tics, representatives of the halogenic, mercurial, silver salt, phenolic, and quaternary ammonium compounds. The most favorable findings among the aqueous solutions of antiseptics were obtained with iodine (aqueous 2 per cent). The toxicity index was 0.0125. Among the tinctures, iodine (2 per cent in diluted alcohol) revealed the lowest toxicity index, 0.18. The mercurial and phenolic compounds displayed high toxicity indices.


Thermophilic cellulose-decomposing bacteria have been obtained in pure culture. They have maintained their ability to digest cellulose through many transfers in cellulose as well as in sugar media. One culture ferments cellulose and cellobiose but does not utilize glucose. The nutritional requirements and the fermentation products have been studied.


The collection of airborne particles impinged upon an agar surface by a high-velocity jet has been carefully studied by Bourdillon, Lidwell, and Thomas, in designing their slit sampler. The smallest bacteria-laden particles are collected with jet velocity of 270 feet per second. Efficiency decreases rapidly with decreasing jet velocity. An air centrifuge attached to the sieve modification of the slit sampler collected from the air passing the sieve many times more droplet nuclei, atomized from a 1 per cent suspension of a broth culture by a Walton humidifier, than were collected by the sieve. The efficiency of the sieve relative to the centrifuge, however, was increased by increasing air flow one-half. Droplet nuclei produced by natural sneezing have been studied by the sieve-centrifuge combination. Alpha hemolytic streptococci normally present in a nasopharynx were collected by the sieve on gentian violet blood agar plates and by the centrifuge in Difco air enrichment broth. Aliquots of the broth were divided into sterile tubes and the streptococci estimated by the dilution method, as in water analysis. Bacteria-laden nuclei sneezed into the air are somewhat larger than Escherichia coli suspended by the water humidifier. For field work in sanitary ventilation, this instrument is now being built with a small fan blower. Apparatus and results are exhibited.


In order to determine the antibacterial activity of intranasally applied antibacterial agents, it is necessary to employ a uniform method of sampling the nasal flora at repeated intervals. The usual method of studying the types of
bacteria in the nasal cavity by inserting and culturing a cotton swab is not satisfactory for a quantitative determination of the number of bacteria present. To obtain a more accurate enumeration of bacteria in the nasal cavity the following method was used: A simple sampling device consisting of a test tube with an opening in the bottom which is connected to a nasal "olive" by rubber tubing was used to irrigate the nasal cavity with Ringer's solution. The washings were diluted quantitatively and cultured in blood agar plates. Each sample consisted of three consecutive washings made at 5-minute intervals which were pooled. The reliability of the method was tested by determining the nasal bacterial counts of five healthy subjects before, during, and after the application of penicillin nose drops. No carrying over of penicillin into the culture plates was detected. Although considerable daily variations in the counts on the same subject were found, the method was satisfactory for demonstrating the effectiveness of intranasally applied antibacterial agents. This method permits a qualitative as well as a quantitative examination of the nasal flora. While penicillin nose drops produced a marked reduction in the nasal bacteria of healthy subjects, this is not to be construed as a direct or implied indication of the efficacy of penicillin nose drops in nasal, sinus, or other respiratory infections.


Mary Ruth Smith and W. Barry Wood, Jr., Washington University School of Medicine and Oscar Johnson Institute for Medical Research, Department of Medicine, St. Louis, Mo.

Results of a recent study of experimental pneumonia reveal, contrary to the accepted immunological concept, that phagocytosis of virulent encapsulated bacteria can occur in the absence of antibody, with subsequent bactericidal effect. This nonantibody mechanism operates in pneumonic, normal, and formalin-fixed lungs, on surfaces of tissues and inert materials as filter paper, but not on smooth surfaces such as glass. No intermediary opsonin is involved, the phagocytosis depending upon the physical properties of the surface contacted by the leucocytes. Direct visualization reveals that the "surface phagocytosis" depends upon leucocytes' trapping the bacteria against the surface. Intercellular "surface phagocytosis" can be demonstrated by merely concentrating leucocyte-bacterial mixtures by centrifugation. Under these conditions direct visualization reveals that the encapsulated bacteria are trapped between the surfaces of adjacent cells and are thus phagocyted in absence of opsonin. "Surface phagocytosis" of pneumococci and Friedlander's bacilli brings about rapid killing of the organisms. Digestion of intracellular pneumococci can be observed directly, and phagocyted Friedlander's bacilli, even when liberated from the cytoplasm of the leucocytes within 30 minutes, are nonviable. The nonantibody phagocytic mechanism appears to play a significant part in the recovery mechanism in both pneumococcal and Friedlander's pneumonia. Preliminary experiments indicate that "surface phagocytosis" operates not only in the lungs but also in other organs, thus constituting an important natural defense of body tissues in general against invasion by encapsulated bacteria.
M4. The Relationship Between Ascorbic Acid and Phagocytic Activity. Ada May Ames and W. J. Nungester, University of Michigan, Department of Bacteriology, Ann Arbor, Mich.

