PROCEEDINGS OF LOCAL BRANCHES OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

MICHIGAN BRANCH

DETROIT, MICHIGAN, JUNE 12, 1947

THE INCIDENCE OF ENTEROCOCCI IN HUMAN FECES. *Morris F. White, Joseph A. Kasper, and Elizabeth J. Cope.*

Fecal specimens received in the laboratory of the Detroit Department of Health served as the source of material studied in this investigation. The culture medium and methods as outlined by Winter and Sandholzer were adopted; however, the medium was modified to the extent that penicillin was omitted from the confirmation broth.

In this series of cultures there was a total of 200 fecal specimens examined. Positive presumptive findings were shown for all of the samples. Of the total number, 115 showed the confirmatory findings for the presence of enterococci, but 85 cultures were considered negative. Thus, organisms of the enterococcus group were recovered from 57.5 per cent of the cultures in this series.

The repeated failure to isolate enterococci from the feces of 7 persons in this study indicates that some humans may not be carriers of enterococci at all times.

These findings seemingly indicate that enterococci are not always present in the feces of humans. Implicit reliance upon the finding of enterococci as a single indicator of human fecal pollution of water cannot, as yet, be accepted without question.

AN ACTINOPHAGE IN STREPTOMYCIN-PRODUCING CULTURES OF STREPTOMYCES GRISEUS. *R. M. Smith, W. H. Kuhn, and G. R. M. Miesel.*

An actinophage which affects cells of *Streptomyces griseus* has been found. Its presence has been noted in stock cultures and in fermentation beers of various types. Plaques typical of bacteriophage action were found when infected cultures were grown on agar media and lysis was noted in cultures grown by submerged methods. The lytic agent increases in quantity upon cultivation of the infected cultures, passes through Seitz and other bacteriological filters, and is relatively heat-stable.

Examination of stock cultures revealed that most of them were infected, and attempts were made to render cultures phage-resistant. Exposure of the susceptible cultures to the phage under various conditions resulted in the development of resistant strains. These strains, thus far, have shown no tendency to revert to susceptibility. The streptomycin-producing capacity of the strains which we have rendered resistant has not differed appreciably from that of the parent cultures.

NORTHERN CALIFORNIA-HAWAIIAN BRANCH

STANFORD UNIVERSITY, CALIFORNIA, JUNE 14, 1947

SELECTIVE BLOOD FACTORS AFFECTING BACTERIAL VARIATION. *Werner Braun*, Division of Veterinary Science, University of California, Berkeley, California.

The selective factor suppressing the establishment of nonsmooth variants of *Brucella abortus*, previously demonstrated in normal serum of various *Brucella*-susceptible animals, has been found in the gamma globulin fraction. *In vivo*, modifications of the gamma globulin, which occur after vaccination, alter the selective activity of normal gamma globulin. *In vitro*, preliminary tests have indicated that the selective activity of normal gamma globulin disappears in the presence of sufficient anti-gamma-globulin (produced by inoculation of bovine gamma globulin into rabbits). Similarly, in the presence of high albumin concentrations, corresponding to approximately twice

the normal blood concentrations, gamma globulin fails to express its selective activity. It is hoped that this information will lead to the creation of *in vivo* conditions which will favor the establishment of non-smooth, avirulent variants.

PENICILLIN STABILITY IN PHOSPHATE, ACETATE, AND CITRATE BUFFERS. *John O. Thomas*, Biological Research Department, Cutter Laboratories, Berkeley, California.

The stability of crystalline potassium penicillin G (1,530 units per mg) in NaH_2PO_4-Na_2HPO_4 buffers (pH 6.0), of final molarities M/16, M/50, M/100, and M/200, and in M/50 acetate and M/50 citrate buffers was studied for a maximum of 86 days, the initial potencies of the sterile mixtures being approximately 10,000 units per ml. Sealed 5-ml volumes of each mixture were kept at 37, 24, and 2 C, one set of mixtures in a temperature group being cup-assayed against *Staphylococcus aureus* (NRRL 318), and the pH's being measured, on a particular day. Residual activities were computed as percentages of zero time potencies.

