
Recent investigations indicate that starches are interfering in the microbiological assay for riboflavin, and that such factors can be removed by enzymatic treatment of the material to be assayed. Since assays run on enzyme-treated distillers' dried solubles (from which starch has been previously removed) indicated that a reduction in assay values occurred, it would appear that enzyme treatment has an effect other than the removal of starch.

Experiments were conducted in which different samples of distillers' dried solubles were treated with varying amounts of taka-diastase or pangestin and the extracts then assayed for riboflavin by the method of Snell and Strong.

Extracts treated with taka-diastase for 24 hours assayed lower than untreated extracts. The assay values were inversely proportional to the enzyme concentration (within certain limits). This reduction was possibly due to the fact that bacterial contamination occurred during the digestion period.

When pangestin treatment was employed, higher assay values were obtained with the dried solubles of one distillery and lower values with the product of another distillery. This variation has been attributed to the different methods employed in producing these solubles.

The conclusion drawn from this work is that enzyme treatment removes interfering factors other than starch.

Multiplication of Tobacco Leaf Spot Bacteria on Roots of Other Species. Stephen Diachun, W. D. Valleeau, and E. M. Johnson, Agronomy Department of the Experiment Station, University of Kentucky, Lexington, Kentucky.

Roots of seedlings of several unrelated species of plants were dipped in a 1:1000 dilution of a broth culture of Bacterium tabacum, and were potted in sterile soil. Ten plants of each species were removed from the soil immediately. The roots were washed in running tap water, crushed in water, and poured on water-soaked tobacco leaves. Very few, if any, wildfire spots developed on the leaves. The roots of 10 plants of each species were then tested in 24 hours, and another set 96 hours after they had been dipped in the bacterial suspension. In each case large numbers of wildfire spots developed on the leaves, showing that the tobacco leaf spot bacteria multiply rapidly on the roots of plants, including wheat, oats, barley, rye, cowpeas, soybeans, castor beans, alfalfa, vetch, and crimson clover.

Some Implications of Histamine Production in Experimental Trichinosis. C. B. Hamann, Department of Bacteriology, University of Kentucky.

The author has found that rats and guinea pigs experimentally infested with Trichinella spiralis show an increase of histamine in the blood and intestinal tissue. The greatest increase occurs during the migratory and localization phase of the life cycle of the parasite. It is believed that this increase in histamine may be a contributing factor in the symptomatology of the disease. Walpole, Varco, Code and Wangensteen (1940) have shown that ulceration of the stomach and intestine may result from chronic histamine intoxication and it seems likely that the puzzling ulceration in the intestine of animals infested with Trichinella may result from the increased histamine production. Edema and lowered blood pressure are no doubt influenced by the presence of additional
histamine. No correlation was found between the eosinophilia and histamine concentration in the blood.

Evaluation of Methods Used for Surface Growth of Obligate Anaerobes. (Preliminary Report.) Holmes T. Knighton, School of Medicine, University of Louisville.

Broth cultures of anaerobes were serially diluted in multiples of ten. A standard loopful of each dilution was streaked on each of six blood agar plates. Different anaerobic techniques were used for each plate, and all cultures were incubated for 48 hours at 37°C.

Results for each technique are expressed according to highest dilution from which growth was constantly obtained in at least four tests:

Both McIntosh-Fildes' and Brewer's jar techniques using hydrogen: Clostridium welchii\textsuperscript{10-4}, Clostridium botulinum and Clostridium tetani\textsuperscript{10-4}, Clostridium oedematis-maligni, Fusiformis dentium and Fusiformis nucleatus\textsuperscript{10-4}.

Rosenthal's technic: C. welchii\textsuperscript{10-4}, C. botulinum and C. tetani\textsuperscript{10-4}, C. oedematis-maligni and F. dentium\textsuperscript{10-4}, F. nucleatus\textsuperscript{10-4}.

Brewer's technic using illuminating gas: C. welchii\textsuperscript{10-4}, C. botulinum and C. tetani concentrated broth, C. oedematis-maligni and F. dentium\textsuperscript{10-4}, F. nucleatus\textsuperscript{10-4}.

Brewer's "petri dish-top" over medium containing reducing substances: C. welchii and C. botulinum\textsuperscript{10-4}, C. tetani concentrate broth, C. oedematis-maligni\textsuperscript{10-4}. No growth was obtained from concentrated broths of either fusiform culture.

Spray dish using pyrogallic acid and NaOH: C. welchii\textsuperscript{10-4}. Growth was constant from concentrated broth of other cultures.

VIRGINIA BRANCH
RICHMOND, VA., NOVEMBER 14, 1942


The Virginia Premarital Examination Law states that a serologic test required for marriage shall be made in a laboratory approved by the State Health Commissioner. It was decided that approval would be based largely upon the demonstration by the various laboratories of a satisfactory test performance. In determining this the recommendations of the National Committee on Evaluation of Serodiagnostic Tests for Syphilis were followed.

The Virginia State Department of Health has completed three intrastate serodiagnostic evaluation studies.

