BACTERIOLOGY

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H. A. DETTWILER, Eli Lilly and Company, was elected chairman for 1955

ABSTRACTS

Antigen-Antibody Reactions in Agar. ROBERT K. JENNINGS, Northwestern University, Chicago.—Antigen molecules diffuse from a mixed source into gel with remarkable independence. The distribution of each component resembles that which would be obtained if other components were absent, and may be made apparent by the precipitin reaction. Within the past six years, half a dozen methods of applying this principle to the analysis of complex biological materials have been reported.

"Simple diffusion" methods, such as Oudin's, use antibody in agar as indicator for antigen diffusing from a superimposed solution. The apparent migration of the precipitate as equivalence is established at points increasingly remote from the interface occurs at rates which are diagnostic of the concentration and diffusion coefficient of the antigen. "Double diffusion" methods use an arena of clear gel, between antigen and antibody, within which overlapping gradients establish equivalence. Applied in plate methods such as Ouchterlony's, the resulting precipitate patterns demonstrate identity or non-identity of unknown and reference reagents.

Applications reported include studies of chemical purity, of the antigen spectra of tissues from animals or insects and of bacterial culture fluids, as well as methods of screening animals for antibody production, bacteria for antigen production and many others. Even greater versatility may be anticipated as the methods are further developed and refined.

Techniques for Studying Bacterial Population Changes and Some Recent Applications. WERNER BRAUN, Camp Detrick, Frederick, Maryland.—A number of useful techniques for analyzing the occurrence of bacterial population changes and factors controlling such changes will be reviewed. The application of such techniques will be illustrated by reference to recent studies on the effects of DNA upon population changes in *Brucella*. These studies revealed two entirely different effects of DNA: (1) transformation-like phenomena which occur when competent strains are exposed to highly polymerized heterologous DNA, and (2) a non-specific selective effect, which involves an inhibition of growth of the parent type cells while favoring continued growth and the rapid establishment of mutant type cells. The latter effect can be observed with certain strains following addition of any bacterial DNA so far tested after it has been briefly exposed to DNase (Braun and Whallon, Proc. Nat'l Acad. Sci., March, 1954). The intensity of the selective effect depends greatly upon the production of a compound produced by mutant cells in the presence of enzyme-treated DNA. Some information on the general nature of this compound, indicating that it is a nucleotide, has been obtained in studies on the selective effects of variously treated filtrates obtained from mutant

cultures containing DNA + DNase. In certain media similar selective effects also occur when cultures are supplemented with *only* DNA or *only* DNase. This was found to be associated with the natural accumulation of DNA and DNase in most media; the differences in effectiveness of various media in producing the selective effects are due to medium-dependent differences in the ability of DNase to act upon DNA.

An acetyl-coenzyme A and succinate requiring enzyme from Azotobacter. R. REPASKE, Indiana University.—When studying the characteristics of a partially purified succinic dehydrogenase from *Azotobacter vinelandii (o)*, it was found that acetyl-coenzyme A had a stimulatory effect on the reaction. The stimulatory reaction was the result of a second enzyme(s) which was separated from succinic dehydrogenase and required succinate and acetyl-coenzyme A as substrates. The reaction as measured by 2,6-dichlorophenol indophenol reduction appears to occur in at least two steps. The first reaction occurs in the absence of dye and involves the acyl bond of acetyl-CoA. The second step is associated with dye reduction; when the reactants are preincubated without dye, the subsequent rate of dye reduction is directly proportional to the preincubation time. The overall reaction has a pH optimum of 6.6, and the enzyme(s) is equally stable at pH 6.5 and 8.5.

The enzyme has been purified 9 fold from the spinco supernatant fluid from alumina ground cells. Purification and properties of this enzyme(s) will be discussed.

The Mutual Effects of Ca^{++} and Antimicrobial Drugs on Coagulation of Plasma. E. D. WEINBERG and M. B. TOLZMANN, Indiana University.—Ca^{++} combines chemically with oxytetracycline, reverses bacterial toxicity of streptomycin, and is more readily assimilated by chicks on a diet containing penicillin. Each of these antibiotics, in high concentration *in vitro*, inhibits the coagulation of blood. The present study was performed to determine if the anti-coagulant activity of oxytetracycline, streptomycin, and penicillin is reversed by the addition of Ca^{++}. Also included in the study were chlortetracycline, chloramphenicol, and sulfanilamide; and the sodium salts of citrate, oxalate, and ethylene diamine tetraacetate.

The 9 test substances were added individually to various samples of citrated rabbit plasma (Bacto-coagulase) in the presence of varying amounts of Ca^{++}. Addition of chloramphenicol or sulfanilamide had no effect on the amount of Ca^{++} required for optimum clotting; addition of each of the other test substances increased the amount of Ca^{++} needed. With the 7 active test substances, two general effects were obtained: inhibition of coagulation in the presence of "normal" concentrations of Ca^{++}, and stimulation of coagulation in the presence of excess concentrations of Ca^{++}. In addition, the anticoagulant activity of *high* concentrations of 3 of the 7 substances (oxytetracycline, chlortetracycline, and penicillin) was not reversed by Ca^{++}. Apparently these 3 drugs inhibit coagulation by an additional mechanism as well as by combining with Ca^{++}.

