AN EVALUATION OF THE KAHN TEST PROEDURE IN 31 ST. LOUIS MEDICAL INSTITUTIONS. Nathan Nagle and J. C. Willett, Laboratory Section of the St. Louis Health Division, St. Louis, Mo.

With the passage of new venereal disease legislation, it became necessary to standardize the serologic test. Information obtained by questionnaires showed that 32 institutions did serological tests for syphilis. Of these, 78% did the Kahn test and, since this test was one of the six officially approved, it was adopted as the standard in St. Louis.

Surveys were made of the Kahn test procedure in the 25 institutions doing the Kahn test and in the remaining 7 after this test had been adopted. It was found that the reading factor caused the greatest variation in Kahn reports. Next in importance was the variation found in the Kahn antigen used. Variations in heating temperatures for serum and using incorrect technique in shaking the tests caused further discrepancies.

After the surveys were completed and satisfactory standards attained, split identical blood specimens were submitted. Twenty laboratories were successful in securing correct results on one series of about 80 check specimens and were approved.

Eleven laboratories failed to agree with the correct Kahn report on the first series of specimens. These showed from 3 to 15 per cent discrepancies. After faulty technique had been eliminated in these 11 laboratories, a second series of check specimens were submitted. All laboratories showed satisfactory agreement and were approved.

These 31 St. Louis institutions are now all reading more uniformly, and are using the standard technique, the same lot of standard Kahn antigen made in the Health Division Laboratory and approved by Dr. Kahn, standard glassware, thermostatically controlled water baths and standard mechanical Kahn shakers.

A NOTE ON THE CULTIVATION OF E. HISTOLYTICA. Simon Russi and George A. Hunt, Snodgrass Laboratory, City Hospital, St. Louis, Mo.

Tsuchiya* has described a method for culture of ameba using Dorset slants covered with extract broth containing rice starch and charcoal. This method, so successful in Tsuchiya's hands, is of value in public health laboratories for several reasons. It is reliable; it is probably less time consuming than an adequate direct microscopic examination of a stool specimen; it may be used with stool specimens ordinarily considered too old for satisfactory direct microscopic examination; stained preparations are easier to make from cultures and more satisfactory in many respects.

Several attempts to maintain

Tsuihaya's cultures in this laboratory or to culture ameba from stool specimens met with failure until, by chance, it was found that prolific growth of the ameba was obtained when an adequate amount of buffer (0.03% phosphate at pH 7) was added to Loeffler slants and extract broth containing rice starch and animal charcoal. Similar results could be obtained by the addition of precipitated CaCO₃. Growth failed in every case when the media did not contain adequate buffer. Cultures which failed to grow in the first 48 hours were made to grow by subsequent addition of buffer (phosphate or CaCO₃ or both).

A culture received from Tsuihaya has since been maintained for nearly two months using buffers.

Of 127 specimens received for examination in this laboratory, five have shown E. histolytica by repeated direct microscopic examination and by culture. All five cultures have been carried for periods varying from 3 weeks to several months.

Application of the Fibrinolysin Test to the Identification of Streptococci. L. Royal Christensen, Department of Bacteriology, St. Louis University School of Medicine, St. Louis, Mo.

Using Fuller's formamide method* for the preparation of antigens and commercial group-specific antisera,† 125 strains of beta-hemolytic streptococci have been grouped (Lancefield's).

The fibrinolysin reactions of these strains were studied using a dried fibrinogen preparation,‡ with the results seen in table 1.

The agreement between group and fibrinolysin reactions seen above has suggested the following procedure as convenient when grouping large numbers of streptococci:

A preliminary fibrinolysin test is performed with the culture. The results are usually available within 30 minutes, never over two hours. If the culture is fibrinolytic, it is tested against A, C and G antisera. If there is no lysis at the end of two hours, it is tested against all other antisera available.

Such a procedure has resulted in saving in time and material in that it has been necessary to perform only about one-half the precipitin tests which would otherwise be necessary.

<table>
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<th>GROUP</th>
<th>NUMBER OF STRAINS</th>
<th>FIBRINO-</th>
<th>NON-FIBRINO-</th>
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<tr>
<td>A</td>
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<td>81</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>13</td>
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<td>13</td>
</tr>
<tr>
<td>C</td>
<td>3</td>
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<tr>
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<td>4</td>
<td>0</td>
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</tr>
<tr>
<td>G</td>
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<td>0</td>
</tr>
<tr>
<td>Others</td>
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</tr>
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</table>

Demonstration of the Microfilms of the Bibliofilm Service of the American Documentation Institute. George A. Hunt, Snodgrass Laboratory, City Hospital, St. Louis, Missouri.


