THE IMPORTANCE OF BIOTIN IN MICROBIOLOGY. Maurice Landy, S. M. A. Corporation, Chagrin Falls, Ohio.

THE GERMICIDAL ACTION OF CERTAIN SUBSTANCES IN THE PRESENCE OF FUNCTIONING AND NON-FUNCTIONING TISSUE. Raymond W. Sarber, Cincinnati College of Pharmacy, Cincinnati, Ohio.

OCURRENCE OF SULFONAMIDE-RESISTANT PNEUMOCOCCI. L. H. Schmidt and Clara L. Sester, Institute for Medical Research, Christ Hospital; Morton Hamburger, Jr., Department of Medicine, College of Medicine, University of Cincinnati, Cincinnati, Ohio.

Experimentally, sulfonamide-sensitive pneumococci may be converted readily into organisms that are highly resistant to these drugs. Clinical studies of cases of pneumococcal pneumonia and meningitis have shown that in some instances pneumococci isolated during or subsequent to sulfonamide therapy are more resistant to the drug used in treatment than the pneumococci isolated prior to therapy. For the most part, however, the changes in resistance were small as compared with those observed experimentally.

In the majority of the above cases, the period of sulfonamide treatment did not exceed 10 to 14 days. However in one case, that of a woman with a type VII bacteremia, sulfapyrazine was administered intermittently over a period of five months. In this case, a change in sensitivity of the pneumococci to sulfapyrazine was observed which was as great as the largest change observed experimentally. The pneumococci isolated from this patient prior to treatment were unable to multiply in media containing more than 2.5 mg. per cent sulfapyrazine, whereas the organisms isolated after five months of therapy multiplied readily in media containing 160 mg. per cent of this drug. It is noteworthy that the development of resistance was paralleled by the failure of sulfapyrazine to control the patient's bacteremia.

A METHOD FOR DETERMINING THE TISSUE TOXICITY OF DISINFECTANTS. T. W. Green and J. M. Birkeland, Department of Bacteriology, Ohio State University, Columbus, Ohio.

A new method for determining the tissue toxicity of disinfectants has been developed. It is based on their effect on pulsating embryonic chick heart fragments and is as follows:

Hearts from two twelve-day-old chick embryos are minced to a fragment size of approximately one mm. and suspended in four ml. of a mixture containing one-fourth sheep serum and three-fourths Tyrode's solution. One-half ml. quantities of this suspension are placed in the walls of six tissue culture slides and serial dilutions of the disinfectant are added. After an exposure of ten minutes the fragments are washed once and resuspended in Tyrode's solution. Epinephrine is added and the fragments are observed for the presence or absence of pulsation.

The results were in general comparable to those obtained by the use of Salle's tissue culture method. However, certain quaternary ammonium derivatives appeared to be much less toxic by the former method than by the latter.

The advantages of the pulsation method are its simplicity, rapidity, and reproducibility. Aseptic technique is not necessary and the results are obtained in thirty minutes.
The effect of separate inoculation of serum and virus on the protection test in vaccinia was determined, using intradermal inoculation of rabbits. In the first series of experiments, the skin was infiltrated over a wide area with 10 per cent solution of immune serum in saline, and at varying intervals thereafter serial dilutions of vaccinia virus suspension were inoculated into the treated area. The apparent titer of virus in the treated animals was compared with a simultaneous control titration. With an interval of six hours, good protection (100 infectious units) was obtained, decreasing with time until after four days no protection was demonstrated. In the second series the virus was inoculated first, followed by serum. In this case also, good protection was secured with an interval of six hours, the degree of effect of the immune serum decreasing with the passage of time.

Behavior of St. Louis Encephalitis Virus in Rats. Carl E. Duffy and Albert B. Sabin, The Children's Hospital Research Foundation, and the Department of Pediatrics of the College of Medicine, University of Cincinnati, Cincinnati, Ohio.