Results of a study of the relationship between ascorbic acid content of exudative polymorphonuclear leucocytes from guinea pigs and the fragility and phagocytic activity of these cells are presented. A parallel is also drawn between the level of vitamin C in the exudative cells and the quantity and quality of exudate obtained following injection of an irritating substance into the peritoneal cavities of guinea pigs. During these studies the vitamin C content of the exudate varied between 0 and 1.25 mg per 100 ml of exudate. Within these limits, with serum present in the system, the percentage of cells showing phagocytosis bears a direct ratio to ascorbic acid content. With levels from 0 to 0.25 mg per 100 ml, only 30 to 35 per cent of the cells show phagocytosis; with 1 to 1.25 mg per 100 ml of exudate, 80 to 90 per cent of the cells are active. Serum in the system, not only promotes phagocytic activity, but also tends to protect the cells from rupture. The amount necessary to afford such protection to the cells bears an inverse relationship to the vitamin C content of the cells. When the ascorbic level equals 0 to 0.25 mg per 100 ml, at least 15 per cent serum is necessary; with 1 to 1.25 mg only 5 per cent is needed to prevent rupture of the cells.

When serum is lacking from the system, the fragility of the cells bears an inverse relationship to the ascorbic acid level of the exudate. With 0 to 0.25 mg present 90 to 95 per cent of the leucocytes were ruptured, while a 1 to 1.25 mg level resulted in only a 5 to 10 per cent rupture of the phagocytic cells.

M5. The Inhibitory Action of Saliva on the Diphtheria Bacillus: Hydrogen Peroxide, the Antibiotic Agent of Salivary Streptococi. Richard Thompson and Ann Johnson, University of Colorado School of Medicine, Department of Bacteriology, Denver 7, Colorado.

Since the inhibitory action of saliva on Corynebacterium diphtheriae was shown to be due to viridans streptococci, we studied the role of hydrogen peroxide in this inhibition and the role of catalase in the staphylococcal antagonism of it. Drops of materials to be tested were placed on four plates containing diphtheria bacilli. Hydrogen peroxide was detected by benzidine or o-tolidine and potato. Catalase was detected by breakdown of hydrogen peroxide and recognized by gas production or by titration by permanganate. Seventy-one strains of salivary streptococci produced peroxide and inhibited diphtheria bacilli. The degree of inhibition was correlated with the concentration of peroxide produced. Several strains grown in deep broth produced no peroxide and filtrates of these cultures were not inhibitory. The same cultures, shaken for 10 minutes while exposed to air, produced large amounts of peroxide and their filtrates inhibited the bacilli. Seventeen strains of streptococci produced neither detectable peroxide nor appreciable inhibition. Thirty-four strains of staphylococci produced neither inhibition nor peroxide but produced catalase and antagonized the inhibitory actions of saliva and streptococci. Potato juice and lysed erythrocytes also antagonized these actions. Additional strains of diphtheria bacilli, pathogenic and nonpathogenic staphylococci, and enteric bacilli were tested.
for inhibition by saliva, streptococci, and peroxide. The order of sensitivity was the same to all three agents, diphtheria bacilli being the most sensitive and enteric bacilli the least. The inhibitory action of saliva against diphtheria bacilli is largely due to hydrogen peroxide produced by the salivary streptococci.

M6. Pork as a Factor in the Antigenicity of Avirulent Diphtheria Bacilli. 

ELAINE 
L. UPDYKE AND MARTIN FRIBISHER, JR., Johns Hopkins University, Department of Bacteriology, School of Hygiene and Public Health, Baltimore, Md.

In several experiments at different times 94 rabbits were repeatedly inoculated with living cultures of avirulent Corynebacterium diphtheriae. Twenty-two animals received organisms which had been cultivated in pork infusion broth. Of these 22, 8 (36 per cent) survived a subsequent challenge dose of virulent C. diphtheriae which was uniformly fatal to nonimmunized animals. The other 14 animals survived an average time of 5.3 days as contrasted to the 2.9 average survival time of 31 control (nonimmunized) animals. Seventy-two of the 94 animals received inoculations of avirulent C. diphtheriae cultivated on media not made with fresh pork, especially veal infusion broth. Of these 72 animals, none survived the challenge dose of virulent C. diphtheriae, and their average survival time (3.2 days) was essentially the same as that (2.9 days) of the 31 control animals. These results suggested that pork contains some factor which is critical for the protective antigenicity of avirulent diphtheria bacilli in regard to virulent diphtheria bacilli. Further experiments demonstrated that this factor is apparently not thiamine. The average survival time of 20 rabbits receiving low thiamine antigen was 3.4 days; that of 13 rabbits receiving high thiamine antigen was 2.5 days.

M7. Fractionation of Hemolytic Streptococci by High-Speed Centrifugation, and Complement-Fixation Tests Between Nucleoprotein Constituents and Sera of Rheumatic Patients. 

T. N. HARRIS, University of Pennsylvania (The Children's Hospital of Philadelphia), Department of Pediatrics, Philadelphia, Pa.

As the beginning of an analysis of the relation of the hemolytic streptococcus to rheumatic fever, group A hemolytic streptococci have been disintegrated by sonic vibration and then separated into chemically and serologically distinct parts by centrifugation at appropriate speeds, without use of acid or alkali. Three fractions have been obtained: (1) the low-speed sediment containing the hulls of the organisms, with the type-specific M-protein present; (2) cytoplasmic particles, largely sedimented at 15,000 rpm, which contain species-specific nucleoprotein and the group-specific carbohydrate; (3) a nucleoprotein, distinct from that of the cytoplasmic particles, not sedimented by speeds of 32,000 rpm. Each of the two somatic nucleoproteins have been separated into protein and nucleic acids, and the latter, which are serologically inert, have been identified. Both of the nucleoproteins are antigenic, and complement-fixation tests have been carried out with each against sera of immunized rabbits,
normal human beings, patients with acute streptococcal disease, and patients with rheumatic fever in various stages of activity. Parallel determinations of the antistreptolysin titers have been made with these sera.