† The author wishes to express his appreciation to Lederle Laboratories, Inc. for the antisera used in this study.

EASTERN PENNSYLVANIA CHAPTER

ONE HUNDRED AND FORTY-THIRD MEETING, PHILADELPHIA COUNTY MEDICAL SOCIETY BUILDING, FEBRUARY 27, 1940, PHILADELPHIA, PENNA.


On February 24, 1920, a group of persons interested in bacteriology and pathology met in the Laboratory of Hygiene at the University of Pennsylvania at the invitation of Dr. David H. Bergey. Most of those present were former members of the Microbiological Club ("Philadelphia Bug Club") and it was proposed to form a local chapter of the Society of American Bacteriologists around that nucleus. The Chapter started with 44 members, dropped to a low of 25 during the depression and now numbers 192 on its active list. The Chapter has met continuously seven times each year since its organization. During the first two decades 546 papers were presented at its meetings by 297 authors. Seventeen of the original members still take an active part in the affairs of the Chapter.

SWINE INFLUENZA. Joseph P. Scott, School of Animal Pathology, University of Pennsylvania, Philadelphia, Pa.

ENZOOTIC TULAREMIA. J. F. Bell, School of Animal Pathology, University of Pennsylvania, Philadelphia, Pa.

Increasing reports of tularemia in man in various parts of the United States have served to emphasize the necessity for a more thorough understanding of the natural incidence of this disease. Since knowledge of the epidemiology of tularemia must come from an investigation of its occurrence in the wild, where it is subject to natural phenomena which in some cases occur at infrequent intervals, it has been necessary to institute such long-time programs as those of the Minnesota Wildlife Disease Investigation, under the direction of Dr. R. G. Green, and the recently initiated project at the School of Animal Pathology of the University of Pennsylvania. These programs have begun to bring into prominence the relative importance of refractory and non-refractory hosts in the spread of tularemia, the reciprocal effects of host and ectoparasite-vector populations, and the role of another disease (shock disease of snowshoe hares) in rendering a normally resistant animal host susceptible to tularemia; and have also demonstrated that knowledge of seasonal distribution of the disease can be used effectively to reduce its incidence in man.

SEROLOGICAL STUDIES WITH BLACK-LEG AGGRESSIN. I. Live, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pa.

Sero logical studies (phagocytosis, agglutination, complement-fixation and precipitation tests) were undertaken to determine whether the immunizing potency of black-leg aggressins could be ascertained in vitro. The phagocytosis experiments showed that the aggressins had an inhibitory influence which was due to anti-opsonic action and not to direct effect upon the leucocytes. The reduction in phagocytosis was proportional to the quan-
tity of aggressin used. The action of aggressin upon an immune serum did not impair the agglutinating property of the serum. Complement-fixation and precipitation tests demonstrated that the quantity of autolyzed bacterial substance present in an aggressin could be determined by the use of antisera produced in response to injections of washed live, as well as formalinkilled, suspensions of Clostridium chauvoei and of aggressin.

Injections of a filtrate of sonically disintegrated C. chauvoei into guinea pigs protected the animals against subsequent infection. These findings, coupled with the fact that killed bacterial cells, free of any aggressin, are known to immunize efficiently against black-leg, would strongly suggest that the autolysates of the organisms present in black-leg aggressins are responsible for the immunizing quality of the latter. Thus, the concentration of dissolved bacterial substance in black-leg aggressins could be evaluated by means of the complement-fixation and precipitin tests.

**Periodic Ophthalmia.** E. L. Stubbs and W. G. Love, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pa.

**One Hundred and Forty-fourth Meeting, Philadelphia County Medical Society Building, March 26, 1940, Philadelphia, Penna.**


An antigen for use in rapid slide agglutinations was prepared from a suspension of Pasteurella tularensis #38 in 12% saline containing 0.5% formalin. The rapid antigen was tested on approximately 600 consecutive sera submitted for agglutination with various antigens. Rapid as well as slow or tube tests were made on 55 of 75 sera from 41 persons diagnosed clinically as having tularemia. Of the 55, 53 were positive and 2 early bleedings negative, by both rapid and slow methods. In no case was the slow method
positive when the rapid test was not. In two patients the rapid test was positive before the slow. Of the total 75 sera, 38 were positive when tested against Brucella abortus or typhoid antigens. Of 528 persons not known to have tularemia, 5 or 0.94% showed a strongly positive reaction with the tularemia rapid antigen. Thirty-two or 6% gave a moderately strong reaction. Of these 37 presumably false positives, 4 could be accounted for on the basis of cross agglutination in that the sera showed titers of 1-640 or over for abortus or typhoid antigens. Lack of clinical data makes it difficult properly to evaluate this group of "false positives."

