PROCEEDINGS OF LOCAL BRANCHES OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

EASTERN NEW YORK BRANCH

DIVISION OF LABORATORIES AND RESEARCH, NEW SCOTLAND AVENUE, ALBANY, APRIL 3, 1937

AN IMPROVED MEDIUM FOR THE DEMONSTRATION OF HYDROLYSIS OF SODIUM HIPPURATE BY STREPTOCOCCI. Julia M. Coffey and George E. Foley, Division of Laboratories and Research, New York State Department of Health, Albany.

A medium is described which yields uniform results in tests for hydrolysis of sodium hippurate by hemolytic streptococci. It substitutes 0.1-per-cent asparagine for 1-per-cent peptone in pepsin broth of the following composition adjusted with N/1 sodium hydroxide to pH 7.1:

Asparagine, (C.P.), Eimer & Amend.............0.1%
Pepsin, (U.S.P.), Eimer & Amend...................0.5%
Calcium chloride, (C.P.)........................0.003%
Sodium hippurate, (C.P.), Eimer & Amend...........1%
Distilled water..........................1000 cc.

THE RELATIVE POTENCY OF MONOVALENT AND POLYVALENT ANTIMENINGOCOCCUS SERA. Mary B. Kirkbride and Sophia M. Cohen, Division of Laboratories and Research, New York State Department of Health, Albany.

A reappraisal was made of the relative group potency and valency of monovalent antimeningococcus sera produced with recently isolated and stock meningococcus strains and of the routine six-strain therapeutic serum. Eight horses were immunized in pairs with recently isolated or stock strains of group I–III or group II. The potencies of the sera were determined by protection tests in mice and precipitation reactions with carbohydrate fractions as well as by agglutination tests with representative strains of the different groups.

As previously, no evidence was obtained of the antigenic superiority of recently isolated strains nor did the degree of virulence appear to be an index of the antigenic activity of the strains under study. The valency of the monovalent group I–III sera, within the homologous group, approximated that of the polyvalent serum; the potency, in general, fell below, especially as determined by agglutination and protection tests. In the case of the group-II sera, according to the agglutinative titers, the valency within the group appeared to be narrower; the potency approximated that of the polyvalent serum. The precipitative and protective activities were, however, definitely greater against the strains tested. Data were obtained which suggested the importance in the case of the polyvalent product of an optimum balance between groups I–III and II antigens.
ANTIGENS FOR THE COMPLEMENT-FIXATION TEST WITH ANTIGONOCOCCUS SERUM. A PRELIMINARY REPORT. Christine E. Rice, Division of Laboratories and Research, New York State Department of Health, Albany.

Titrations of complement-fixing activity with antigonococcus rabbit serum were made by the precise, quantitative method of Wadsworth, Maltaner, and Maltaner. Extracts of frozen and thawed gonococci or broth culture filtrates of these organisms, purified and concentrated by ultrafiltration on a 4.5 per cent nitrocellulose (Parlodion) membrane, when used as antigens, were less antigenic, but of the same order in antigenic potency, than "nucleo-protein" fractions of the same strains prepared as described by Price and Menzies, and were equally specific. Although the antigens and immune sera produced with recently isolated strains of gonococci were somewhat more specific than stock cultures of the old Torrey strains, all antigens gave marked cross reactions with antimeningococcus horse and rabbit sera and relatively weaker reactions with sera from rabbits immunized with Neisseria flavescens or Micrococcus catarrhalis. Correspondingly, all the gonococcus antisera fixed complement with extracts, filtrates, and "nucleoproteins" of meningococci.

THE EFFECT OF PHENOL AND 'MERTHIOLATE' ON THE ANTIGENIC POTENCY OF PURIFIED DIPHTHERIA TOXOIDS. Gretchen R. Sickles, Division of Laboratories and Research, New York State Department of Health, Albany.

Stored in the cold room with either phenol (0.4 per cent) or 'Mertihiolate', 1:10,000, diphtheria toxoids purified by acetone precipitation retained their antigenic potency for six years; others purified by adsorption on calcium and alum precipitates, have been observed for two years and have retained their antigenic potency during this period. At room temperature, however, one preparation of the precipitated material stored with 'Mertihiolate' had diminished in potency after two years, while another was unchanged.