M8. Experimental Streptococcal Infections. II. A Study of Spreading Factors Produced by Hemolytic Streptococci. Noble P. Sherwood and Barbara E. Russell, University of Kansas, Department of Bacteriology, Lawrence, Kans.

In a previous communication one of us reported upon the ability of representative strains of the various Lancefield groups of hemolytic streptococci to spread within the mesoderm of the C-A membrane of the embryonic chick. The present paper is a report of our studies of the filtrates of these and other hemolytic streptococci, representing nine Lancefield groups, to determine whether they could reduce the viscosity of hyaluronic acid and could spread within the dermis of albino rabbits. The effect on the streptococci of animal passage was investigated. The results may be summarized as follows: Filtrates possessing the property of reducing the viscosity of hyaluronic acid solutions were found only among cultures of some members of Lancefield groups A, B, and C, the majority of group A strains giving negative results. All filtrates that reduced the viscosity of hyaluronic acid spread rapidly when injected into the skin of rabbits. A number of filtrates of organisms belonging to groups A, B, C, and D did not reduce viscosity of hyaluronic acid solutions but did spread slowly in the skin of rabbits. When strains were passed through mice and rats, it was observed that occasionally there was an apparent loss of ability to reduce the viscosity of hyaluronic acid solution although there was frequently an apparent acquired ability to spread within the skin. Cyanide, iodine, fluoride, peroxide, and shaking did not inactivate hyaluronidase. Heating to 50 C for 1 hour completely inactivated the bacterial enzyme and reduced the activity of the testicular enzyme.

M9. The Collagenase Activity of Culture Filtrates of Clostridium histolyticum. Marshall W. Jennison, Syracuse University, Department of Plant Sciences, Division of Bacteriology, Syracuse, N. Y.

In a previous paper on the action of several bacterial species on collagen, it was noted that bacteria-free filtrates from cultures of Clostridium histolyticum showed a high collagenase activity. The present report deals with this activity quantitatively, with regard to age of culture and rate of digestion of the collagen. Culture filtrates of different ages were prepared by growing the organism in liquid thioglycolate medium (without glucose or agar) at 37 C for 24, 48, 72, and 96 hours, and filtering through a Seitz filter. The collagen substrate consisted of fine strands of clean, unprocessed beef tendon (about 85 per cent collagen), both unsterilized and sterilized (dry heat). Ten ml of culture filtrate (pH 7.2) and about 300 mg of tendon were used for each activity determination. The mixtures were incubated with and without toluol at 37 C for 24, 48, 72, and 96 hours, then filtered through quantitative filter paper, and the undissolved
collagen was weighed after drying. Enzyme activity was measured by digestion of the collagen, expressed as percentage loss in weight. The results show that filtrates from 24-hour cultures have a greater collagenase activity than those from older cultures. With 24-hour culture filtrates, a typical series gave 60, 83, 90, and 90 per cent of the collagen digested after 24, 48, 72, and 96 hours, respectively.

M10. Streptococcal Content of Lungs of White Mice Infected with Streptococcus hemolyticus After Influenza Virus. HAROLD N. CARLISLE AND N. PAUL HUDSON, The Ohio State University, Department of Bacteriology, Columbus 10, Ohio.

The observation that influenza virus infection increased the susceptibility of white mice to Streptococcus hemolyticus (group C) was confirmed. Mice that had been inoculated with killed or viable influenza virus were inoculated subsequently with hemolytic streptococci and sacrificed 24 hours later. The bacterial content of their lungs was determined by plate count. In one set of experiments, the interval between viral and streptococcal inoculations was 2 days, the virus dosages were 0.2, 1.0, and 100 MLD, and the coccal dosage was 100 MLD. Both inocula were given by intranasal instillation under ether anesthesia. The streptococcal count was 10 times greater in those mice that previously received active virus than in those inoculated with killed virus, without respect to dosage of virus. In another set of experiments, the interval between inoculations was varied from 0 to 24 days, the virus dose was 0.2 MLD, and the streptococcal inoculum was 100 MLD. Tenfold differences in the bacterial count were found in those groups of mice inoculated at intervals of 4, 8, and 12 days between agents; and fewer or no differences were found in those inoculated at other intervals. These data provide additional information relative to the period of increased susceptibility to the streptococcus arising as a result of sublethal influenza virus infection.

M11. An Experimental Investigation of the Pathogenicity of Diphtheroids Isolated from the Human Conjunctiva. CHARLES WEISS AND MARION C. SHEVKY, Laboratory for Ophthalmic Research, Mount Zion Hospital, San Francisco, Calif.

Diphtheroids resembling Corynebacterium xerose that were isolated from the human conjunctiva grew well in a menstruum of mucin. Similarly, when suspended in this medium and inoculated into the anterior chamber of the eyes of albino rabbits, cultures retained their viability for several days, while in salt solution they were rapidly destroyed. Intracocular injections of albino rabbits with diphtheroids suspended in saline produced moderate inflammation of the uveal tract. When suspended in mucin, the reaction lasted longer, was much more severe, and was associated with an acute keratitis. The lesions usually regressed spontaneously within 2 or 3 weeks. While killed cultures of diphtheroids in saline produced mild inflammatory changes in the ciliary process which were seen in histologic sections, none were visible grossly. In a menstruum of mucin the inflammatory reaction was more severe. Since mucin by itself was relatively
innocuous, it is suggested on the basis of these studies and those of others that it protects bacteria from the digestive action of endocellular proteolytic enzymes and other immunologic defense mechanisms. Living diphtheroids are thus permitted to grow and exert their pathogenic activity. By applying the recently developed principles of Mueller and Miller and Pappenheimer and Johnson, it was possible to demonstrate that a filtrate which is produced by growing diphtheroids in a medium of very low iron concentration is injurious to the uvea and cornea, but not to the skin or conjunctiva, of albino rabbits. On the basis of these investigations, it may be concluded that diphtheroids which are present on the normal or the inflamed human conjunctiva may be considered as potential pathogens which may exert injury when they are introduced into the interior of the eye.

