure) for 20 minutes and cooled down to 40°–42° C. One agar slant of a 24-hours old culture of the test organism is washed with 5 ml of saline, and 0.2 ml of this emulsion is added to 1 litre of the medium, which is then distributed into tubes to a height of approximately 5 cm, the diameter of the tube being of no importance. The tubes are placed immediately in the refrigerator and are ready for use after two hours, but can be kept for at least one week.

The solutions to be tested, which need not be sterile, should not be excessively acid or alkaline. They can be diluted with water or any suitable buffer between pH 5 and 8. They must not contain ether or chloroform and not more than 10 per cent. alcohol or acetone. Approximately 0.5 ml of each solution (the exact volume does not matter) is pipetted on top of the agar stab. Standard solutions are treated in the same way. After overnight incubation, the degree of inhibition can be measured by placing a transparent millimeter scale on the wall of the tube or by the use of a pointed caliper.

The assay of penicillin is carried out in the same way as described for the cylinder method, *i.e.*, several dilutions of a standard are set up simultaneously with the unknown, and a graph is constructed by plotting the zone of inhibition in mm against the number of units per ml. Using amounts of penicillin between 0.2 and 2 units/ml a curve similar in shape to that given by Heatley is obtained.

Being based on the same principle as the cup assay, the method is subject to the same limitations, *i.e.*, the diffusibility of the inhibiting substance and a number of other factors are apt to influence the results.

During the past year the method has been successfully applied to the study of a number of other antibiotics derived from moulds and has proved particularly useful in the survey of large numbers of mould

² Agar, powdered, natural. Agar Products Company, 616–618 North Robertson Blvd., Los Angeles 46, Calif.

cultures and in the follow-up of the developing antibiotic activity.

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SEPARATORY FUNNELS AS EXPERIMENTAL CHAMBERS IN STUDIES OF INSECT PHYSIOLOGY

In studies of insect physiology an array of experimental chambers is frequently required within which the animals can be exposed to various gases or vapors. Preferably, such chambers should be not only durable and inexpensive, but also transparent, air-tight and capable of being easily washed out with and sealed to contain the gas mixtures.

I wish to call attention to the fact that separatory funnels fulfil these requirements in every respect, although they have apparently not been previously used for this purpose. The gas mixtures are readily introduced through the stem of the funnel, the chamber, by virtue of its smooth contours, easily washed out, and, most important, the experimental conditions then maintained by lubricated ground-glass seals at both ends. The further fact that separatory funnels in a variety of sizes and shapes are already at hand in most laboratories is not the least advantage at the present time.

In experiments in which one desires to test a large number of insects within a single chamber, it may prove convenient to enclose each animal within a gelatin capsule of suitable size. In order to insure continuous equilibration with the gas mixture in the funnel, numerous perforations should previously be made with a hot needle in both ends of each capsule. The capsules can then be numbered with ink and, if so desired, arranged in a continuous strip by placing them transversely between two longitudinal lengths of Scotch tape.

In prolonged experiments the atmosphere in the funnels can be washed out and renewed at suitable intervals. In studies not involving gas mixtures containing carbon dioxide, a loosely plugged glass vial containing soda lime should always be enclosed in the chamber.

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SCIENTIFIC BOOKS

IMMUNO-CATALYSIS

Immuno-Catalysis. By M. G. SEVAG. Pp. xv + 272. Illustrated. Springfield (Ill.) and Baltimore: Charles C Thomas. 1945. \$4.50.

THE scope of this book is well stated in the preface

by Dr. Stuart Mudd: "The fullness of the integration possible between the fields of enzyme chemistry, immunochemistry and the mechanisms of infectious disease, has, indeed, in the writer's belief, been indicated for the first time in this volume . . ." As stated, the