PROCEEDINGS OF LOCAL BRANCHES OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS

TEXAS BRANCH

DALLAS, TEXAS, NOVEMBER 16 AND 17, 1946

POLIOMYELITIS (LANSING) CONTACT INFECTION IN MICE. *Christine Zarafonetis, S. Edward Sulkin, and Cleo Hausman Terry*, Department of Bacteriology and Immunology and Virus Research Laboratory, Southwestern Medical College, Dallas.

During 63 days of observation, only 1 of 59 white Swiss mice in contact with a similar number of animals inoculated intracerebrally with the virus of mouse-adapted Lansing poliomyelitis developed a paralytic infection. Virus was recovered from this animal but not from 3 other contact mice which died. The appearance of diarrhea among some of the animals in the cage shortly before the paralytic infection occurred suggested the importance of nonspecific factors of resistance; these possibilities are under further investigation. Subsequent challenge inoculations of virus revealed cerebral resistance among contact mice significantly more marked than that among controls of similar age and weight, but somewhat less marked than that among mice surviving previous intracerebral injection of the same virus. The virus recovered from the paralyzed animal, and subsequently inoculated into other mice, differed from the virus of spontaneous mouse encephalomyelitis on the basis of incubation period, site and extent of paralysis, and mortality rate among paralyzed animals. Immunologic studies to identify the agent as mouse-adapted Lansing poliomyelitis virus are still in progress.

FURTHER OBSERVATIONS ON THE INTESTINAL BACTERIA OF GUINEA PIGS FED WITH PARA-AMINOBENZOIC ACID. *Dorothy M. Whitney*, Department of Preventive Medicine and Public Health, The University of Texas School of Medicine, Galveston.

The investigation of the intestinal flora of guinea pigs before and after feeding *p*-aminobenzoic acid is the subject of this study.

Animal feces were suspended in 1:10 dilutions, and serial tenfold dilutions were inoculated into various media. In "normal" animals, the gram-positive bacteria predominated. In this group, bacilli, streptococci, and spore-bearing anaerobes occurred in the order named. Of the gram-negative bacilli, *Aerobacter aerogenes* and *Escherichia coli* dominated. In addition, non-lactose-fermenters such as *Alcaligenes*, *Pseudomonas*, and *Proteus* were found.

Guinea pigs treated with 2 per cent PABA in the diet for 48 hours showed a marked decrease in the number of gram-positive and gram-negative bacilli. In certain cases no reduction was noted, but a definite suppressive and apparently selective action was observed. The 48-hour treatment with PABA resulted in an almost complete inhibition of all gram-negative bacilli with the exception of *E. coli*.

A prolonged feeding with PABA (6, 8, and 10 days) resulted in a return to the normal flora. A small dosage of PABA (0.1 per cent) over 6 days resulted in increased streptococci counts.

PERMEABILITY OF THE LACTATING BOVINE MAMMARY GLAND TO SULFONAMIDES. *V. T. Schuhardt, T. B. Carroll, L. J. Rode, and Helen Lacy*, The Brucellosis Research Project of The Clayton Foundation and The University of Texas.

In preliminary and repeat experiments, sulfanilamide, sulfathiazole, sulfadiazine, sulfamerazine, sulfapyrazine, 2-sulfanilamido-5-bromo pyrimidine, and 2-sulfanilamido 5-chloro pyrimidine each were administered orally in capsules to 2 cows. Sulfapyridine was administered to 4 cows. Each cow received 90 grams of the ap-

propriate drug in 3 equal doses at intervals of 4 hours. Blood and milk samples, collected from each cow at 6, 10, 22, 34, and 46 hours after the initial drug dose, were tested for free sulfonamide concentrations, and the last three samples of both blood and milk were tested for total sulfonamide concentrations.

Sulfathiazole, sulfapyrazine, and sulfadiazine attained maximum blood levels of less than 5 mg per cent of the free drug, and only the latter was demonstrable in the milk, attaining a concentration of 1.1 mg per cent. All the other drugs attained maximum blood levels ranging from 5 to 13 mg per cent free drug. Only sulfanilamide and sulfapyridine, however, came through the mammary gland in concentrations approaching those found in the blood, and sulfapyridine reached higher levels in both blood and milk.

FURTHER STUDIES ON THE EFFECT OF STERILIZING GLUCOSE IN CULTURE MEDIA ON GROWTH OF MICROORGANISMS: UTILIZATION OF CYSTINE BY LACTIC ACID BACTERIA. *C. E. Lankford, Cathryn Swausch, and Joanne Macow Ravel*, University of Texas School of Medicine, Galveston.

Previous studies have shown that autoclaving glucose or other reducing sugars in certain culture media for the gonococcus renders the peptone cyst(e)ine partially unavailable for growth. With the H_2O_2-treated peptone media of Lyman *et al.* (Arch. Biochem., **10**, 427), the growths of *Lactobacillus arabinosus*, *Lactobacillus casei*, and *Leuconostoc mesenteroides* were compared at different dosage levels of cystine with glucose autoclaved separately and in the medium. When glucose was autoclaved in the medium at pH 6.8 for 15 minutes at 15 pounds, there was a decrease in response to cystine ranging from 50 to 95 per cent. Increasing pH or autoclaving time markedly increased the extent of inactivation of cystine. With glucose autoclaved separately, smooth and reproducible curves were obtained, particularly with *L. mesenteroides*, which produces one-half maximum response at 0.3 μg per ml. Lower maxima with glucose sterilized in the medium suggests partial "inactivation" of other essential nutrilite(s), possibly as a result of combination of the reactive functional groups with aldehyde degradation products of glucose.

PRELIMINARY STUDIES ON THE POTENTIAL PATHOGENICITY OF BACILLUS CEREUS. *Kenneth L. Burdon*, Department of Bacteriology and Immunology, Baylor University College of Medicine, Houston.

PENICILLIN PRODUCTION BY A THERMOPHILIC FUNGUS. *L. J. Rode, Jackson W. Foster, Helen Lacy, and V. T. Schuhardt*, Department of Bacteriology and The Brucellosis Research Project of The Clayton Foundation, The University of Texas, Austin.

THE EFFECT OF BISMUTH ON THE ANTIBIOTIC ACTIVITY OF ASPERGILLIC ACID. *Andres Goth*, Department of Physiology and Pharmacology, Southwestern Medical College, Dallas.