The Nucleic Acid Pneumococcic Horse Serum Reaction. David Lackman, Stuart Mudd and M. G. Sevag, Department of Bacteriology, University of Pennsylvania, School of Medicine, Philadelphia, Pa.

Nucleic acids from several sources give a precipitate with certain anti-pneumococcic horse sera (J. Bact., 39, 32, 1940). Homologous pneumococcic carbohydrate precipitins can be absorbed from the serum, leaving the nucleic acid "precipitins" intact; likewise, the nucleic acid "precipitins" can be absorbed, leaving the carbohydrate precipitins intact.

The reaction is very sensitive as regards ionic strength, formation of a precipitate being completely prevented when the ionic strength reaches 0.256.

The reaction between nucleic acid and the "precipitin" was specifically inhibited by certain nucleic acid derivatives and related compounds: purine base (adenine), methyl purines (caffeine and theophylline), purine nucleotide (adenylic acid). Slight inhibition was obtained with a pyrimidine base (uracil). None of these reagents affected the pneumococcic carbohydrate reaction.

In October 1939 Winkerwerder, Buell and Howard (Science, 90, 356, 1939), subsequent to our original observation of serological reactivity of nucleic acid (J. Biol. Chem., 124, 425, 1938), reported that positive skin reactions were obtained in ragweed sensitive individuals when they were tested with nucleic acid, nucleotides, purine bases, uracil (pyrimidine base) and certain other compounds. Their list is similar although differing in certain respects from our list of inhibitors for the in vitro nucleic acid pneumococcic horse serum reaction.

The Inhibition of Proteolytic Enzymes of Pathogenic and Non-pathogenic Clostridia. Louis DeSpain Smith, The Biochemical Research Foundation of the Franklin Institute, Philadelphia, Pa.

Normal rabbit, rat, guinea pig and human sera were found to inhibit the proteinases in filtrates of 24-hour cultures of Clostridium aerofetidum, C. botulinum, C. fallax, C. putrificum, C. sporogenes and a rough non-pathogenic strain of C. histolyticum. The proteinases of pathogenic strains of C. oedematis-maligni, C. welchii and a smooth strain of C. histolyticum were not so inhibited. The organisms that produced proteinases which were inhibited by normal serum also produced peptidases which were inhibited. This inhibition of the proteolytic enzymes was due mainly to the albumin of the serum, although some inhibition was due to the beta globulin. Trypsin was inhibited only by the albumin.

Quantitating Gordon's Bacterial Test for Estimating Pollution of
Air. William Firth Wells and Dorothy Wells, Laboratories for the Study of Air-borne Infections, University of Pennsylvania, School of Medicine, Philadelphia, Pa.

Last year, Prof. C.-E. A. Winslow suggested to the Sub-committee on Bacteriologic Procedures of the Committee on Ventilation and Atmospheric Pollution, American Public Health Association, investigation of the practicability of enrichment media for the isolation of nasopharyngeal streptococci from air. We undertook to quantitate, by means of the air centrifuge, Gordon's bacterial test for air pollution (Gordon, M. H., Report on a Bacterial Test for Estimating Pollution of Air, Report Med. Off., Local Gov. Bd., London, 32, 421, 1903-4). Five series of four "dilutions" each in lactose broth, representing 5, 1, \( \frac{1}{2} \) and \( \frac{1}{4} \) cubic feet of air were collected for each sample, incubated at 37° C. for 24 hours, and tested for acid formation with brom-thymol blue. Computations of the number of cubic feet of air per lactose-fermenting organism were obtained by the average-dilution-positive method from a simple table. Sixteen samples of outdoor country air averaged 14.5 cu. ft. of air per lactose-fermenting organism; 62 samples of outdoor city air averaged 10.2 cu. ft.; 55 samples of air from an ultra-violet irradiated hospital ward averaged 5.0 cu. ft. per organism as contrasted with an average of 0.93 cu. ft. in a similar unirradiated control ward; 36 samples of air in various rooms of a medical school averaged 1.2 cu. ft. per organism. Since the common nasopharyngeal streptococci ferment lactose, correlation between lactose-fermenting organisms in the air and opportunities for human pollution would be expected. Apparently the presence of lactose-fermenting organisms in a majority of cubic foot volumes indicates low values for sanitary ventilation.