M12. The Production of Salmonella Osteomyelitis in White Rats. J. Emerson Kempp and Theodore A. Fox, University of Illinois College of Medicine, Department of Bacteriology and Public Health and Department of Orthopedic Surgery, Chicago, Ill.

Osteomyelitis in man caused by Salmonella typhimurium has been reported with increasing frequency. Therefore, it seemed advisable to produce salmonella osteomyelitis in a laboratory animal such as the white rat in order that more extensive studies of the disease might be made. The culture of Salmonella typhimurium used in these experiments was isolated from a bone lesion of a patient with chronic osteomyelitis and identified by serological methods. An incision was made over the proximal portion of the tibia of a white rat and a 2-mm opening was made into the bone marrow. A small pledget of cotton which had been immersed in a 24-hour broth culture of S. typhimurium was inserted into the bone and the incision was closed. Control animals received an insertion of cotton and sterile broth into the tibial bone marrow. The animals were observed for 21 days, at the end of which period the animals were sacrificed, and the lesions, the liver, and spleen were cultured; gross and microscopic examinations of the tibia were also made. Of 15 rats inoculated, 14 developed typical microscopical lesions of osteomyelitis and positive cultures of S. typhimurium. The spleens of three animals yielded the same organism. Twelve rats used as controls had no bone lesions, 11 had negative cultures, and 1 had a gram-positive coccus. It is concluded that osteomyelitis caused by S. typhimurium can be readily produced in white rats and that the rat is a suitable animal for the study of this disease.


Monkeys were fed cultures of mouse-virulent strains of Shigella dysenteriae (Shiga) in doses of 2 to 100 billion bacteria. Shiga organisms were recovered from stools on the following day only. No monkeys seemed ill. Other monkeys were injected intraperitoneally with Shiga cultures in mucin in doses of 10
million to 20 billion bacteria. Those receiving 10 million were unaffected. Those receiving 100 million showed malaise for 2 days but recovered. Those receiving 20 billion developed an acute hemorrhagic peritonitis and died within 24 to 72 hours. *S. dysenteriae* was recovered from heart blood and various organs with these fatal doses, but not from the blood of any that survived. Other monkeys were fed undiluted Shiga toxin. An increased excitability, leg muscle weakness, and, in one case, tetanic convulsions were the only effects produced. Monkeys injected with relatively high dilutions of toxin intravenously showed a characteristic picture. An incubation period of at least 48 hours was followed by weakness of the legs and by tetanic convulsions if the animal was exercised. In fatal intoxications the state of convulsions was followed by an ascending spastic paralysis, prostration, and death. Death occurred in from 48 hours to 4 to 5 days as a rule. At autopsy these animals showed hemorrhagic petechiae on the skin and hemorrhage into the lymph nodes, peritoneum, and walls of large intestine. Most conspicuous was hemorrhage into the heart muscle. Histological sections showed focal hemorrhages into many tissues, especially into heart muscle and adrenals. Sections from brain and cord were essentially negative. Apparently Shiga toxin is relatively innocuous to the unbroken intestinal mucosa but a small amount parenterally can produce a characteristic and rapidly fatal syndrome.

**M14. The Potential Pathogenicity of Bacillus cereus and Its Relationship to Bacillus anthracis.** KENNETH L. BURDON, Baylor University College of Medicine, Department of Bacteriology and Immunology, Houston, Texas.

The close similarity between *Bacillus anthracis* and *Bacillus cereus* extends to the possession of a definite pathogenicity for laboratory animals on the part of the latter organism. There is evidence also that *B. cereus* may cause anthrax-like lesions in man. Nevertheless, distinctive properties of *B. cereus* serve to differentiate it from both virulent and nonvirulent strains of the anthrax bacillus. Moderate doses of active, young cultures of *B. cereus* (which are markedly hemolytic) may cause local hemorrhagic and granulomatous lesions, or death within a few hours, in rabbits and guinea pigs. When broth cultures of such active strains are inoculated intraperitoneally into mice, in very small doses, corresponding to the minimal dose of freshly isolated, virulent anthrax bacilli required to cause death in 18 to 24 hours, they may produce an overwhelming septicemia, so that the mice die within 6 hours. Mice injected subcutaneously usually develop a large ulcer at the site of inoculation. These pathologic effects of *B. cereus* are enhanced by the hemolytic activity of the cultures, but they are due primarily to a true infective process; virulence is increased by animal passage. However, this pathogenic action still differs from the characteristic course of anthrax infection, and at autopsy the organisms, though numerous in the tissues, fail to show capsules. Attenuated strains of *B. anthracis* may not be identifiable by animal inoculation, but they are still distinguishable from *B. cereus* by differences in growth temperature limits, sensitivity to penicillin, hemolytic power, and several other easily demonstrated properties.