ERADICATION OR SUPPRESSION OF BORRELIA RECURRENTIS IN THE VECTOR O. TURICATA BY PENICILLIN. *W. M. Fisher*, Department of Public Health and Preventive Medicine, Baylor University College of Medicine, Houston.

BIOCHEMISTRY OF ACTINOMYCETES. *C. Willard Lewis and Orville Wyss*, Department of Bacteriology, The University of Texas, Austin.

PROTEOLYTIC ENZYMES OF BACTERIA. *Dorothy L. Wallace, J. R. Stockton, and Orville Wyss*, Department of Bacteriology, The University of Texas, Austin.

THE EFFECT OF VARIOUS ESSENTIAL OILS ON MICROORGANISMS. *A. Packchanian*, Laboratory of Microbiology, The University of Texas Medical School, Galveston.

RESULTS OF COMPLEMENT FIXATION TESTS FOR ENDEMIC TYPHUS ON RATS FROM DDT-DUSTED AREAS. *Billie Jo Colquitt, J. N. Murphy, Jr., and J. V. Irons*, Bureau of Laboratories, Texas State Department of Health, Austin.

MICHIGAN BRANCH

EAST LANSING, MICHIGAN, NOVEMBER 20, 1946

NEWCASTLE DISEASE. *Charles H. Cunningham.*

Accurate differential diagnosis requires laboratory procedures for the isolation of the virus, serum neutralization tests, and hemagglutination and hemagglutination inhibition tests. Embryonated chicken eggs provide an excellent cultural medium. Early clinical cases are the best for the recovery of the virus. Brain and spleen are the organs of choice, although other organs, body discharges, and egg yolk may be used. With the onset of nervous symptoms the virus is usually no longer recoverable.

The virus retains its infectivity under the usual methods of storage. Inactivation by formalin, ultraviolet light, and heat does not materially alter its hemagglutination activity.

Several types of vaccines elicit immunity response, but the protection is of short duration. Vaccinated birds may resist challenge exposure from 2 to 4 days following vaccination, although demonstrable antibodies are not present until the sixth or eighth day. Refractivity to infection and immunity response are associated with growth and maturity.

Congenital passive immunity may be demonstrated in embryos and chicks from recovered or vaccinated hens. Immunity in such chicks closely parallels the period of yolk absorption.

OBSERVATIONS ON CANINE LEPTOSPIROSIS IN THE LANSING AREA. *J. P. Newman,* Department of Bacteriology and Public Health, Michigan State College.

During the past 15 months approximately 500 samples of canine blood have been examined for a present or past infection of leptospirosis. The following laboratory methods have been employed: darkfield examination, Giemsa stain preparations of whole blood and serum, macroscopic agglutination test, microscopic agglutination test, guinea pig inoculation, and culture using Verwoort's medium (Schuffner's modification).

It is evident in the work done thus far that, because of the individual limitations and pitfalls encountered with the foregoing laboratory diagnostic procedures, one must utilize two or more in an attempt to arrive at an accurate diagnosis of leptospira infection in the canine. The two procedures which will give the most accurate picture of present or past infection are the microscopic agglutination test and culture.

Equine, bovine, ovine, and porcine serums have been used in an attempt to find a more readily available serum to replace the rabbit serum employed in Verwoort's medium, with little if any success. Growth was very poor, if any, in the medium containing the foregoing serums.

Serological studies employing the microscopic agglutination test were made, indicating an approximate 29 per cent canine infection in the Lansing area, of which 27 per cent are *Leptospira canicola* infections and 2 per cent *Leptospira icterohemorrhagiae.*

OBSERVATIONS ON THE VARIANT TYPES OF CORYNEBACTERIUM DIPHTHERIAE: ANTIGENIC ANALYSIS. *Howard E. Lind,* Dow Chemical Company, Midland, Michigan.

Antigenic analysis, consisting of two phases of investigation—(1) agglutinin production against bacterial cultures, and (2) precipitin production against alkali-soluble protein extracts of the microorganisms—were made in an attempt to show some degree of inherent relationships or common identity when the other characteristics differed. It concerned the original smooth virulent parent colony, 4 nonvirulent parent type variants, and 4 nonvirulent small type variants.

Antigenically, the antiprotein and antibacterial serum of the parent type indicated similarity with the parent type variant but a distinct dissimilarity with the small variants. The parent type variants in demonstrating a low degree of cross reaction suggest alteration in the composition of the

protein. This substance in the small variants is altered beyond detection or is destroyed completely, inasmuch as no cross reaction with the parent antiserum was observed.

Preliminary findings suggest that the quantity of protein in the alkali-soluble fraction of *Corynebacterium diphtheriae* is directly correlated with those strains that maintain virulence.

NORTHWEST BRANCH

STATE COLLEGE OF WASHINGTON, PULLMAN, WASHINGTON, NOVEMBER 23, 1946

THE REAL AND THE APPARENT BACTERICIDAL EFFICIENCIES OF THE QUATERNARY AMMONIUM COMPOUNDS. *Ernest C. McCulloch*, State College of Washington, Pullman, Washington.

Marked commercial interest now is being shown in the quaternary ammonium compounds, which are used for the disinfection of skin and mucous membranes, for the cold "sterilization" of minor surgical instruments, as bactericides for eating utensils and drinking glasses, and as general disinfectants.

Plate counts of bacterial suspensions exposed to quaternary ammonium compounds show a very rapid initial decrease in plate count numbers, followed by a much less rapid decrease. The velocity of

$$\text{disinfection, } K = \frac{I}{\text{time}^2 - \text{time}^1} \cdot \frac{\text{Log plate count at time}^1}{\text{Log plate count at time}^2}$$

has been observed as 0.43 during the first minute, as 0.37 during the second minute, and as 0.0004 between the second and twenty-fourth hours. In milk, the addition of a quaternary ammonium compound produces a very rapid decrease in plate count numbers, which after several days' incubation is followed by a marked increase which may exceed the original inoculum.

The hypothesis is advanced that the very rapid initial decrease in plate count numbers reflects the agglomeration of the exposed organisms and their adherence to the sides of the tube, as well as actual killing. When the FDA technique is used, the agglomerated organisms may not be picked up by the 1:100-ml loop; they may adhere to the loop and not remain in the subculture medium; and in the absence of particulate material in the subculture medium they may stay coated with the quaternary ammonium compound and remain in a condition of bacteriostasis.