When St. Louis encephalitis virus, which hitherto was considered non-pathogenic for rats, was injected into the brain or dropped into the nose of 7- to 8-day-old rats, a fatal encephalitis developed in all the animals tested. To date the virus has been carried through 10 serial intracerebral passages in 7- to 8-day-old rats. The virus of the 10th rat passage when titrated in mice was active in a dilution of $1 \times 10^{-4}$ and was neutralized by a St. Louis encephalitis immune serum. Rats 21 days of age or older showed no clinical signs of disease following intracerebral injection or nasal instillation of the virus. However, nasally instilled virus was found to produce an inapparent infection in 21-day-old rats in that the virus invaded and multiplied in the central nervous system. The nasally instilled virus could not be recovered from the nasal mucosa 2 days later but was present on the 3rd, 4th, and 6th, but not on the 10th days following instillation. The virus was not recovered from the blood of 21-day-old rats which had had the virus dropped into the nose but was demonstrated in the blood of 7-day-old rats similarly infected.

Depending upon their age, rats may develop apparent or inapparent infections with the virus of St. Louis encephalitis, a fact which is worthy of consideration in the final elucidation of the epidemiology of this disease.

Connecticut Valley Branch
Yale Medical School, November 22, 1941

Immune Reactions with Anti-tubercle Bacillus Serum. John T. Riordan, Section of Preventive Medicine, Yale University School of Medicine.

A single 15-year-old sample of anti-H37 tubercle-bacillus serum #8807, tested with various extracts and derivatives of acid-fast bacilli, yielded precipitin reactions and colloidion particle agglutinations in high dilutions with four samples of Old Tuberculin and four samples of polysaccharides from acid-fast bacilli.

Reactions were also encountered in low dilutions of crude aqueous extracts of tubercle bacilli and in aqueous extracts of tuberculous tissue from monkeys and guinea pigs and from lesions produced in the chorio-allantoic membrane of the developing chick embryo. No precipitin reactions or colloidion-particle agglutinations were obtained with protein derivatives of tubercle bacilli.

These findings confirm the work of previous investigators in that the antibody content of this particular serum is
composed entirely of anti-polysaccharide antibodies.

The use of the collodion-particle method renders tests for detection of tubercle bacillus polysaccharides slightly more delicate.

**The Effect of Potassium Tellurite on the Dehydrogenases of Escherichia coli.** *Emily H. Kelly*, Department of Bacteriology, Yale University.

In seeking the cause for the bacteriostatic effect of potassium tellurite on *Escherichia coli* an investigation of the action of this substance on the dehydrogenases of the organism was undertaken.

Formate and lactate dehydrogenases were found to be tellurite-resistant; galactose, levulose, and glucose dehydrogenases were inhibited, at least by the higher concentration of tellurite. Citrase, pyruvase, glycerophosphorase, glycerase and acetase were completely sensitive to tellurite whenever they were demonstrable in the culture.

The tellurite resistance of the trained strains differed very little from that of the untrained, and tellurite-tolerant strains appeared to possess enzymes which were no more resistant to the action of tellurite than were those of the parent strains; nor had they developed any new dehydrogenases. They had, however, in many instances, lost the dehydrogenases which were most strongly inhibited by tellurite.

The results would indicate that the primary toxic action of tellurite is not on the dehydrogenases, since tolerant and susceptible strains were alike in their response to tellurite.

**Latent Feline Tularemia Apparently Responsible for a Human Infection.** *Erwin Jungherr*, Department of Animal Diseases, University of Connecticut, Storrs, Conn.

The first reported case of human tularemia in Connecticut occurred near Colchester in Nov. 1940, and was characterized by ulcero-glandular lesions, sero-positiveness, and cerebral edema, according to Gibbons *et al.* (Conn. State Med. J. 1941, 5: 679). The second case, for the clinical data of which we are indebted to Dr. E. Ottenheimer, occurred in Columbia, about 10 miles distance from Colchester, in Sept. 1941. Twenty-two days after having been bitten on the hand by a young house cat, the patient died with evidence of sero-positiveness, caseous glandular lesions in the exposed extremities, pneumonia, and caseous focal lesions in spleen, liver and kidneys. Direct culture of human material yielded a mixed growth presumably containing *Pasteurella tularense*; inoculation of lymph glands, spleen and lung into guinea pigs caused death within 6 days accompanied by typical tularemia lesions from which the organism was isolated in pure culture. The implicated cat and a sib were negative on clinical, gross, histopathologic and cultural examination but were sero-positive in dilutions of 1:640 and 320, respectively. Guinea-pig inoculation of liver-spleen suspension of the implicated cat failed to cause disease but that of the sib induced tularemia lesions from which the organism was recovered.