A true linear relationship has been shown to exist between the concentration of virus inoculated into the yolk sac of embryonated eggs and the resulting average day of death (A.D.D.) of the embryos. From composite data it has been possible to establish a plotted line from which an estimate of the LD₅₀ value of a virus suspension can be made by determining the A.D.D. of embryos inoculated with a single dilution and candled at regular 24-hour intervals. Lines have thus been plotted for two psittacosis viruses (6BC and Gleason strains), meningopneumonitis virus (Cal 10 strain), and Louisiana pneumonitis virus (Borg strain). The error of LD₅₀ estimates by this method is shown to be approximately the same as that which obtains in the standard titration method employing 10 to 20 eggs per dilution. Either 8- or 9-day-old embryos may be used with no significant change in titer resulting. The advantages of this method are: (1) fewer eggs and less time are required; (2) titrations are usually completed within 4 to 6 days instead of the usual 10; and (3) end points are not missed through improper choice of dilutions.


Earlier studies had shown that a complement-fixing yolk sac antigen for lymphogranuloma venereum was soluble in ether. This study is concerned with the purification and the properties of the various fractions obtained by ether extraction of yolk sac antigens for the psittacosis lymphogranuloma venereum group of viruses. A high degree of purification was effected by successive extraction of the ether extract with acetone and methyl alcohol. The acetone- and alcohol-soluble fractions were inactive. The acetone-insoluble, alcohol-insoluble fraction, inactive as such, was fully activated by the addition of optimal amounts of lecithin of ether origin. The ether-soluble antigen in the form of a saline suspension showed no loss in reactivity when stored at refrigerator temperature for more than 18 months. Chloroform extracts of infected yolk sac suspensions were similar to the ether extracts in that most of the activity was in the extract. Benzene and petroleum ether extracts of yolk sac suspensions showed little or no activity, whereas the suspensions after extraction showed markedly enhanced activity. No specificity within the psittacosis lymphogranuloma venereum group could be demonstrated with any of the fractions studied.

M17. The Transmission of the Virus of Lymphocytic Choriomeningitis by Trichinella spiralis. J. T. Syvertson, O. R. McCoy, and J. Koomen, Jr., University of Rochester School of Medicine and Dentistry, Department of Bacteriology, Rochester 7, N. Y.

The purpose of this laboratory investigation was to open further avenues for
the epidemiological investigation of natural virus transmission and of virus survival during interepidemic periods. The experiments were designed to learn whether the nematodal parasite, *Trichinella spiralis*, could act as an intermediary for the maintenance and transfer of the virus of lymphocytic choriomeningitis from one host to another. *T. spiralis* and lymphocytic choriomeningitis were selected because each is representative of a large class of closely related agents, each has a wide host range that includes man, and each is cosmopolitan in distribution. Conclusive results were obtained in three out of six experiments in which guinea pigs were infected concurrently with the virus of lymphocytic choriomeningitis and *T. spiralis*. They showed that trichinella larvae, after maturation in the muscles, had acquired the virus and were capable of transmitting it to new hosts. Transmission resulted when living larvae were fed to normal guinea pigs and when triturated dead larvae were injected. Virus harbored within the body of the larvae rather than virus on their outer surfaces was responsible for transmitting the infection. The results indicate that *T. spiralis* may act under experimental conditions as an efficient vehicle for the maintenance of the virus of lymphocytic choriomeningitis, for its penetration through natural barriers, and for its transmission to new hosts. The relationship of these results to the natural spread of lymphocytic choriomeningitis is unknown.

**M18. Isolation of the Virus of Herpes Simplex from Six Cases of Kaposi's Varicelliform Eruption.** ISAAC RUCHMAN, KATHARINE DODD, AND ASHTON WELSH, The Children's Hospital Research Foundation and The University of Cincinnati College of Medicine, Departments of Bacteriology, Pediatrics and Dermatology and Syphilology, Cincinnati, Ohio.

Herpes simplex virus was recovered by inoculation of the rabbit's cornea with material obtained from the skin lesions of three children and three adults presenting the syndrome of Kaposi's varicelliform eruption. The presence of intranuclear inclusion bodies of the herpetic type in the lesions of the rabbit cornea and the demonstration of cross immunity in animals with a known strain of herpes virus (H. F. strain) proved the identity of the agent recovered. An increase in antibodies against the homologous strain of virus was demonstrated in the sera of all three children and of one adult patient during convalescence. Antibodies were absent in the serum of one fatal adult case. In the serum of the third adult antibodies were found during the acute phase of the illness; no rise in antibody titer subsequently occurred. All six patients had been in contact with individuals suffering from labial herpes 5 to 10 days preceding the onset of this varicelliform eruption but gave no history of exposure to vaccine virus.

**M19. A Quantitative Method for the Assay of Influenza Virus Vaccine, Influenza Serum, and Complement.** JAMES W. FISHER, Department of National Health and Welfare, Laboratory of Hygiene, Ottawa, Canada.

Studies were made to develop a method that may be employed for the quanti-
tative evaluation of influenza virus vaccine, influenza serum, and complement. The activity of one or more specimens of these materials may be assayed in terms of standard preparations by the use of an in vitro assay method. When influenza virus vaccines or sera are titrated by the method of Hirst and Pickels, a straight line is obtained by plotting either probit or logit percentage maximum sedimentation of the cells against the logarithm of the dilution of the test reagents. By this procedure one may determine the relative value of two preparations and estimate the degree of precision of the results. For example, a serum having a theoretical strength of one-third of the reference standard was found by actual test to have a potency of 35.0 ± 4.1 per cent of the latter at P = 0.05. Similarly, in the assay of complement in the complement-fixation test, a linear dosage response relationship may be used by plotting either probit or logit percentage hemolysis against the logarithm of the amount of complement. By experiment a preparation of complement was found to be 50.7 ± 1.4 per cent (P = 0.05) of the standard rather than the theoretical 50 per cent. The hemolysin was assayed by the slope ratio method. In tests using positive and negative typhus or encephalitis antigens and sera, parallel lines were obtained that differed significantly in position. Smaller amounts of antibody were assayed by this method than by other complement-fixation techniques.