Penicillin destruction at 37 C was rapid, first-order curves resulting. Similar less steep curves were encountered at 24 C. At both temperatures protection efficiency followed buffer capacity, with the exception of citrate, which was the most efficient.

At 2 C, a first-order inactivation curve resulted for the saline control. The buffered mixtures' curves, however, all showed periods, from 10 days (M/100 phosphate) to 72 days (acetate), when the activities did not drop below 100 per cent. These indicate activity potentiation because maximal potencies, for example, of 150 per cent and 138 per cent (assay error about 10 per cent) occurred in the acetate and M/200 phosphate buffers, respectively, and these in spite of corresponding pH drops to 5.50 and 5.30.

Except for saline and M/200 phosphate, all 2 C curves showed an initial rise, a moderate fall, and a second rise before final drops, the rises being independent of pH drops, though pH's remained practically constant in citrate and M/16 phosphate. No second rise occurred in M/200 phosphate, the pH of which (4.70) was the lowest of the buffers, at 50 days. Acetate provided the best protection, despite a pH fall to 5.20 at 86 days. The buffer ions are apparently concerned with these phenomena.

AN IMPROVED TECHNIQUE FOR BACTERIOLOGICAL CULTURE STUDIES. *Phillip J. Brady and Paul Esau*, Research Laboratories, California Packing Corporation, San Francisco, California.

A simple, convenient, and inexpensive double compartment culture tube for fermentations and aerobic and anaerobic culture studies has been designed. Its uses can be enumerated as follows:

(1) A liquid medium or agar is put in the long arm and pyrogallol in the short arm of the tube. Anaerobes can be cultured by closing the tube with a rubber stopper after a cotton plug.

(2) The nature of a gas produced by bacteria (usually CO_2) can be detected by putting lime water (filtered) in the short arm. Precipitation of calcium carbonate designates CO_2.

(3) Partial neutralization of acid media by hydrolysis during sterilization is avoided by placing the neutral medium in the long arm and the acid medium in the short arm and mixing together after autoclaving and cooling.

(4) Carbohydrate media for fermentation studies can be prepared by placing the sugar solution in the short arm, and the peptone broth with indicator and gas vial in the long arm. After being autoclaved for 10 to 15 minutes at 10 to 15 pounds' pressure, the medium is cooled and the ingredients combined in the long arm. The short arm can now be used for the detection of gas or for creating anaerobic conditions.

DECOMPOSITION OF TARTRATES BY SOME MESOPHILIC, SPOREFORMING, OBLIGATE ANAEROBES. *Joseph Tabachnick*, Division of Food Technology, University of California, Berkeley, California.

Since the classical experiment performed by Pasteur in 1863 in which he demonstrated the existence of obligate anaerobes (with calcium tartrate as a substrate), very little work has been done with the obligatory anaerobic bacteria which decompose tartrate. None of the later investigations were made with pure cultures.

Twenty-three strains of tartrate-fermenting clostridia were isolated by an enrichment technique from calcium tartrate recovery equipment and spoiled calcium tartrate, as well as from soils.

With the exception of their ability to utilize glycerol and tartrate, the majority of the strains isolated were closely related to the type species, *Clostridium butyricum*, as described in Bergey *et al.* (1939). Glucose was fermented with the production of carbon dioxide, hydrogen, butyric and acetic acids, and very small amounts of neutral volatile products. Tartrate was fermented with the production of carbon dioxide, hydrogen, and acetic acid, and small amounts of butyric acid and ethanol. Trace amounts of pyruvic acid from the tartrate fermentation were isolated and identified.

With the exception of *l*-malic acid, four carbon dicarboxylic acids other than *d*-tartaric acid were not attacked by the cultures investigated.

The enzymes involved in the decomposition of tartrate were shown to be adaptive in character. Attempts to adapt other cultures of the common saccharolytic clostridia to the utilization of tartrate were unsuccessful.