This report seems to represent the first isolation of *P. tularense* from guinea pigs, inoculated with human and feline material, in Connecticut.

Attempts have been made in cooperation with the State Department of Fisheries and Game to locate other animal carriers. During 1936-38, 342 Connecticut cottontail rabbits were examined for parasites (Clancy *et al.* J. Wildlife Manag. 1940, 4: 162), without observing evidence of tularemia. In 1940, 19 rabbits from the Colchester vicinity, and in 1941, 6 rabbits, 23 mice, 9 rats, 1 hawk, 2 grouse, and 24 cats from the Columbia area, were examined by guinea pig inoculation and serologic tests for tularemia, with negative results.

**Bacterial Decomposition of Vitamin C.** *Raymond M. Young*, Department of Bacteriology, Yale University.

A general survey was made of the ability of a number of species of bacteria to decompose vitamin C. To one-per-cent buffered peptone broth was added as-
corbic acid to give a concentration of .4 to .5 mg. per ml. broth. All cultures and an uninoculated control were incubated at 37°C. under anaerobic conditions. Analysis for vitamin C was made frequently, using Tillman's sodium 2:6-dichlorophenolindophenol method as adapted by Bessey to the Evelyn photoelectric colorimeter.

Thirty-two strains of Escherichia coli and twenty strains of enterococci completely decomposed the vitamin C present in the medium after 12 to 24 hours' incubation. Several members of the Salmonella genus, including S. paratyphi, S. schottmülleri, S. aertrycke, S. enteritidis and S. gallinarum, completely oxidized the vitamin in 24-30 hours; however, S. pullorum had no destructive effect upon ascorbic acid. The human pathogens, Streptococcus pyogenes, Encapsulatus pneumoniae, Eberthella typhosa, and Vibrio cholerae were active in the decomposition. On the other hand Staphylococcus aureus, Staphylococcus albus, members of the Brucella genus and the dysentery group did not attack the vitamin; nor did the X-19, X-2 and OXK strains of Proteus have any effect upon the substance. However two strains of Proteus morganii isolated from rat feces were very active in the oxidation, as were a number of strains of Aerobacter aerogenes. Members of other genera, including Erythrobacillus, Flavobacterium, Achromobacter, and Alcaligenes had no effect whatever upon ascorbic acid, even though moderate to luxuriant growth of the test organism was evident. Decomposition of ascorbic acid was usually accompanied by marked acceleration in growth when the medium was buffered to a suitable pH.

AN EPIDEMIC OF SCARLET FEVER IN A KINDERGARTEN. Dr. Paul L. Boisvert, Department of Pediatrics, Yale School of Medicine.


NEW JERSEY BRANCH

FIFTH MEETING, RUTGERS UNIVERSITY, NEW BRUNSWICK, N. J., DECEMBER 4, 1941

BACTERIOLOGY IN FRANCE TODAY. Dr. Francis F. Schwenker, International Health Division, Rockefeller Foundation.

THE SYNERGISTIC ACTION OF SULFONAMIDES, AZOCHLORAMID, AND WETTING AGENTS. Dr. Oreille Wyse and Dr. F. C. Schmelkes, Research Department, Wallace & Tiernan Products, Inc., Belleville, N. J.

The enhancement of the bactericidal action of sulfanilamide by concentrations of azochloramid in themselves too small to cause killing, was discussed. Evidence was offered to substantiate the theory that the potentiating effect of the small concentrations of azochloramid is due to their ability to inactivate the inhibitors of sulfanamide action. This phenomenon was demonstrated with a variety of sulfonamide derivatives and several species of microorganisms.
SIXTH MEETING, Rutgers University, New Brunswick, N. J., December 11, 1941

A Bacterial Growth Inhibitant—Penicillin, Its Properties, Uses

SEVENTH MEETING, Princeton University, Princeton, N. J., February 12, 1942

Use of Luminous Bacteria as a Tool in Fundamental Research. Dr. Frank H. Johnson, Department of Biology, Princeton University.