With the desire to use the techniques of chick embryo propagation and the Hirst and Pickels’ method of determination of influenza virus for studies on virus inhibitors, a study of these methods has been made. To increase the accuracy of the virus determination and to speed the calculation of results, it has been found desirable to use a daily virus hemagglutination standard curve. With a standardized procedure for the production of influenza virus in the chick embryo, the standard deviation of titer values ranged from 25.5 to 69 per cent of the average titer for replicate embryos, and was 16 per cent of the average titer obtained from pooled lots of allantoic fluid from groups of 10 to 20 embryos. The mechanism of virus multiplication in the embryo is remarkably unaffected by the addition of foreign substances. The addition of relatively high concentrations of malonate and thiourea into the allantoic cavity of the embryo caused a reduction in virus multiplication, while cyanide, fluoride, and oxalate were ineffective. The large inherent variation in replicate embryos is demonstrated by the large variations in pH, volume, and uric acid content of the allantoic fluid. The variation in pH is partially explained by the low buffer power of the fluid between pH 7.5 to 8.5. The retarding of embryo development by virus multiplication is indicated by the lower uric acid concentration in the allantoic fluid of infected embryos. The relationship between pH and virus concentration of infected allantoic fluid will be discussed.

Active and inactive influenza virus was used for interaction studies with chick red cells and human red cell ghosts. Observations with the electron microscope were made during the various stages of interaction, demonstrating absorption of virus particles on red cell surface and agglutination phenomena. Studies were made also on aggregation and disintegration of virus particles as a function of time. It was observed that virus particles in filament form (possibly due to polymerization) were absorbed on the red cell surface.

M22. Preliminary Studies on the Identification of the Principle in Chorioallantoic Fluid Responsible for the Agglutination of Some Strains of Staphylococcus aureus. EDWARD W. SHRIGLEY and ESTHER S. MACULLAR, Yale University School of Medicine, Departments of Bacteriology and of Pathology, New Haven 11, Conn.

Chorioallantoic fluid from normal hens' eggs as well as that from eggs infected with influenza virus A (PR8) will agglutinate certain strains of Staphylococcus aureus. The present study is an effort to determine the nature of the agglutinating principle in these fluids. It has been demonstrated that virus-infected fluids possess a greater capacity to elicit the phenomenon than normal (non-virus-infected) materials. However adsorption and elution studies suggest that the virus per se has nothing to do with this reaction. The agglutinating principle is thermolabile, being destroyed at 56°C in 2 to 5 minutes. Its activity is favored by an alkaline reaction, but acidification does not destroy the principle, since an increase in pH will restore activity. The substance is not dialyzable either through cellophane or collodion membranes. It is insoluble in ether and acetone, and appears to be inactivated by alcohol. Evidence suggests that the agglutinating principle will precipitate out with ammonium sulfate saturation of the fluid; it appears to be present in the white sediment which forms either on long standing or following freezing and thawing. It is possible to concentrate the substance by lyophilization and ultrafiltration. The effect of proteolytic enzymes on the principle is now under study.

M23. Nonspecific Inhibition of Virus Hemagglutination. W. F. FRIEDEWALD, E. S. MILLER, and L. R. WHATLEY, Emory University School of Medicine, Department of Bacteriology and Immunology, Atlanta, Ga.

The agglutination of red blood cells by certain viruses may be inhibited by a variety of materials not containing specific antibody, such as normal animal sera and allantoic fluid. In a study of this nonspecific inhibition reaction, sera and tissue extracts of human and animal origin were tested by means of a modified Salk method, using mumps virus and the PR8 and Lee strains of influenza virus. Inhibition of hemagglutination was obtained in high titer with saline extracts of organs (lungs, liver, kidney, spleen) from human autopsies and from normal rabbits. The serum inhibition titers were invariably less.
Saline extracts of human and chicken red blood cells caused inhibition in high titer, in contrast to low titers found in sheep and rabbit red cell extracts. When the virus receptor substance was removed from human and chicken red cells by Hirst's method of adsorption and elution, extracts of these cells no longer contained the inhibitory substance. Furthermore, some virus was released from its union with the inhibitory substance after incubation for 6 hours at 22 C or 37 C. The substance did not neutralize influenza virus in mice and it failed to fix complement in mixture with influenza or mumps virus. The findings indicate that the inhibitory factor is the virus receptor substance which has been released from cells.


The Hirst method for determination of influenza virus has been primarily applied to the quantitative assay of the virus and the estimation of antibody titers. In the search for substances possessing qualities of inhibiting influenza virus, the Hirst method has been adapted and amended for the evaluation of such virus-inhibiting substances in the chick embryo. The tests were conducted with a substance so far not isolated or identified, and in concentrations which are unknown and probably were not constant in the various determinations. Inhibition both in vitro and in vivo has been quantitatively determined in a significant number of instances. A quantitative relation has been established and measured by this method between the inhibitive qualities of an antiviral substance and influenza virus at various degrees of concentration. The unknown substance is obtained as the metabolic fermentation product of a microorganism isolated from soil, but not as yet identified. Results are reported in tables and graphs from more than 250 fermentations.