A surface-active bacteriostat, which forms a persistent film and has low toxicity to tissues, may have definite value in clinical medicine. Also, in sanitizing certain types of food-handling equipment, such a bacteriostat might have definite, but limited, use. As bactericides, these compounds need to be reinvestigated.

CELLULOSE-DECOMPOSING BACTERIA FROM THE RUMEN OF CATTLE. *R. E. Hungate*, State College of Washington, Pullman.

THE BACTERIOLOGY OF SPRAY-DRIED WHOLE MILK POWDER. *J. F. Coal*, State College of Washington, Pullman.

THE OXYGEN REQUIREMENTS OF MOLDS. *N. S. Golding*, State College of Washington, Pullman.

STUDIES ON A SPECIES OF TRICHOSPORON. *C. H. Drake*, State College of Washington, Pullman.

STUDIES ON THE EPIDEMIOLOGY OF SPOTTED FEVER. *W. L. Jellison*, USPHS, Rocky Mountain Laboratory, Hamilton, Montana.

SOME ASPECTS OF THE POLIOMYELITIS PROBLEM. *C. A. Evans*, University of Washington, Seattle.

KENTUCKY-TENNESSEE BRANCH

UNIVERSITY OF TENNESSEE, KNOXVILLE, TENNESSEE, NOVEMBER 16, 1946

MICROBIAL FOULING OF ZEOLITE WATER SOFTENERS. *W. L. Williams*, Louisville Water Company, Louisville, Kentucky.

A water treatment plant treating a highly contaminated river water with lime and ferric sulfate for carbonate hardness reduction, followed by zeolite softening, used insufficient chlorination on the initial runs, thereby seeding the softener beds with *Aerobacter aerogenes*. By the application of break-point chlorination to the raw water intake, sterile conditions were thereafter maintained throughout the treatment until the water reached the softeners. The carbonaceous zeolite in the softeners quickly absorbed the chlorine residual, and bacterial growth flourished throughout the softeners, finally causing the beds to jell and channel on regeneration, with the result that the softeners failed to function. Water leaving the filters appeared to be clear, but the Sedgwick-Rafter concentration of samples, followed by microscopic examination, showed that dead organic matter was passing through the filters, thus constantly supplying nutrient material for the growth of bacteria. Bacterial counts on water leaving the softeners were extremely high, *Aerobacter aerogenes* being the predominating organism. It was always present when short softener runs were experienced. Several methods of backwash and two sterilizing compounds were effective only temporarily in removing contamination from softener beds.

THE USE OF SODIUM AZIDE FOR DETERMINING THE FERMENTATIVE ABILITY OF YEAST DEVELOPED UNDER DIFFERENT OXYGEN TENSIONS. *M. C. Brockmann*, Joseph E. Seagram and Sons, Inc., Louisville, Kentucky, *and T. J. B. Stier*, Department of Physiology, Indiana University, Bloomington.

If the rate of fermentation by yeast is limited primarily by overaccumulation of high energy phosphate bonds, conventional procedures are inadequate for evaluating fermentative ability. In the presence of 0.002 M azide there is a marked elevation in the rate of glucose utilization per unit cell population. This elevated rate remains constant for relatively long periods, presumably because azide interferes with the generation of high energy phosphate bonds and thereby releases the fermentative processes from the consequences of overaccumulation of these bonds.

On the basis of rates of glucose utilization in CO_2-sparged glucose yeast extract KH_2PO_4 medium containing azide (0.002 M), yeast cells developed under aeration have about one-half the fermentative activity of cells developed in CO_2-sparged medium. Cells grown in cotton-plugged flasks develop 70 to 80 per cent of the activity of cells grown under CO_2 sparging.

For yeast developed under different oxygen tensions, the rate of glucose utilization per unit cell population appears to be a more adequate expression of activity than a corresponding rate based on unit cell weight.

THE COMPLEMENT FIXATION TEST FOR LYMPHOGRANULOMA AS A DIAGNOSTIC PROCEDURE. *Anna Dean Dulaney and Henry Packer.*

The complement fixation test for lymphogranuloma venereum was conducted on 649 sera from diversified sources. Positive reactions in serum dilutions of 1:5 were obtained with 8.7 per cent of 148 Wassermann-negative individuals with no evidence of venereal infection, 2 per cent of 42 children with febrile disease, none of 22 children with congenital syphilis, 57 per cent of 81 patients with neurosyphilis, and 70 per cent of 214 patients with anogenital lesions of various types.

The nonspecific reactions in the nonvenereal group were correlated in most instances with upper respiratory infections.

Titration studies showed that positive reactions in the nonvenereal groups were characterized by low titers (1:5 to 1:20) whereas 56 per cent of the sera from the venereal groups yielded titers of 1:40 and above.

It is concluded that the complement

fixation test for lymphogranuloma venereum is of diagnostic value if sera are tested routinely in dilutions of 1:40. Such titers are in general diagnostic of lymphogranuloma venereum and tend to screen out nonspecific reactions due to early syphilis or other infectious diseases.

PHASE VARIATION IN PARACOLON ORGANISMS. *Mary G. West*, Department of Animal Pathology, Kentucky Agricultural Experiment Station, Lexington, Kentucky.

The phase variation of Andrewes was found in a group of lactose-fermenting organisms bearing slight serological relationship to the *Salmonella* genus.

In some instances one phase of these paracolons could be well represented by *Salmonella* antigenic factors. In other instances the phases were found to bear only slight antigenic relationships to *Salmonella* organisms.

The discovery of phase variation among a group of organisms outside the *Salmonella* genus is significant in that this phenomenon is a factor which should be considered in the serological classification of paracolon and coliform organisms.

A STUDY OF VARIABILITY IN DUPLICATE STANDARD PLATE COUNTS AS APPLIED TO MILK. *J. L. Courtney*, Oak Ridge Department of Health, Oak Ridge, Tennessee.