A discussion of the application of the unique properties of bacterial luminescence in the study of certain problems of general importance was reviewed, accompanied by demonstrations with the living organisms. Recent advances in the general understanding of the biological effects of temperature, hydrostatic pressure and various narcotics were described and their relation to the problem of both sulfanilamide and para-aminobenzoic acid action discussed.

EIGHTH MEETING, Rutgers University, New Brunswick, March 27, 1942

Biological Experiments with Penicillin. Clara M. McKee and Geoffrey Rake, Division of Microbiology, The Squibb Institute for Medical Research, New Brunswick, N. J.

Five cultures of Penicillium notatum produced penicillin in markedly varying degree. Cultures derived from morphologically different colonies of the same strain also varied in their penicillin production. Various media were tried but a modified Czapek-Dox containing 4% brown sugar and no glucose gave the best results. Highly active filtrates were obtained in 10 days after original inoculation and in 6 to 7 days after reflooding the mat of mold with fresh media. These filtrates had a potency approximately ten times as great as that reported by the Oxford group for their crude filtrates.

A tube dilution test has been used for determining potency and the results obtained have been referred to a stable calcium salt of penicillin having 30 Florey units per mg., used as a control standard in all tests. Streptococcus pyogenes, Staphylococcus aureus, Streptococcus viridans and the Pneumococcus Types I, II, III were susceptible to the action of penicillin, while Escherichia coli, the Friedlander bacillus, Salmonella, Streptococcus faecalis and Aerobacter aerogenes were not.

Mice infected with a Type III pneumococcus can be protected by treatment with penicillin. Less penicillin and fewer injections are needed for protection when treatment is given at the same site as the infection. The number of active units of penicillin needed for protection is independent of the degree of purity of the preparations tested.

Mice infected with sulfanamide-resistant strains of pneumococci have been protected with penicillin.

Smaller amounts of penicillin were necessary to protect mice against streptococcus C303 than were needed for one tenth the number of lethal doses of the Type III pneumococcus.

Toxicity tests in mice show that purified penicillin is relatively non-toxic. A highly purified sodium salt of penicillin containing 240 Florey units per mg., was at most only a quarter as toxic as a cruder butanol extract containing only 15 Florey units per mg.

All penicillin preparations we have tested have been non-pyrogenic.


Clorarsen is a mixture of 3-amino-4-hydroxy-phenyl dichlorarsine hydrochloride and sodium citrate. This mixture has been tested for toxicity and...
as a trypanocidal and spirochaetocidal agent in animals.

When injected intraperitoneally in mice or intravenously in rats it was found to have a toxicity (in terms of mg. of Arsenic per kg. of body weight) approximately the same as that of Mapharsen. Similarly its trypanocidal activity against Trypanosoma equiperdum in rats and its spirochaetocidal action, whether in vitro, or in vivo in rabbits was found to be at least as great as that of Mapharsen.

THE DUAL PATHOGENICITY OF PSEUDOMONAS AERUGINOSA. R. P. Elrod and A. C. Braun, Rockefeller Institute for Medical Research, Princeton, N. J.

FORMATION OF BIOLOGICALLY ACTIVE TOBACCO-MOSAIC VIRUS IN VIVO, AS AFFECTED BY NITROGEN SUPPLY. Ernest L. Spencer, Rockefeller Institute for Medical Research, Princeton, N. J.

In a continuation of the study previously reported on the influence of nitrogen on the metabolism of tobacco-mosaic virus (Marmor tabaci H.) in vivo, experiments were carried out to determine the specific biological activity of newly formed virus and to ascertain whether nitrogen limitation would hinder the full development of such virus.

In nitrogen-fed tobacco plants, virus protein, as isolated by ultracentrifugation, continued to increase in the inoculated leaf both in amount and specific activity (activity per unit of weight) for as long as 20 days after inoculation. When nitrogen was withheld shortly after inoculation, the virus-protein content increased at the normal rate for a limited period but the specific activity of this material remained fairly constant at the level reached when nitrogen was last added. These observations suggest that virus in young lesions displays only a fraction of its potential specific biological activity and that an increase in specific activity is dependent on the nitrogen supply.