The calcium and alum-precipitated preparations with 'Mertihiolate', 1:10,000, as a preservative and stored at room temperature for two years inhibited growth of Pseudomonas pyocyanea, but when the samples were diluted to obtain a concentration of 1:20,000 of the preservative, growth was not inhibited. A similar decrease in preservative action was noted in samples of crude toxoids stored for six months at room temperature. Before storage, growth was inhibited by a concentration of 1:100,000. These precipitates appeared darkened, possibly as a result of the presence of mercuric sulphide as a decomposition product of the 'Mertihiolate' compound at room temperature. The same preparation stored with 'Mertihiolate' in the cold for two years, when diluted to obtain a concentration of 1:50,000 of the preservative, inhibited the growth of Pseudomonas pyocyanea.

DECOMPOSITION OF PNEUMOCOCCUS CARBOHYDRATE BY THE COMBINED ACTIVITY OF STRAINS OF TWO BACTERIAL SPECIES. Myrtle Shaw, Division of Laboratories and Research, New York State Department of Health, Albany.

Increased and broadened activity in the decomposition of pneumococcus carbohydrates was noted when two different species of microorganisms isolated from a soil sample were grown together. Both strains are aerobic. One is a Gram-negative coccus which produces smooth round colonies on
sucrose-mineral-medium agar, very slight growth on beef-extract-peptone agar, and no growth on blood agar. The other strain is pleomorphic, Gram-positive, producing extremely mucoid growth on beef extract or blood agar, but dry, irregular, yellow colonies on mineral-medium agar.

Individual colonies inoculated into mineral medium failed to decompose pneumococcus type-II carbohydrate. A mixture of both types inoculated similarly grew more heavily and utilized the carbohydrate. After further cultivation on a sucrose medium, the coccius, in pure culture, utilized the carbohydrate of type II; the other strain did not. However, the amount and rate of decomposition were increased markedly when both were grown together.

Growth of the two strains in association also broadened the activity. The coccius decomposed carbohydrates of pneumococci types II, VII, and VIII. The other strain had no action on any of the carbohydrates tested. In association, the carbohydrates of types II, III, V, VII, and VIII were utilized.

A COMPARISON OF THE EFFECT OF DIFFERENT TOXIC BACTERIAL PRODUCTS UPON THE ADRENALS OF GUINEA PIGS. PRELIMINARY REPORT. Calvin C. Torrance, Division of Laboratories and Research, New York State Department of Health, Albany.

The adrenals of guinea pigs which had died from intoxication with tetanus and botulinus toxins and toxic filtrates of the meningococcus were examined for their ascorbic-acid content by the method of Bessey and King. The results were compared with those obtained in a similar study of these organs from animals injected with diphtheria toxin. The vitamin-C content was found to be diminished approximately 85 per cent by diphtheria, 65 per cent by tetanus, and 50 per cent by botulinus toxins, when compared with uninjected animals of similar weight and dietary history. Toxic filtrates of the meningococcus produced an effect paralleling that of dipheria toxin—84-per-cent reduction. Following the injection of the various bacterial products, the weight of the adrenals increased over that of the controls, diphtheria 85 per cent, tetanus 31 per cent, botulinus 13 per cent, and meningococcus 10 per cent.

From these findings it does not appear that the effect of diphtheria toxin on vitamin C is unique. Attention is drawn to the fact that the ascorbic acid was decreased more than 80 per cent in the adrenals of animals injected with the two toxic products studied which customarily produce hemorrhage; this suggests the well-known effect of generalized depletion of vitamin C following dietary deficiency.

NEW YORK CITY BRANCH

NINTH MEETING, CORNELL MEDICAL COLLEGE, NEW YORK CITY, MAY 11, 1937


Data obtained through the analysis of skim milk inoculated with Pseudomonas fluorescens demonstrate that increase in specific conductivity of the medium is directly proportional to the amino nitrogen and ammonia formed. The rate of the ammonia formation is 42 per cent of the rate of amino nitro-
gen formation. A new lipolytic organism isolated from cream by Anderson was grown in a 1 per cent peptone medium. Analyses made on this medium show that the change in specific conductivity is also directly proportional to the formation of ammonia. There is, however a marked change in the proportionality constant near the end of the growth period. Lack of knowledge of the mechanism of coordination makes empirical relationships between other variables, such as increase in CO₂, pH, growth etc., and specific conductivity difficult to interpret.