In the last study twenty-two laboratories achieved satisfactory ratings. There were twenty-three diagnostic flocculation procedures and eight complement fixation procedures which were satisfactory. Nine laboratories had a satisfactory specificity, but their sensitivity was too low. Twelve laboratories had a satisfactory sensitivity, but failed in specificity. Three had unsatisfactory ratings.

The sources of error in the performance of the various serodiagnostic tests fell into the four major groups as observed in State Laboratories.

There are today forty-eight approved laboratories in the State of Virginia.

A steady improvement in the test performance of laboratories throughout the state has been demonstrated. Better and more dependable results are taking the place of the doubtful, nonspecific and undependable tests of the past.

Results of Kolmer Complement Fixation Tests on Spinal Fluids with and without the Addition of Egg Albumin to the Complement. E. P. Foxhall, Virginia State Department of Health.

Complement-fixation tests for syphilis employing ice-box incubation frequently give nonspecific reactions with spinal fluids. This phenomenon was first observed in quantitative tests with serum. Frequently the first two tubes carrying 0.2 ml. or 0.1 ml. of negative serum would show complete hemolysis, whereas, the following tubes carrying smaller amounts of serum would give a one, two, or four-plus reaction.
Serum controls carrying no antigen were always completely hemolyzed. The antigen control would either show partial or complete inhibition of hemolysis. These zonal reactions are peculiar to spinal fluids even though other findings are normal.

A lack of sufficient protein suggests itself as a possible factor in the causation of these false positives. Egg albumin has been found to be a very satisfactory substitute for this protein deficiency.

The Virginia State Department of Health Laboratory examined 2,331 specimens of spinal fluid, both with and without the addition of egg albumin to the complement. Positive reactions with albumin numbered 615. Positive reactions without albumin but negative with albumin were 1,590. Anticomplementary reactions with albumin were 49 compared to 138 anticomplementary reactions with plain complement.

STUDIES ON THE PENETRATION OF GERMI-CIDES. F. J. von Gutfeld and L. G. May, Medical College of Virginia.

The methods used in testing germicides consist generally in bringing the germs in direct contact with the killing agent. By such methods the germicides have the greatest chance to exert their killing power. Addition of protein or dried feces in many cases diminishes the bactericidal power. For practical purposes it seems necessary to take into consideration the ability of the germicide to penetrate substances in which the germs may be embedded. The cup-plate method partly takes care of this problem. It seems, however, that a method should be worked out which approaches natural conditions still more closely. We present a method which may be suitable for this purpose.

Infected strings are embedded in agar, serum-agar, and blood-agar respectively; and the germicide is poured on top of the “coating layer”. After a suitable time the strings are removed, rinsed with sterile water and transferred to a nutrient medium. These experiments show that much more time is necessary to kill bacteria or fungi under these conditions than under the usual experimental conditions.

A CASE OF LUNG INFECTION WITH ASPERGIL- LUS FUMIGATUS. Francis D. Smith, Department of Clinical Pathology, University of Virginia.

The case reported is that of a 44-year-old white man with a productive cough and frequent hemoptysis for four years. Because of loss of weight and strength the patient was forced to give up his job as a machinist and was admitted to a tuberculosis sanatorium. Repeated sputum examinations over a period of three months were negative for tubercle bacilli. X-ray examinations, however, showed cavities in both upper lung fields, and unidentified fungus structures were found in the sputum.

Subsequent examinations in another hospital and in this hospital have failed to demonstrate tubercle bacilli. Assuming tuberculosis to be ruled out, a consulting surgeon has suggested the condition represents bilateral cystic disease with secondary infection.

Sabouraud’s agar cultures of all sputum specimens, five in number, and one bronchoscopy specimen have been positive for Aspergillus fumigatus, with identification of the fungus confirmed by Dr. C. W. Emmons.

The sputum was greenish in color and, though formed in abscesses, was not malodorous. A distinguishing characteristic of the Aspergillus fumigatus conidiophore is the presence of certain vesicles having the shape of an Erlenmeyer flask modified to possess a hemispherical instead of a flat bottom.

THE BACTERIOSTATIC EFFECT OF GLYCINE ON GROUP A HEMOLYTIC STREPTOCOCCI. G. M. Lawson, J. A. Patterson, K. C. Bass, and A. L. Jones, University of Virginia School of Medicine.

Compounds similar in structure to the sulfonamides but lacking the sulfon group may be bacteriostatic. Para-amino hip-puric acid, the theoretical product formed by the combination of glycine and para-amino benzoic acid, was tested for bacteriostasis but was found instead to have as great or greater growth-stimulating action than para-amino benzoic acid on Group A hemolytic streptococci. However, glycine alone or in combination with para-amino benzoic acid produced definite bacteriostasis and
occasionally showed bactericidal powers when the former ingredient was used in concentrations of 500–1000 mg. per cent. In several media devoid of antagonists to the action of sodium sulfadiazine, glycine alone or in the mentioned combination proved a more effective bacteriostatic agent than sodium sulfadiazine in 15 mg. per cent. concentrations as measured in vitro.