Further experiments, carried out to determine whether differences other than specific biological activity could be detected between virus from young and old lesions, indicated that virus preparations from young lesions may contain particles considerably larger than those characteristic of virus preparations from older lesions.

Study of the Virus of Pseudorabies on the Chick Embryo. Frederick Bang, Department of Animal and Plant Pathology, Rockefeller Institute for Medical Research, Princeton, N. J.

Infection of the chick embryo with the virus of pseudorabies results in the production of pocks on the chorioallantoic membrane and destruction of the central nervous system. All of four strains were found to produce this reaction. Neutralization of the virus by specific hyperimmune sera can be demonstrated by inoculation on the membrane. The reaction of the chick to the virus varies with age of the embryo.

WASHINGTON BRANCH

U. S. Naval Medical School, Washington, D. C., January 27, 1942


AEROBACTER AEROGENES AND ESCHERICHIA COLI VAR. ACIDILACTICI AS CAUSES OF MORTALITY IN TURKEYS. Hubert Bunyea and Angus D. MacDonald, Pathological Division, Bureau of Animal Industry, U. S. Department of Agriculture, Washington, D. C.

An increasingly high mortality occurred for the 4 years beginning in 1936 in the turkey flock at the Animal Husbandry poultry station at Beltsville,
Maryland. Laboratory examinations failed to determine the cause.

Agglutination tests for pullorum disease were applied to the flock of 339 breeders in 1940, using a special stained antigen made from turkey strains, a regular chicken stained antigen, and also the tube agglutination test.

With the tube test 9 birds gave positive reactions at 1 to 50, 22 at 1 to 25, and 24 were partial at 1 to 25. Five of the reactors at 1 to 50 reacted positively to the rapid stained antigen (turkey strains). Two that reacted at 1 to 25 in the tube test were positive to the turkey rapid stained antigen. Afterwards the mortality among the turkeys was greatly reduced, probably as a result of the removal of the reactors.

The ovaries and testes of the reactors were examined bacteriologically. Salmonella pullorum was not recovered in any instance. Two organisms predominated, namely, Aerobacter aerogenes and Escherichia coli, var acidilactici. They were identified by biological and tinctorial characteristics. Aerobacter aerogenes was negative on the methyl-red test and positive on the Voges-Proskauer test. E. coli, var acidilactici was positive on the methyl-red test and negative on the Voges-Proskauer test.

Pathogenicity tests showed that both organisms when recently isolated from turkeys were capable, by intravenous or intraperitoneal inoculation, of producing disease and death in young or adult turkeys. Turkeys when dying of the inoculation yielded the same organism on post mortem examination. Laboratory strains of these organisms of bovine origin were not pathogenic for turkeys.

Antigenic response of bovine calf scour antiserum, equine S. pullorum antiserum, Salmonella typhimurium antiserum (rabbit), and the sera of turkeys that reacted to S. pullorum was shown to exist for antigens made from bovine and turkey strains of E. coli, var acidilactici, and also (except S. typhimurium antiserum) for antigens made from Aerobacter aerogenes (turkey strain) and S. pullorum. The serum of one adult turkey inoculated with E. coli, var acidilactici (turkey origin) responded positively to antigens made from bovine and turkey strains of E. coli, var acidilactici and Aerobacter aerogenes (turkey origin), but not S. pullorum.

Results and Implications of a Tuberculosis Case-Finding Program Among Medical Students at the George Washington University. Leland W. Parr, The George Washington University, Washington, D. C.