WASHINGTON BRANCH

112th Meeting, Washington, D. C., February 27, 1940


In previous work (J. Wash. Acad. Sci., 30, 125, 1940) a number of sulfur compounds were found to have fungicidal and fungistatic action. Accordingly, some fifty organic sulfur compounds were tested, relative to their effect on the growth of various streptococci. A number of compounds were found to have more or less bacteriostatic or bactericidal power at a level of 100 parts per million in peptone broth. The most promising compounds are phenothioxin which showed marked bacteriostatic action on Streptococcus hemolyticus (Oyler strain) and Streptococcus hemolyticus (epidemicus) group A and considerable inhibition of Streptococcus viridans number two; and mercaptobenzothiazole which showed bactericidal action on Streptococcus hemolyticus (Oyler strain) and on Streptococcus hemolyticus (epidemicus) group A, but not on Streptococcus viridans number two. The criterion of biological action of the various compounds was the degree of growth on
blood agar slants after a period on peptone broth culture with and without the compound under study.

**Factors Influencing the Production of Acetyl-Methyl-Carbinol by the Aerobic Spore-Formers.** Nathan R. Smith, Bureau of Plant Industry.

The production of acetyl-methyl-carbinol in the medium recommended in the Manual of Pure Culture Studies of Bacteria, was found to be variable with certain species of the genus *Bacillus*. Sterilization of the medium by filtration improved it for some species but not for all. Leaving out the phosphate entirely was still better but did not suit *Bacillus alvei*. By substituting 0.5% sodium chloride for the phosphate a very good medium for the production of acetyl-methyl-carbinol resulted. This medium may be autoclaved without damage to its efficiency. Tests were made as to the proper length of incubation and the proper temperature. Owing to considerable variation in rate of growth in this genus, it was found that observations should be made at 2, 4, and 6 days, the majority being positive at 2 days. Temperature should be 32°C. or somewhat lower. The proposed modified medium is also suitable for *Aerobacter aerogenes*.

**WASHINGTON BRANCH**

**113TH MEETING, WASHINGTON, D. C., MARCH 26, 1940**

**The Possible Utilization of Bacteriophage in the Classification of the Aerobic Spore-Formers.** Ruth E. Gordon, Bureau of Plant Industry.

The use of bacteriophage in classifying the aerobic spore-formers is now being investigated. At present, a pure line strain of bacteriophage for each of the three following species; *Bacillus cereus*, *Bacillus megatherium* and *Bacillus mesentericus*, has been obtained. Each strain of bacteriophage has, so far, proved to be specific for all cultures of its particular species.

This specificity was further tested by determining the susceptibility of *B. megatherium* to the "nascent bacteriophage" of *B. cereus*. The inhibition of *B. megatherium*, first considered as evidence of susceptibility, was found due to the antibiotic effect of the culture of *B. cereus* rather than to the lytic effects of the *B. cereus* bacteriophage.

Since nearly fifty per cent of the cultures of collections of aerobic spore-formers studied in this laboratory belong to the three species, *B. cereus*, *B. megatherium*, and *B. mesentericus*, bacteriophages for these three species may be of greater use than the smallness of their number indicates.

**The Incidence of Aerobacter Aerogenes in Human Fecal Excreta.** R. J. Reedy and J. F. Puncochar, Technological Laboratory, U. S. Bureau of Fisheries, College Park, Maryland.

Two hundred and fifty three samples of human feces obtained from 253 normal adult women were tested for the presence of *Aerobacter aerogenes* and the approximate ratio of aerogenes, or citrate utilizers, to coli was determined.

Enrichment in citrate was used followed by streaking on EMB. Typical colonies were picked and planted into
citrate. All organisms were considered to be aerogenes that were gram-negative non-spore-forming rods, MR-, VP+, citrate +, and indol+. Of 253 samples of feces, aerogenes was detected in 220, an incidence of 87%.

The fecal samples were diluted to 1:100,000,000, duplicate plates being inoculated from 1:10,000 to 1:100,000,000. One set was poured with Simmons citrate agar, the other with EMB. Counts were determined and the ratio of aerogenes, or citrate utilizers, to coli obtained. The ratio was divided into 5 groups: (1) from 1 to 1:500 occurred 90 times averaging 1:110; (2) from 1:500 to 1:1,000 occurred 18 times averaging 1:707; (3) from 1:1,000 to 1:10,000 occurred 38 times averaging 1:3657; (4) from 1:10,000 and higher occurred 16 times averaging 1:28249. Group 5, in which aerogenes occurred more frequently than coli, was found 4 times averaging 1.5:1.

In 4 samples no coli could be found in the 1:10,000 dilution. In 82 samples aerogenes, or citrate utilizers, could not be found in the 1:10,000 dilution; however, of these 82, aerogenes was found 54 times in citrate.

In 166 samples aerogenes, or citrate utilizers, were present in quantities of at least 10,000 per gram.