A total of 299 duplicate counts were made on raw milk. Of these, 278 varied less than 50 per cent; 18 between 50 and 100 per cent; 3 over 100 per cent, the highest being 191 per cent. The average variation was less than 20 per cent. It seems that many of the extreme variations in standard agar plate counts are a result of failure to appreciate the importance of care in every detail. This conclusion seems more obvious when we recall statements often made that there is no point in being careful with certain phases of the technique, or that there is no point in doing something a certain way because the error inherent in the method is greater than the error which will be introduced.

Our limited study indicates that the human error may introduce extreme variations, and yet the result is seemingly accepted as the normal variation of the method. In the light of the abuse so often heaped upon this procedure, we feel that an improvement in the technique will be rewarded by more accurate results.

PROPOSED CHANGES IN INCUBATION TEMPERATURES FOR STANDARD AGAR PLATE COUNTS. *James D. Brew*, University of Tennessee.

The temperature of incubation for estimating bacteria by the agar plate method has long been a controversial issue. Forty years ago incubation was 5 days at 20 C, and 2 additional days at 37 C. The theory was that since these temperatures represented the optimum for most bacteria, including the pathogens, more accurate results would be obtained. A 7-day incubation period, however, proved to be impractical. The incubator space in most laboratories was limited; another objection was the long wait for results. Incubation at 37 C for 48 hours was finally agreed upon as being most satisfactory.

Recently, workers in milk control laboratories observed greater variabilities in estimates made at 37 C than at lower temperatures. Apparently, 37 C is close to or possibly above the maximum growth range for some bacteria; also the temperatures in different incubators vary more widely in the range of 37 C than at lower temperatures. Not only do temperatures of incubators of different manufacturers vary, but there may be variations at different points inside any incubator. Some 37 C incubators may run as high as 45 C, which is sufficiently high to inhibit the growth or possibly kill some organisms. The proposed change is to lower the incubation temperature to 32 C for 48 hours. Pederson and others found the variability in estimates at 32 C to be about 4 per cent, whereas at 37 C it was about 25 per cent. Furthermore, the total number estimated at 37 C averages about 50 per cent lower than that obtained at 32 C.

THE FUNGISTATIC EFFECTS OF THE FATTY ACIDS ON SPECIES OF TRICHOPHYTON. *Emanuel Grunberg.*

The fungistatic action of the series of fatty acids (formic acid to undecylenic acid)

at various pH levels was investigated. It was determined that at pH 5.5 and pH 6.5 the potency of each acid increased with the addition of each methyl group according to Traube's rule. At pH 7.5 the potency of the acids from formic acid to valeric acid increased also according to Traube's rule; however, between valeric acid and caproic acid, there seems to be a break, with caproic and the subsequent acids much more potent than would be expected if potency increased according to this same constant. The potency of the acids at pH 8.5 is essentially the same as it is at pH 7.5.

Four strains of *Trichophyton gypseum* and four strains of *Trichophyton purpureum* were employed. Although it has been claimed that there is a difference in resistance to fungistatic agents between the two species, none was apparent in *in vitro* tests with the fatty acids.

A consideration of the results seems to indicate that the fungistatic activity of the fatty acids can be correlated with the undissociated fraction.

A CAPSULE-DISSOLVING FACTOR. *James C. Humphries*, Department of Bacteriology, University of Kentucky, Lexington, Kentucky.

Phage lysates of *Klebsiella pneumoniae*, type A cultures, freed from phage by ultrafiltration, contain a factor which removes the capsule from type A cells. This factor is specific, failing to remove the capsule from the types B and C Friedlander and *Aerobacter aerogenes* strains tested.

Capsule removal was demonstrated as follows: (1) Ultrafiltrates applied to the surface of agar plate culture produce greatly reduced opacity in the growth film. (2) Ultrafiltrate-treated growing cells or formalin-killed cells are no longer agglutinable by type-specific "M" sera, are agglutinable by "S" phase sera, and are greatly reduced in cell volume (Hopkins tube technique). (3) Ultrafiltrate-treated growing cells are rendered susceptible to lysis by an "S" culture phase bacteriophage.

In these crude preparations the principle is (1) stable near neutrality but rapidly inactivated in weak acid solutions; (2) destroyed by 85 C for 10 minutes, but resistant to 75 C for 30 minutes; (3) nondialyzable through cellophane, and not precipitated by the dialyzing process; and (4) precipitated following 50 per cent ammonium sulfate saturation.

SOME OBSERVATIONS DEALING WITH CORRELATIONS OF RESULTS OF STANDARD KAHN TEST AND THE QUANTITATIVE KAHN TEST. *Cooper Brougher, George M. Cameron, Rufus Buchanan, and Kent Roark*, State Department of Health, Nashville, Tennessee.

THERMAL SHOCK IN THE ISOLATION OF STREPTOMYCES. *A. L. Pollard*, University of Tennessee, Knoxville, Tennessee.

THE OCCURRENCE OF SALMONELLA ANTIGENS IN A COLIFORM BACTERIUM. *Alice B. Moran*, Department of Animal Pathology, Kentucky Agricultural Experiment Station, Lexington, Kentucky.

EASTERN PENNSYLVANIA CHAPTER

ONE HUNDRED AND NINETIETH MEETING, PHILADELPHIA COUNTY MEDICAL SOCIETY BUILDING, PHILADELPHIA, PENNSYLVANIA, NOVEMBER 26, 1946

THE EFFECT OF ULTRAVIOLET IRRADIATION ON VARIOUS PROPERTIES OF INFLUENZA VIRUSES. *Werner Henle and Gertrude Henle*, Children's Hospital, Philadelphia, Pennsylvania.

The effect of ultraviolet irradiation on various properties of the influenza viruses types A and B has been analyzed. The studies involved propagation and interference in the allantoic sac of the chick embryo, inhibition of embryonic development, toxicity for white mice, hemagglutination including the adsorption-elution mechanism, immunizing capacity for mice, and, finally, complement fixation activities in the presence of antibodies to the 600S antigen (human convalescent and post-vaccination sera) and the 30S antigen (convalescent sera only). It could be shown that the various activities of the

influenza viruses were affected by irradiation at different rates, indicating that they are based, at least in part, on different constituents of the virus particle. On account of these differences in the susceptibility of the various properties to ultraviolet light it was possible (a) to differentiate between the interference phenomenon as observed in the allantoic sac and the development of nonagglutinability in red cells by either homologous or heterologous fresh virus, and (b) to separate individual steps involved in the mechanism of infection of susceptible host cells. The implications of these findings were discussed.