Eleven consecutive classes of medical students have been tested for their sensitivity to tuberculin. Beginning in 1932 the class of 1935 was 92.8% positive. In 1941 the class of 1945 was 34.0% positive and the average for all 762 students was 60.1%. Early in 1939 a case-finding program involving four steps—(a) tuberculin testing; (b) x-ray examination; (c) careful examination of suspects by physician and laboratory; and (d) sanatorium treatment was set up and seven students have since been found with minimal tuberculosis. During this time 39 students have changed from tuberculin negative to distinct positive and of these 22 made the change before entering the junior year and none during the senior year. In these three and one-half years 192 students have been graduated and of these 31 were tuberculin negative throughout their course. Of 1061 students tested in this interval 26% of those reacting to tuberculin did so only to the second injection (0.005 mg. P.P.D.). The evidence seems to show that the white adult has a very considerable resistance to tuberculosis disease, even to sensitizing infection. This resistance is not, however, the possession of all for there are students who become sensitized in the relatively benign environment of the Freshman class in contrast with those who resist infection even while juniors and seniors. The importance of tuberculosis and the fact that thus far serum, vaccine and chemotherapy approaches to its control are disappointing, make inquiry into factors of resistance unusually important.
STUDIES IN SKIN DISINFECTION: PRICE'S METHOD FOR THE EVALUATION OF MERCURIALS. A. P. Casman, U. S. Naval Medical School, Bethesda, Md.

CAPSULE FORMATION IN GENUS BRUCELLA. Cornelia Cotton, Maryland

PRODUCTION OF EXPERIMENTAL INFLUENZA IN MICE BY MEANS OF EXPOSURE TO AIR-BORNE VIRUS AND ITS PREVENTION BY PROPYLENE GLYCOL VAPOR. Clayton G. Loosl, O. H. Robertson, and Theodore T. Puck, Department of Medicine, University of Chicago.

Influenza pneumonia can be readily produced in mice by exposing them to atmospheres containing small quantities of virus (PR-8 strain). The pneumatic lesions resemble in every way those resulting from intranasal instillation of the virus. Three hundred and fifty-two mice placed in 60-liter chambers, from five minutes to one hour, into which were sprayed 0.2 to 1.5 ml. of a 10^-3 dilution of lung virus suspension, all died from three to 13 days later with extensive consolidation of the lungs. The average survival time for 112 mice exposed to 0.6 to 1.5 ml. of virus was 5 days, while that of the 240 mice which were exposed to 0.2 to 0.4 ml. of virus, was 7 days. Mice exposed for 2 minutes to 60-liter atmospheres, containing approximately 0.30 ml. of 10^-3 dilution of virus, all died within 7 to 10 days. Some of the mice survived when exposed to the same quantity of air-borne virus for shorter intervals. Fifteen-second exposures, however, resulted in extensive consolidations in all the animals, and death in a few cases. The majority of mice exposed to 0.30 ml. amounts of a 10^-3 dilution of virus for 15 minutes, survived. No deaths occurred among the mice exposed to a similar quantity of 10^-4, 10^-3 and 10^-2 dilutions of virus, and increasing numbers also showed no lesions. The virus remained viable in the air for one hour after spraying. Propylene glycol vapor in concentrations of one part by weight to two to seven million parts by volume of air completely protected mice against lesions as well as death.

PREVENTIVE MEDICINE IN THE ARMY. Hugh R. Gilmore, Sixth Corps Area Laboratory, Fort Sheridan.

Preventive medicine is one of the important activities of the medical department of the army. Colonel James S. Simmons, M.C., is in charge of this work and has organized the preventive medicine section of the Office of the Surgeon General.

All troops are being immunized against typhoid fever, small pox, tetanus and yellow fever. Troops are also being immunized against typhus fever, cholera and plague before being sent to areas where these diseases are prevalent.

The typhoid vaccine is made by the Army Medical School in one of the largest laboratories of its kind in the world. The yellow fever vaccine is prepared in the laboratories of the Rockefeller Foundation. The typhus fever vaccine is prepared by the yolk sac method of Cox.

The laboratories of the Army Medical School also prepare a vaccine for protection of army horses against equine encephalomyelitis.

Epidemiological laboratories have been established in each corps area and at every army post a medical inspector is responsible for making recommenda-
tions concerning the sanitation of the post and the prevention of disease in the personnel.

The Surgeon General has appointed a civilian Board for the Control of Influenza and other Communicable Diseases in the Army, headed by Dr. Francis G. Blake. Commissions for the study of various communicable diseases will function under this board. By this means it is hoped to secure the best talent available for the study of epidemiological problems. Methods of recruiting commissioned medical personnel were also discussed by the speaker.