M25. Inhibition of Hemagglutination and of Multiplication of Influenza Virus by Certain Polysaccharides. D. W. Woolley and R. H. Green, Rockefeller Institute for Medical Research, Department of Physiology, New York 21, N. Y.

If one assumes, as Hirst has done, that in causing hemagglutination influenza virus behaves as an enzyme which attacks a special substrate in the red cell, then it should be possible to inhibit the action of the virus on the erythrocyte by adding a suitable structural analog of this substrate. Since present evidence indicated that this substrate in the cell was carbohydrate in nature, a number of carbohydrates were tested for ability to prevent hemagglutination by influenza A virus. When judged by the appearance of the sedimented erythrocytes, several polysaccharides such as apple and citrus pectins, flax seed mucilage, myrrh, and gum acacia prevented the formation by the virus of characteristic patterns of agglutinated erythrocytes. Many other complex or simple carbohydrates were unable to do this, e.g., starch, oxidized starches, galacturonic or cellobiuronic acids, etc. The most effective agents were polygalacturonides, although complexes containing glucuronic or mannuronic acids had some potency. The
action appeared to be due to an exclusion of the virus from the erythrocyte, presumably by competition. Since it was assumed that hemagglutination is analogous to the initial stage of infection, the study was extended to a more complex system, namely, the infection of embryonated eggs. Apple pectin was found to inhibit or prevent multiplication of influenza A virus in the allantoic sacs of 10-day eggs. Thus, embryos injected with pectin and virus showed little or no multiplication of virus, as measured by both hemagglutination and infectivity.

M26. Effect of Acridines on the Growth of Influenza A and B Viruses. A. F. Rasmussen, Jr., Julia C. Stokes, Harry A. Feldman, and Joseph E. Smadel, Army Medical Department Research and Graduate School, Department of Virus and Rickettsial Diseases, Washington 12, D. C.

A preliminary report from this laboratory has been published on the inhibiting action of 2,3-dimethoxy-6-nitro-9-(diethyl-amino-oxypropyl)aminoacridinedihydrochloride, nitroakridin 3582, on the growth of type B (Lee) influenza virus in embryonated eggs. Further experiments in which parallel infectivity and red cell agglutination titrations were made on allantoic fluids taken from treated eggs at intervals after inoculation with Lee influenza virus confirmed and extended the earlier observations. The administration of 0.5 to 1.0 mg of nitroakridin 3582 prior to inoculation with 10 minimal infecting doses of virus resulted in varying degrees of suppression of virus growth. Virus was either absent or present in low concentrations in the allantoic fluids of most treated eggs throughout the period of observation, 48 to 72 hours. In certain eggs the appearance of virus was delayed 8 or more hours, but it ultimately reached a concentration equal to that in untreated controls. Nitroakridin 3582 has a similar but less pronounced effect on type A (PR8) influenza virus infections in embryonated eggs. A group of related compounds have also been investigated and rutenol, 2-nitro-5-aminoacridine, and atabrine have been found to have some viristatic activity when tested against the influenza viruses. Nitroakridin 3582 is somewhat viricidal in vitro but this is insufficient to account for the degree of inhibition observed in vivo.

M27. Nonbacillary Forms of Mycobacterium tuberculosis and Mycobacterium leprae. Eleanor Alexander-Jackson, Cornell University Medical College, Department of Public Health and Preventive Medicine, New York 21, N. Y.

In 1945 the writer described a hitherto undemonstrated form of Mycobacterium tuberculosis as revealed in both unstained cultures and material stained by a new differential triple stain technique. A study of skin biopsies from leprosy lesions stained by this method revealed not only non-acid-fast rods and granules but non-acid-fast and semi-acid-fast zoogleal forms identical to those observed for Mycobacterium tuberculosis. Recently, a comparison of slides from treated and untreated leprosy lesions disclosed that in treated lesions, both rod and
zoogeleal forms—if not fragmented or destroyed—become condensed by degrees into large numbers of non-acid-fast or semi-acid-fast globoi bodies. Similar globoi condensation bodies were also observed in both stained and unstained preparations from cultures of H37RV tubercle bacilli in Dubos medium containing various concentrations of streptomycin, in fluid from streptomycin-treated tuberculous patients, and in preparations from tissues and fluids of immunized tuberculous guinea pigs. The interrelationship of the various forms of these mycobacteria is being studied.

M28. The Effect of Tissue Extracts on Experimental Tuberculose Infections in Mice. Girard W. Thomas and Leo G. Nutini, Institutum Divi Thomae, Department of Experimental Medicine, Cincinnati 6, Ohio.

Studies using deproteinized aqueous extracts of beef brain, heart, liver, kidney, and spleen, previously shown to have bacteriostatic action on the growth of the tubercle bacillus in Youmans' liquid synthetic medium, were extended to the H37Rv strain. Heart extract, which in 5 per cent concentration had shown bactericidal activity for the H37 strain, was likewise bactericidal for the H37Rv type within 21 days. For in vivo experiments, 20 mice were used as control animals, and 20 for each extract, with the exception of brain extract, for which 10 mice were used. One-tenth mg of the H37Rv strain was injected intravenously. After 28 days' treatment with spleen and heart extract (20 mg per day subcutaneously) the mortality rate was 15 and 10 per cent respectively, and 60 per cent in the control series. Mortality with the brain, kidney, and liver extracts was 40, 35, and 45 per cent respectively. In untreated control mice, the lungs showed massive tubercle formation and marked enlargement. In those treated with heart and spleen extracts, there was slight enlargement with little evidence of tubercle formation. Acid-fast bacilli were found in the lungs of the control animals in large numbers. They were slightly reduced in those treated with spleen extract, whereas the lungs of the mice receiving the heart extract contained only a few isolated organisms. Kidney, brain, and liver extracts appeared to have no influence on the number of organisms present in the lung tissue.

