

breaking its surface. We find that with the brand of agar recommended<sup>2</sup> 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

<sup>2</sup> Agar, powdered, natural. Agar Products Company, 616-618 North Robertson Blvd., Los Angeles 46, Calif.

cultures and in the follow-up of the developing anti-biotic 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

large common denominator of enzymology and immunology has long been perceived. The basic theoretical considerations have been formulated, but hitherto they have not been extensively explored in the light of modern developments in these fields, and with a view to elucidation of concrete mechanisms.

The subject is considered in five sections: (1) "Antigens as Biocatalysts"; (2) "Antibody as a Specific Enzyme Inhibitor"; (3) "Anti-Enzyme Immunity"; (4) "Immunity against Bacterial Enzymes," and (5) "The Problem of Antibody Formation against Respiratory Enzymes."

The first section deals with the characteristics of catalysis in general and reviews the data showing that an antigen functions as a catalyst: (a) one molecule of an antigen induces the formation of many molecules of antibody; (b) the antigen (catalyst) forms no part of the resulting antibody (reaction product); (c) the reaction catalyzed is thermodynamically possible regardless of the presence of the catalyst. The nature of antibodies and current theories of the mechanism of their formation are summarized. Of especial interest is a discussion of the directive effects of optically active catalysts upon certain simple chemical reactions.

In the second section, the hypothesis is advanced that since antigens are catalysts and "since practically all proteins are antigenic, the conclusion appears to be inescapable that all proteins are endowed with catalytic activity." (The possibility that certain non-protein substances are antigenic is considered briefly and set aside on the grounds that it is not proved that such substances do not combine with proteins *in vivo*, to form the complete antigen.) This hypothesis leads to the following analogy:

Antigen + globulin factors → Antibody globulin  
Enzyme + substrate → Reaction products

Hence antigen and enzyme, globulin factors and substrate, and antibodies and reaction products, respectively, are regarded as counterparts, and the "neutralization" of an antigen by its antibody is comparable to the specific inhibition of an enzyme by the reaction products. It is noted that this viewpoint differs from the usual one, wherein the relationship of antibody to antigen has been likened to that of enzyme to substrate.

The remainder of the second section, and of the book, is largely devoted to a summation of experimental data bearing on the above analogy, principally under the headings of the formation of specific inhibitors in enzyme reactions and of antibodies against enzymes. The anomalous failure of the prosthetic groups of respiratory enzymes to function as haptens is considered. Particularly commendable is the author's broad conception of bacterial toxins and the

recognition of bacterial enzymes as toxins, points concerning which too restricted a view has been too often taken.

In the formulation of his ideas, Dr. Sevag has consulted more than a thousand publications, of which 482 constitute the bibliography of his book. Immunologists and enzymologists will be indebted to him for the critical collation of so much literature, much of it not readily accessible previously.

The author has successfully avoided the pitfall of unnecessary duplication of basic material adequately treated elsewhere. As a consequence, this is no book for the neophyte. The reader will profit most if he has a solid foundation in immunology and enzymology. Furthermore, he who desires a didactic presentation will be disappointed, for Dr. Sevag does not pretend to provide the ultimate solution of the problems he discusses—quite the contrary, he attempts only to point the way. Each reader will find many opportunities to take issue with the author, who, one feels, was fully aware of the alternatives when he chose particular conclusions. The present reviewer surmises that the usefulness of Dr. Sevag's book will derive as much from the disagreement and consequent experimentation that it will catalyze, as from its factual content.

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### MOSQUITOES OF NEW JERSEY

*The Mosquitoes of New Jersey and Their Control.*

By THOMAS J. HEADLEE. 9 × 6 inches. x + 326 p. 16 pl. 87 figs. New Brunswick: Rutgers University Press. 1945. \$4.00.

In early times the travelers who wrote books frequently paid their respects in uncomplimentary terms to the mosquitoes of New Jersey and elsewhere. But in spite of the mosquito problem that persisted for years to the great annoyance of large numbers of our population, the entomologists of this country did not pay much attention to it until around 1900. According to the "Bibliography of American Economic Entomology," the 2,418 separate titles by B. D. Walsh and C. V. Riley from 1860 to 1888 include only two that deal with mosquitoes. During the same period the 3,006 titles by state and other entomologists include only 4 on mosquitoes. From 1888 to 1896, the 3,956 references include 19 on mosquitoes, 10 of which are either by L. O. Howard or by Riley and Howard. From 1896 to 1900, out of 1,882 titles, 55 are on mosquitoes. During this long period economic entomologists were concerned mainly with insects injurious to crops.

Such a condition no longer exists and we now have