FAMILIAL NONSPECIFIC SEROLOGIC REACTION FOR SYPHILIS. *Arthur G. Singer, Jr., and Fred Boerner*, Graduate Hospital and Lankenau Hospital, Philadelphia, Pennsylvania.

The existence of nonspecific positive reactions to a serologic test for syphilis is now generally accepted. The majority of false positive serologic reactions are traceable to an infectious disease, an injection, or treatment of some sort. However, the case in question deals with a family, several members of which show persistent false positive tests that cannot be attributed to any of the usual causes.

The routine prenatal serologic tests for syphilis on a 35-year-old white female of Italian birth and four to five months pregnant repeatedly showed a positive Kline test but a negative complement fixation. The husband was found to give entirely negative serologic results; the serum of the three oldest children, however, gave reactions identical with that of the mother, i.e., positive Kline, negative complement fixation. Neonatal serologic studies on the youngest child were entirely negative. The entire family were Rh-positive, but the father and youngest child were found to be in blood type group O, whereas the mother and three oldest children belonged to group A.

Quantitative serological tests were conducted on the mother and three oldest children; positive results were obtained in the flocculation test, but the complement fixation test gave negative results. In each case, the results fell into class 5, group 2, a doubtful classification according to Boerner's system of classification. The father and youngest child remained negative in both tests, falling into class 1, group 1, negative.

These studies, conducted over a period of two years, suggest the existence of a possible mechanism of hereditary transmission of the factor responsible for the nonspecific positive reaction.

INHIBITION OF DIVISION IN THE PROTOZOAN TETRAHYMENA BY ANTISERA AND BACTERIA. *Elizabeth H. Fowler and James A. Harrison*, Department of Biology, Temple University, Philadelphia, Pennsylvania.

Further study has been made of the interesting serologic reaction with the free-living ciliate, *Tetrahymena*, reported by the authors in 1945. The antibody which interferes with division without interrupting other growth processes and leads to the abnormal formation of pairs and chains of cells, as well as monsters, may be removed by absorption. An unsuccessful attempt was made to produce a similar reaction in these organisms by exposure to 195 strains of coliform bacilli by the method of Chatton and Chatton reported in 1925.

EASTERN NEW YORK BRANCH

ALBANY, NEW YORK, DECEMBER 6, 1946

THE RELATION BETWEEN INDUCED RESISTANCE TO PENICILLIN AND OXYGEN UTILIZATION. *W. D. Bellamy and J. W. Klimek*, Sterling-Winthrop Research Institute, Rensselaer, New York.

In this laboratory the resistance to penicillin of *Staphylococcus aureus* 209P has been increased over 60,000 times by 64 serial transfers in broth containing penicillin, the amounts of which were increased from 0.00006 mg per ml to 4 mg per ml (Klimek *et al.*: J. Bact., **51**, 550). We have found that this resistant strain has lost the ability to grow anaerobically. The rate of aerobic

growth is from one-half to two-thirds of that of the penicillin-sensitive parent strain.

Under similar conditions, three other organisms, which normally obtain energy for growth by anaerobic processes, e.g., *Streptococcus faecalis* 10Cl, *Streptococcus agalactiae* 68C, and *Clostridium perfringens* M, were found to develop little resistance to penicillin. The resistance of *S. faecalis* increased 11 times in 47 transfers, that of *S. agalactiae* 6 times in 24 transfers, and that of *C. perfringens* M 10 times in 25 transfers.

It appears that penicillin interferes with an essential step or steps in anaerobic metabolism. Those organisms which were unable to develop or utilize an alternative energy mechanism did not develop resistance to penicillin.

INACTIVATION OF THE GERMICIDAL ACTION OF QUATERNARY AMMONIUM COMPOUNDS. *C. A. Lawrence*, Winthrop Chemical Company, Inc., Rensselaer, New York.

To be considered a suitable inactivator for the antibacterial action of quaternary ammonium germicides the agent or process used must meet certain prerequisites: (1) the method should, as completely as possible, neutralize the bacteriostatic and bactericidal actions of the quaternaries; (2) it should maintain the state of inactivation for a prolonged period of time; and (3) the neutralizing agent itself should not possess antibacterial properties. Evidence is presented to show that several anionic detergents, including soaps, would not meet the requirements given. In addition, a series of compounds known to give a precipitate in the presence of quaternary ammonium solutions failed to inactivate completely the germicidal action of the latter. A sulfonic acid derivative, the sodium salt of symmetric bis(*meta*-amino-benzoyl-*meta*-amino - *para* - methylbenzoyl - 1 -naphthylamino - 4,6,8 - trisulfonic acid) carbamide, more closely meet the requirements for an inactivator for squaternary ammonium compounds.

ACCESSORY GROWTH SUBSTANCES INFLUENCING COLONY CHARACTERISTICS AND MACROCONIDIAL FORMATION OF MICROSPORUM AUDOUINI. *Elizabeth L. Hazen*, Branch Laboratory, Division of Laboratories and Research, New York City.

THE PROTECTIVE VALUE OF THE VOLE BACILLUS (WELLS) AS COMPARED WITH BCG AGAINST TUBERCULOUS INFECTION. *Konrad Birkhaug*, Division of Laboratories and Research, Albany.

CONNECTICUT VALLEY BRANCH

YALE UNIVERSITY, NEW HAVEN, CONNECTICUT, DECEMBER 7, 1946

GENETIC EVIDENCE FOR SEX IN BACTERIA. *Joshua Lederberg and E. L. Tatum*, Osborn Botanical Laboratory, Yale University, New Haven, Connecticut.

Following a discussion of a previous publication (Nature, **158**, 558) a demonstration was presented illustrating the recombination of genetic factors in *Escherichia coli*.

THERAPY OF TRYPANOSOMIASIS. *Benjamin A. Rubin*, Department of Bacteriology, Yale University School of Medicine, New Haven, Connecticut.

Prodigiosin and synthetic pyrrole derivatives were found to have selective trypanocidal activity *in vitro*, but not *in vivo*. No antibacterial effects were demonstrable.