Measurements made on skim milk inoculated with Lactobacillus odontolyticus demonstrate that specific conductivity increases approximately in direct proportion to a decreasing pH to the isoelectric point. The relationship between these two variables after the isoelectric point is reached depends in part upon the physical nature of the curd which is formed.

**Cultivation of Throat Inclusion Bodies in Vitro.** Joan Broadhurst, Teachers College and Gladys Cameron, New York University.

Human throat inclusion bodies, previously reported as occurring in the human throat, have been cultivated in chick and human tissues, heavy growths being obtained in several types of tissue cultures, including squamous epithelial cells and white corpuscles as well as fibroblasts.

**The Pathogenic Staphylococcus:** Its Isolation and Differentiation from Non-pathogenic Types. George H. Chapman, Conrad Berens Lillian Curcio and Edith L. Nilson, Clinical Research Laboratory, and laboratory of the Lighthouse Eye Clinic, New York, N. Y. Aided by grants from the Ophthalmological Foundation, Inc.

Phenol-red mannitol agar and alkaline bromthymol-blue lactose agar were inoculated with material to be tested for the presence of pathogenic staphylococci. After 10 hours incubation, yellow-zoned colonies on PRM agar were isolated and their pathogenicity confirmed by in vitro tests. After 48 hours incubation colonies on BTB agar were also isolated and confirmed. The results were similar in 56 per cent of the comparisons but PRM plates contained more in vitro positive (mannitol fermenting) colonies in 43 per cent. Both media had certain advantages.

Strains from pathologic conditions were tested for pigment production, hemolysis, coagulase, crystal-violet agar, BTB agar, mannitol and lactose fermenting and dermonecrotic properties. Typical pathogenic strains reacted positively to all. The commonest form of degeneration was loss of hemolytic power. Pigment production and coagulating power were more stable. When interpreted according to the criteria of Chapman et al. (J. B., 28, 343, 1934) the results of these three tests paralleled those expected from the source and dermonecrotic properties. In some strains, regarded as intermediate in pathogenicity, these tests were negative but the other in vitro tests were positive.

**Monilia Albicans Infection of the Human Gall Bladder and Biliary Tract with Report on Three Cases.** Frederick R. Weedon, Marie E. Shirk and Dorothy Kenney, Bureau of Laboratories, Department of Public Health, Yonkers, N. Y.

Record of infection of the biliary tract including the gall bladder by Monilia albicans does not appear in
the literature, although *Monilia krusei* was reported in 1933 by Mirman. Examination of bile obtained by Rehfuss tube from 14 cases of typical gall bladder disease resulted in finding *Monilia albicans* in three cases. Since the first, or "A" bile may be contaminated by organisms from the duodenum, little importance is attached to its examination except that it in turn may contaminate the "B" and "C" bile fractions from the gall bladder and hepatic ducts which follow it. In all these cases "A" bile was obtained which did not contain yeast while the "B" and "C" bile contained organisms which were typical of *Monilia albicans*, morphologically, culturally and in respect to pathogenicity for rabbits.

**LABORATORY STUDIES IN ACTINOMYCES.**

**PRODUCTION OF THE DISEASE IN WHITE MICE WITH PUS FROM HUMAN CASES.** Frederick R. Weedon and Florence E. D. Knacke, Bureau of Laboratories, Department of Public Health, Yonkers, N. Y.

Pus from each of five human cases of actinomycosis has been injected intraperitoneally into adult white mice and has produced a condition very similar to that seen in visceral actinomycosis in man, including the production of typical "sulphur-granules," sinus formation, cachexia and death. From the mouse lesions have been recovered morphologically typical actinomycetes and with the pus from these mouse lesions the condition has been produced in a second group of mice. A third passage has also been successful.

One hundred mice were injected with pus varying in amount from 0.5 cc. to the small quantity which could be recovered from a dried cotton swab. Thirty-five mice acquired the disease, 15 died of acute peritonitis in from one to eight days and 50 were negative. In three human cases characteristic lesions in mice were produced with pus obtained before heavy treatment of the patients with iodines was begun but no such lesions were produced with pus obtained after treatment, even though actinomycetes were still present in the draining fluid. These negative mice are included in the above figures.