A Study of Antibiotic Producing Bacteria From the Soil. C. L. BALDWIN, BETTY PETERS, CATHERINE NORTH, HAROLD A. NASH, Pitman-Moore Co.—The approximate numbers and inhibition spectra of antibiotic pro-

ducing organisms among the gram-positive aerobic spore-forming and gram-negative bacteria occurring in soil were studied.

A total of 284 soil samples from nation-wide sources yielded a total of 1,147 antagonists when screened on agar dilution plates. Gram-positive antagonists were detected by spraying or over-laying with *Brucella bronchiseptica*, *Mycobacterium* sp. #607, and *Trichophyton mentagrophytes*. Gram-negative antagonists were also detected with indicator organisms after the soil bacteria had developed on crystal violet agar or at 5 C. on trypticase soy-agar.

The soil samples which yielded antibiotic producing gram-positive organisms contained on the order of 150,000 antagonists per gram. The samples exhibiting gram-negative antagonists yielded about 25,000 per gram by the crystal violet agar method and 5,000 per gram by the cold incubation method.

Routine determination of antibacterial spectra against ten test organisms revealed the gram-positive isolates to have slightly broader antibacterial spectra than did the gram-negative isolates. Almost half of the former inhibited five or more of the test organisms whereas of the latter group less than one-third did as well. Three per cent of the total isolates were effective against all ten of the test organisms. Antagonists isolated for activity against *Brucella bronchiseptica* and *Mycobacterium* sp. #607 were superior in breadth of antibacterial spectrum to those isolated against *Trichophyton mentagrophytes*. Comparison of the spectrum patterns allowed the most active cultures to be classified into 65 groups, each containing from one to 80 replicates.

The Effects of Fixatives on the Internal Structure of Bacteria. DONALD J. MASON and DOROTHY M. POWELSON, Purdue University.—Changes in the internal structure of cells of *Escherichia coli*, strain 61, and *Bacillus cereus*, strain CR, produced by the action of fixing agents were studied by phase contrast microscopy. The fixatives used were Bouin's solution, a mixture of methyl alcohol, picric acid and formalin, osmium tetroxide and formalin. Special slide preparations permitted the same cell to be photographed before and after fixation. Fixation increased the differences in contrast between the cytoplasm and the "nuclear" areas of the unstained cells. The original appearance of the cells seemed to be disturbed by fixation less with formalin or osmium tetroxide than with Bouin's or fixatives of the methyl alcohol type which caused distortion within the cells. Cells fixed in Bouin's solution contained large granular-like clumps which were probably precipitated proteins.

Fixing in 2% osmium tetroxide solution for longer than two hours produced a latent effect. After the fixed cells were hydrolyzed in N HCl at 60 C, the hydrolysis commonly employed in the Feulgen reaction, the cytoplasm had a retracted appearance.

The Role of Nutrition in the Host-Paraside Relationship. E. D. GARBER, The University of Chicago.—The role of nutrition in the host-parasite relationship has been explored by means of biochemical mutants of an animal and of a plant pathogen. The data indicate that the avirulence of certain biochemical mutants is related to the unavailability of the required nutrilites in the host either at the site of entry or localization. In the case

of the plant pathogen, unavailability of required nutrilites was related to the inability of certain biochemical mutants to produce a proliferating or metabolizing population because of the relatively low concentration of the required nutrilites at the site of inoculation. The probable significance of this interpretation for the virulence of obligate parasites or pathogens with exacting nutritional requirements will be discussed.

Some Applications of Isotopes to the Study of Microbial Physiology. E. C. HEATH, Purdue University.—It has become increasingly evident, with the availability of labeled intermediates from commercial sources, that the purity of these compounds must be critically established before their use experimentally, since a small trace of radioactive impurity can cause error in interpreting results. Experiments with radioactive compounds frequently are performed to follow the incorporation of label into metabolic intermediates. Specific activities of intermediate compounds often offer clues to the sequence of biochemical reactions taking place. Care must be exercised in these determinations to take into account pool amounts of the individual intermediates being studied. Instances arise in which it is desirable to add unlabeled carrier in order to isolate minute amounts of suspected intermediates. The failure of label to appear in the reisolated carrier compound does not necessarily indicate that the compound is not an intermediate, since the added carrier may not equilibrate with the pool of the same compound in the cell due to the latter's presence in a bound state. Respiratory CO_2 yields valuable evidence for metabolic mechanisms but it is necessary to correct specific activities of CO_2 for endogenous CO_2 production. These problems will be discussed and experimental examples of them which have been encountered will be considered.