Oxygen analogues in the form of natura and synthetic lactones were highly effective *in vitro* and in certain cases provided permanent cures for *Trypanosoma equiperdum* infections in mice. The level of this activity depended upon the location of unsaturated linkages and upon the nature of the substituents.

The locus of trypanocidal activity was in one case shown to be different from that of the antibacterial effect. The parent substance of "anemonine," the synthetic 2-pentene 1:4 olid, had equal trypanocidal activity but had none of the substantial antibacterial effect of anemonine.

The *in vitro* trypanocidal activity of some of the lactones (particularly "parasorbic" acid) could be reversed by *beta*-alanin

and to a lesser extent by *alpha*-alanine, but not at all by calcium pantothenate or pantolactone.

VARIATION IN TISSUE SPECIFICITY OF THE ROUS CHICKEN SARCOMA VIRUS FOLLOWING GROWTH OF THE TUMOR IN THE MAMMALIAN EYE. *Edward W. Shrigley*, Bacteriology Department, Yale Medical School, New Haven, Connecticut.

It has been shown that the Rous sarcoma will grow in the anterior chamber of the guinea pig eye. Following residence of 12 days in this host, the causative virus of this tumor has been found to be altered in its tissue specificity as evidenced, on subsequent inoculation into chicks, by the increase in frequency of periosteal tumors.

In the present study it has been possible to grow the Rous sarcoma in the anterior chamber of the mouse eye. Twenty-three of the 31 A strain mice inoculated intraocularly with the tumor tissue showed growth of the neoplasm, as manifest by increase in size and the presence of mitotic figures. All ocular growths remaining in mice up to 15 days produced typical sarcomas when reinoculated into chicks.

The incidence in chicks of bone lesions produced by the injection of the guinea-pig-passage strain of tumor compared with the frequency of similar lesions in birds inoculated with mouse-passage material showed that the virus varied while in the guinea pig environment but was not altered, according to our tests, during mouse passage. The explanation for these results must await further study.

THE ISOLATION OF PARACOCCIDIOIDES BRASILIENSIS FROM A CASE OF SOUTH AMERICAN BLASTOMYCOSIS. *Rosalie Ferguson and Margaret F. Upton*, St. Luke's Hospital, New York City.

The fungus *Paracoccidioides brasiliensis* was isolated from a case of South American blastomycosis in a 23-year-old man who contracted the disease in Colombia about 3 months before coming to this country. At St. Luke's Hospital, New York City, the fungus, showing multiple budding, was observed in sections from an axillary lymph node. The mycelial phase was grown from a lymph gland upon blood veal agar in 4 weeks, incubated at room temperature. Transfers to Bordet-Gengou medium incubated at 37 C were successful in growing, in 7 days, the multiple-budding yeast characteristic of the tissue form. This is probably the first isolation of this fungus reported in this country.

A QUANTITATIVE METHOD FOR THE DETERMINATION OF THE FUNGISTATIC ACTIVITY OF ANTISEPTIC POWDERS. *Marion B. Sherwood*, Wellcome Research Laboratory, Tuckahoe, New York.

NORTH CENTRAL BRANCH

UNIVERSITY OF MINNESOTA, MINNEAPOLIS, MINNESOTA, NOVEMBER 22 AND 23, 1946

SOME OBSERVATIONS ON THE FREE-LIVING LEPTOSPIRAS OF MINNESOTA WATERS. *B. H. Hoyer*, University of Minnesota, Department of Bacteriology and Immunology, Minneapolis, Minnesota.

Attempts were made to isolate free-living leptospiras. Of the enrichment methods tested, Walker's medium was found to give the best results. Walker's medium is 0.3 per cent egg yolk in 0.3 per cent agar. Modifications of this medium consisting of added phosphate buffer, cystine, or tryptose (Difco) gave results no better than, or poorer than, the plain medium.

Positive enrichments for free-living leptospiras were obtained in 17 out of 19 enrichment attempts using Walker's medium and the straw enrichment of Kitaoka. All types of waters were included: algae-filled ponds, muddy waters, clear leafy-bottomed ponds, rivers, and even sulfur springs. Positive enrichments were obtained in equal number at both 23 C and 37 C.

Pure cultures of the free-living leptospiras may be obtained by a preliminary Berkefeld filtration followed by growing the filtrate on Walker's medium. Leptospiras and very small rods and vibrios appear in this stage, and purification may be made by dilution extinction.

Strains of the leptospiras may be separated on a solid tryptose (Difco) agar medium after preliminary purification as described above.

ASSIMILATION OF ACETATE BY YEAST. *A. G. C. White, L. O. Krampitz, and C. H. Werkman*, Industrial Science Research Institute, Iowa State College, Ames, Iowa.

Large increases in the fat content of metabolizing yeast (*Saccharomyces cerevisiae*) in the presence of sodium acetate were shown to be due to the assimilation of the intact acetate molecule. By the use of acetic acid labeled in the carboxyl group with a heavy isotope of carbon (C^{13}), the carbon from the acetate was shown to be present in both the fat and carbohydrate fraction of the yeast cells.

In one experiment normal acetate was supplemented by $NaHC^{13}O_3$; in a second experiment isotopic acetate was supplemented by normal bicarbonate. No isotope was found in the yeast fat in the first experiment, a fact which indicates that the C^{13} was not incorporated into the fat of the yeast cells by CO_2 fixation but rather by utilization of the intact 2-carbon chain.

THE CONVERSION OF 2,3-BUTYLENE GLYCOL TO ACETYLMETHYLCARBINOL IN BACTERIAL FERMENTATIONS OF GLUCOSE. *David Paretsky and C. H. Werkman*, Bacteriology Section, Iowa Agricultural Experiment Station, Ames, Iowa.

The yields of acetylmethylcarbinol (AMC) in the fermentations of glucose by *Aerobacter aerogenes* may be increased at the expense of 2,3-butylene glycol (2,3-BG). Methylene blue added toward the end of aerated glucose fermentations increases the amounts of AMC produced.

Aeration of fermentations under pressure also increases the ratio of AMC to 2,3-BG. The highest conversions of 2,3-BG to AMC are obtained when methylene blue is added to the fermentation in conjunction with pressure aeration.

THE MICROBIOLOGY AND CHEMISTRY OF CANNED BACON. *J. A. Ulrich, H. M. Tsuchiya, and H. O. Halvorson*, The Hormel Institute, University of Minnesota, Austin, Minnesota.