**War Time Immunization.** George McLean Lawson, University of Virginia School of Medicine.

The susceptibility of medical students to diphtheria and to scarlet fever is increasing in Virginia in recent years and may be an indication of a similar trend in the adult population as a whole. Of the students tested over the five-year period 1938 to 1942, inclusive, 33.2% were shown to be susceptible to diphtheria and 42.2% to scarlet fever as determined by Schick and Dick tests.

Immunization of civilian populations is discussed and stress is laid on the necessity of immunization before rather than during civil mass evacuations, influxes of workers to defense areas and war time disasters such as bombing or sabotage. Accepted immunization procedures are outlined as they apply to civil and military populations.

**Washington Branch**

George Washington University School of Medicine, November 24, 1942

A **Summary of Recent Work in the Control of Triple Typhoid Vaccine.** Major George F. Luippold, U. S. Army Medical School, Washington, D. C.

This paper discusses two problems connected with the control of triple typhoid (T.A.B.) vaccine: (1) the selection of strains of vaccine organisms, and (2) the range of immunogenic coverage produced by inoculation of human beings with the T.A.B. product.

Selections of strains of Eberthella typhosa, Salmonella paratyphi, and Salmonella schottmuelleri were based upon their virulence for mice and upon their immunogenic potency in laboratory animals. Antigenic content, as determined by agglutination tests with diagnostic Salmonella sera, does not serve as a reliable index to an organism's immunogenic potency. The decisive criterion for an organism's acceptance should be its performance, as a vaccine, in the animal body.

T.A.B. vaccine prepared at the Army Medical School possesses an effective immunogenic coverage over the component organisms and over antigenically related types of Salmonella. However, it is relatively ineffective against the "Suipestifer" or paratyphoid C group of organisms.

Coliform organisms containing Salmonella antigens were also considered as possible pathogens against which T.A.B. vaccine may be called upon to protect. Inoculation of T.A.B. vaccine produced in the blood serum of human subjects significant
amounts of protective substances active against a coliform strain containing *Salmonella* O-antigens I and II (as in paratyphoid A), and against a second strain containing O-antigens IV and V (as in paratyphoid B).

**The Mechanism of Adaptation of Bacterial Species to High Temperatures.**

R. R. Spencer, National Cancer Institute, Bethesda, Maryland.

Repeated results (lantern slide demonstrations) following the exposure of three species of actively dividing bacteria (*Streptococcus hemolyticus, Enterobacteria typhosa* and *Escherichia coli*) to high temperatures have led to the formulation of two biological generalizations regarding the mechanism of species adjustment. These are:

1. **Continuous exposure** of a species to a harsh environment may be harmless to the individual organism, but in due time, fatal to the species.

2. **Rhythmic exposure** of a species results in successful adaptation to the same and to a greater intensity of the same environment which is fatal when the exposure is continuous.

The first generalization has been shown to hold for species other than bacteria (paramecia and flat worms), subjected to several different environments (dyes and other chemicals).

**The Differentiation of Human and Chicken Strains of *Escherichia coli* on the Basis of Phenol Tolerance.**

J. M. Leise and L. H. James, Department of Bacteriology, University of Maryland, College Park, Maryland.

Human and chicken strains of *Escherichia coli* were isolated from human and chicken fecal matter respectively and growth curves were obtained by growing the strains in phenol-containing broth. An electric nephelometer was used and the results showed the chicken strain to be more resistant than the human strain. The greatest difference was obtained at a phenol dilution of 1:525. Using a second set of freshly isolated human and chicken strains of *E. coli*, growth curves were obtained by means of plate counts. These results checked with the first set in that the best differentiation was again obtained at a phenol dilution of 1:525.

In order to inhibit the weaker human strains further while allowing the chicken strains to grow, a technique in which the strains were cultured twice in phenol-containing broth was used. This method, using phenol dilutions of 1:520 and 1:525, completely inhibited 3 out of 4 human strains while only one chicken strain failed to grow.

**Development of Sulfonamide Resistance (Fastness) in *Staphylococcus aureus* Correlated with Greatly Increased Synthesis of p-Aminobenzoic Acid by the Organism.**

Maurice Landy, Newton W. Larkum, Elizabeth J. Oswald and Frank Streightoff, Division of Bacteriology, Army Medical School, Washington, D. C.

Sulfonamide-resistant strains of *Staphylococcus aureus* grown in a synthetic medium free of p-aminobenzoic acid were found to synthesize greater amounts of p-aminobenzoic acid than did their parent strains. This synthesis was ascertained by the microbiological assay method of Landy and Dicken, employing *Acetobacter suboxydans* as the test organism. p-Aminobenzoic acid made by resistant strains averaged seventy times the amount produced by the parent strains and was produced both in the absence and in the presence of sulfonamides. The quantity of p-aminobenzoic acid elaborated by resistant strains (3-4 micrograms per ml. of culture) appears sufficient to account for the resistance of the staphylococcus to the sulfonamide drugs. It is therefore suggested that the development of ability to synthesize p-aminobenzoic acid in excess of normal metabolic requirements, as a result of continued exposure to sulfonamides, is responsible for sulfonamide-fastness in *Staphylococcus aureus*.