Because "heavy cure" bacon leaves much to be desired in palatability, microbiological and chemical studies have been made of the changes that occur during each of the commercial steps involved in the processing of bacon sides from "green bellies" to the finished "mild cure" product, and on storage at 37.8 C.

The bacterial population decreases during the curing and smoking processes. On storage at 37.8 C (100 F) the total bacterial and the lipolytic bacterial counts of canned bacon samples increased and subsequently decreased. The free fatty acid values and the ammonia N: total N ratios increased, while the nitrite concentrations decreased. The pH and peroxide values remained constant during the period of storage. Vacuum pack samples kept better than nitrogen pack samples, and the latter better than the carbon dioxide pack samples. The usual commercial practice of derinding after the smoking process gave a better keeping product than did derinding before this step.

Samples of "heavy cure" canned bacon withstood storage very well at the storage temperature of 37.8 C.

THE EFFECT OF DOSAGE ON INTERFERENCE BETWEEN DISTEMPER VIRUSES. *Cyril S. Stulberg and Robert G. Green*, Department of Bacteriology and Immunology, University of Minnesota Medical School, Minneapolis, Minnesota.

Interference between animal, plant, and bacterial viruses in their respective hosts has been demonstrated by many workers. The authors have previously described an interference between distemper viruses (Proc. Soc. Exptl. Biol. Med., **61**, 117; Science **103**, 497). A highly fatal canine distemper in young foxes produced by intranasal inoculation could be blocked during the incubation period by the intramuscular injection of a nonvirulent ferret-passage virus.

Further studies have now shown that foxes can be uniformly infected with virulent distemper by intranasal inoculation of 50 mg of infected tissue, and that varying dosages of the modified virus will interfere with the virulent distemper infection. Sixty red fox pups were injected with 50 mg of virulent distemper virus intranasally.

Seven days later 50 of the animals were divided into groups of 10, which were injected intramuscularly with modified distemper virus in dosages of 200, 100, 50, 25, and 10 mg, respectively. Ten animals kept as controls died of virulent distemper. The 50 foxes in the groups inoculated with modified virus exhibited definite symptoms of distemper but subsequently recovered, except that 1 fox died in the group inoculated with 200 mg of modified virus, 1 in the group inoculated with 100 mg, 4 in the group inoculated with 50 mg, and 2 in the group inoculated with 10 mg.

It appears that as little as 10 mg of modified distemper virus injected intramuscularly 1 week after intranasal instillation of virulent distemper virus will interfere with the course of an infection caused by the virulent virus.

EFFECT OF SPECIFIC ANTIBODY ON TRANSPLANTED MOUSE MAMMARY CANCER. *David T. Imagawa and Robert G. Green*, Department of Bacteriology and Immunology, University of Minnesota.

Green, Moosey, and Bittner (Cancer Research, **5**, 538) have shown that the inciting agent of mouse mammary cancer is highly antigenic and, when inoculated into rabbits, produces specific antibodies which inactivate the agent. It has been possible in our laboratory to concentrate these antibodies from 4 to 10 times their original concentration by precipitation of the globulin fraction with 1.39 molar ammonium sulfate.

This concentrated antiserum has been found to have a definite inhibitory effect on the growth of transplanted mouse mammary cancer. Clumps of mouse mammary cancer cells were inoculated subcutaneously into the abdomens of mice. At the beginning of each experiment the tumor-bearing animals were divided into groups so that the average tumor size of each group was approximately the same. Treatment with antiserum was started after the tumors had been established and had attained the size of a small pea. Different routes of injection were used at varying time intervals. At the beginning and at various intervals of the experimental periods, the surface areas of the tumors of both control and treated animals were accurately calculated and compared.

It appeared that the growth of transplanted mouse mammary cancers was partially inhibited by the injection of concentrated mouse cancer antiserum, but the injection of normal rabbit serum and concentrated normal rabbit serum showed no noticeable effect. Injection of the specific antiserum into the tail veins of mice was the most effective method of injection.

SYNERGISM BETWEEN SOME ANIONIC WETTING AGENTS AND AZOCHLORAMID. *B. H. Hoyer and E. J. Ordal*, University of Washington, Department of Microbiology, Seattle, Washington.

In view of the synergistic action of some anionic wetting agents on the germicidal action of undissociated phenols, an attempt was made to demonstrate similar action using an oxidizing disinfectant. Because of its stability, azochloramid (N,N′-dichlorazodicarbonamidine) was used as the oxidizing type disinfectant. Three anionic wetting agents were used: sodium lauryl sulfonate, sodium tetradecyl sulfate, and the dioctyl ester of sodium sulfosuccinate.

Escherichia coli and *Staphylococcus aureus*, used as the test organisms, were grown in shaken flasks and washed. Centrifuged cells were used in all experiments. Attempts to demonstrate synergism were made by using decrease in respiration, as determined by the Warburg respirometer, and by bacterial death, as determined by quantitative plate counts.

No evidence of synergism was found in the respiration studies using oxidation of lactate as the indicator. Quantitative counts gave definite evidence of synergism. The extent of synergism did not follow a definite trend, as in the case of the phenols, suggesting some mechanism in operation other than the action on undissociated hypochlorous acid resulting from the hydrolysis of azochloramid. Synergism apparently occurred to the greatest extent when both of the agents independently exerted some amount of germicidal activity.

ON THE MODE OF ACTION OF PENICILLIN. *L. O. Krampitz, M. N. Green, and C. H. Werkman*, Industrial Science Research

Institute, Iowa State College, Ames, Iowa.

An inhibitory effect of penicillin on the oxidation of neucleotides or nucleic acids by *Staphylococcus aureus* has been demonstrated. Penicillin has no effect on glucose oxidation or on the utilization of the constituents of a synthetic medium on which good growth can be obtained.

The reaction inhibited by penicillin has an RQ of 1.0. The measurable end products are CO_2 and acetic acid in a ratio of 3 to 1. Since 3 moles of oxygen are required for the oxidation, the dissimilation of a pentose, according to the following equation, is postulated.

$$C_5H_{10}O_5 + 3\ O_2 \rightarrow CH_3COOH + 3\ CO_2 + 3\ H_2O$$

Sodium ribonucleinate on oxidation by *S. aureus* shows the same ratio of end products. This reaction is inhibited by penicillin. Streptomycin has also been shown to exhibit an inhibitory action on the same reaction.

YEASTS IN DECOMPOSING FLESHY FUNGI. *K. W. Anderson and C. E. Skinner*, Department of Bacteriology and Immunology, University of Minnesota.

MELIBIOSE BROTH FROM RAFFINOSE BY FERMENTATION IN YEAST TAXONOMY. *R. Bouthilet and C. E. Skinner*, University of Minnesota.

INACTIVATION OF NEUROTROPIC VIRUSES BY MECHANICAL AGITATION. *W. F. McLimans*, Naval Medical Research Institute, Bethesda, Maryland.

EXPERIMENTAL THERAPY OF SCRUB TYPHUS WITH METHYL THIAMINE CHLORIDE. *W. F. McLimans and C. W. Grant*, Naval Medical Research Institute, Bethesda, Maryland, *and W. P. Larson*, University of Minnesota.

COMPARISON OF GROWTH OF LACTOBACILLUS CASEI IN YEAST EXTRACT MEDIA AND SYNTHETIC MEDIA OF VARYING COMPOSITION. *M. R. Muedeking and H. O. Halvorson*, University of Minnesota.

INTRAMURAL DISSEMINATION OF MOLD SPORES. *C. M. Christensen*, Plant Pathology Department, University of Minnesota, St. Paul, Minnesota.

ILLINOIS BRANCH

CHICAGO, ILLINOIS, JANUARY 17, 1947

VARIATION IN MOLDS—NATURAL AND INDUCED. *Kenneth B. Raper*, Fermentation Division, Northern Regional Research Laboratory, Peoria, Illinois.

Saprophytic molds are regularly characterized by marked natural variation, and in the genera *Aspergillus* and *Penicillium* this occurs at all levels of classification. Groups are established as a matter of convenience, but are seldom sharply delimited. Within these groups, species descriptions are centered upon fairly tangible differences, but intermediate and transitional forms are the rule rather than the exception. The more common species in turn are extremely variable, and individual strains or isolates often differ markedly in cultural and morphological characteristics. Cultures derived from single spores, long considered by mycologists as yielding the ultimate in strain individuality, afford little assurance of sustained stability. Such natural variation offers unusual opportunities for the experimental microbiologist, since species and strains vary not only in cultural and morphological characteristics, but in nutritional requirements, biochemical reactions, and in fermentative capacities as well. It also poses some problems, since most variations are in the direction of reduced activity, or, in fermentations, reduced yields. By careful selection, substrains characterized by some increased activity in desired directions can often be developed. This is generally limited, however, and it is necessary to employ external stimuli such as ultraviolet or X-ray radiation to secure further improvements. Variation in colony

appearance and structure may or may not be correlated with changes in physiological activity.

SOME FACTORS AFFECTING THE STABILITY OF TYPE A (PR8) INFLUENZA VIRUS. *George F. Forster, Venus Love, and Esther Carson.*

The viability of this virus has been studied under certain conditions of laboratory maintenance, particularly (1) in storage at −60 to −70 C as a concentrate of allantoic virus, (2) in storage at 5 to 8 C as a lyophilized concentrate of allantoic virus, and (3) in storage at 5 to 8 C as allantoic virus diluted decimally in nutrient broth. Longevity was measured in terms of months under each of these methods of treatment. The chick red cell method of concentration was employed, and the character of the elution fluid was an important factor in the stability of the concentrates whether stored as such at −60 to −70 C, or lyophilized and stored at ordinary refrigerator temperatures. Elution into a 50:50 mixture of normal rabbit serum and nutrient broth or into inactivated allantoic fluid resulted in considerably greater stability than when elution was made into physiological saline or buffered (pH 7.4) saline. When unconcentrated allantoic virus was diluted in nutrient broth (10^{-1} to 10^{-7}) and these dilutions were stored at 5 to 8 C, deterioration was gradual over a period of many weeks. Mouse-killing potency was the criterion of viability.

A STUDY OF THE BACTERIAL FLORA OF THE NORMAL AND PATHOLOGICAL VAGINA AND UTERUS. *K. Eileen Hite, H. Close Hesseltine, and Louis Goldstein,* Department of Bacteriology and Parasitology and the Department of Obstetrics and Gynecology, The University of Chicago and The Chicago Lying-In Hospital.

A study has been made of the aerobic and anaerobic bacterial flora of 248 patients of the Chicago Lying-In Hospital. The study included vaginal cultures from normal prenatal patients and patients having vaginal infections (trichomoniasis, moniliasis, and vaginitis of unknown etiology), and intra-uterine cultures from normal and febrile *post-partum* patients and from a few postabortal puerperae. In general, the flora of the vagina was similar in normal prenatal patients and in those with mycotic and nonspecific vaginitis. Aciduric rods were the predominant organisms. A variety of bacteria were isolated from the vagina of trichomoniasis patients and the uterine cavity of normal and febrile puerperae. The bacterial flora in the latter groups was similar.

AUTOLYZED BRAIN TISSUE AS A MEANS OF FACILITATING TRANSMISSION OF POLIOMYELITIS TO MICE. *Albert Miller, Chester L. Byrd, and Sidney O. Levinson,* Serum Center, Michael Reese Research Foundation, Chicago, Illinois.

Most attempts to infect various animals with poliomyelitis monkey-passage strains or infected human tissues failed until Armstrong was able to adapt the Lansing strain from monkeys to cotton rats and from the latter to white mice. Since then many unsuccessful attempts to establish other monkey-passage strains in various rodents have been made using a variety of techniques such as rapid passage, brain trauma, hyperpyrexia, chilling, spreading factor, and use of immature animals.

Autolyzed brain tissue diluent prepared from normal CFW Swiss mice was found to shorten the incubation period and facilitate the transfer of poliomyelitis virus to CFW mice, hamsters, and rhesus monkeys. By means of this technique the Leon monkey-passage strain of poliomyelitis virus was successfully adapted to CFW mice. Proof of adaptation was shown by successful transfer to monkeys and neutralization in high dilution with human immune serum globulin. More recently several strains of poliomyelitis virus have been isolated in CFW mice from infected human stools and a spinal cord using autolyzed brain diluent.