M29. The Effect of Para-aminosalicylic Acid on Tubercle Bacilli. Guy P. Youmans and Gordon W. Raleigh, Northwestern University Medical School, Department of Bacteriology, Chicago, Ill.

Para-aminosalicylic acid was found to be highly bacteriostatic in vitro for 17 strains of virulent human type tubercle bacilli, including six strains resistant to the bacteriostatic action of streptomycin. This tuberculostatic activity was partially reversed by p-aminobenzoic acid, but was not markedly interfered with by the presence of plasma. When p-aminosalicylic acid, either in the form of the hydrochloride or the free base, was administered by the drug diet method to white mice infected intravenously with 0.1 mg of the virulent H37Rv strain of Mycobacterium tuberculosis, it was found to exert a suppressive action on the
tuberculous process. Toxic reactions were noted in the mice when the compound was administered in the diet in concentrations of 2.0 and 4.0 per cent, but were not evident when administered in a concentration of 1.0 per cent.

**M30. Comparative Susceptibilities of Different Strains of Mice to Experimental Infection with Mammalian Tubercle Bacilli. CYNTHIA PIERCE, RENE J. DUBOS, AND GARDNER MIDDLEBROOK, Rockefeller Institute of Medical Research, Department of Pathology and Bacteriology, New York, N. Y.**

Mice of known genetic backgrounds were infected with young cultures of tubercle bacilli growing diffusely in the tween-albumin liquid medium. Striking and reproducible differences were observed in the course and outcome of the infection, resulting from injection of small amounts of bacilli by the intraperitoneal, intravenous, or intracranial routes. Of the 20 strains tested, mice derived from the Albino Swiss exhibited highest resistance, whereas all pigmented strains were more susceptible.

**M31. The Effect of Diet on the Susceptibility of Mice to Experimental Infection with Mammalian Tubercle Bacilli. RENE J. DUBOS AND CYNTHIA PIERCE, Rockefeller Institute for Medical Research, Department of Pathology and Bacteriology, New York, N. Y.**

The susceptibility of mice to experimental tuberculous infection varies greatly, depending upon the composition of the diet. Highest susceptibility was observed when the animals were maintained for 2 weeks on a mixture of corn meal, gelatin, butter fat, nicotinic acid, and minerals. Resistance was much increased when certain natural food products were added to this mixture.

**M32. Differential Characteristics of Virulent and Avirulent Variants of Mammalian Tubercle Bacilli. GARDNER MIDDLEBROOK, RENE J. DUBOS, AND CYNTHIA PIERCE, Rockefeller Institute for Medical Research, Department of Pathology and Bacteriology, New York, N. Y.**

Virulent mammalian tubercle bacilli multiplying in infected tissues or in tween-albumin liquid medium grow in the form of long strands consisting of strongly acid-fast cells oriented in parallel along their longitudinal axis. The avirulent bacilli, on the contrary, are less uniformly acid-fast and occur in clumps without any polarity; the individual cells in these clumps exhibit random orientation or, at most, a suggestion of rosette formation. These differences in microscopic cellular arrangement are reflected in the gross macroscopic appearance of the growth of the cultures. On the surface of liquid media, the virulent culture spreads to form a thin veil which rapidly and uniformly covers the whole surface, whereas the avirulent culture gives rise to isolated islands of growth, presenting a granular and clumpy appearance. On tween-albumin agar, the colonies of the virulent form are flat, translucent, and spreading; the avirulent form, on the other hand, gives raised, opaque colonies with little tendency to spread peripherally.
M33. Resistance of Tubercle Bacilli to Streptomycin in Guinea Pigs After Administration of the Drug: The Effect on Response to Treatment with Streptomycin. ALFRED G. KARLSON and WILLIAM H. FELDMAN, Mayo Foundation, Division of Experimental Medicine, Rochester, Minn.

Twenty-eight guinea pigs were each inoculated with 0.1 mg of tubercle bacilli resistant to only 0.15 micrograms of streptomycin per milliliter of medium. Twenty days later four were killed and found to have visible lesions of tuberculosis. At this time treatment of ten of the remaining guinea pigs with streptomycin was started. Each received 1.5 mg four times daily. The other 14 animals served as controls. Treatment had continued for 146 days when the experiment was ended on the one hundred and sixty-sixth day of infection. The untreated guinea pigs survived an average of only 70.6 ± 22 (standard deviation of the mean) days. All had widespread lesions of tuberculosis. When cultures from their spleens were made, the same sensitivity to streptomycin was noted as in the original culture. Two of the treated animals died on the one hundred and sixtieth and one hundred and sixty-fourth day, respectively, from causes other than tuberculosis. The other eight were apparently well on the one hundred and sixty-sixth day after having been treated for 146 days. They were then killed and three were found to have visible lesions. Organisms in cultures from the spleens of these were resistant to more than 2,000 micrograms of streptomycin per milliliter of medium. Seven of the treated animals had no lesions of tuberculosis. On culture of the spleens from two, no growth was observed in 60 days. Organisms in cultures from the other five were resistant to only 0.15 micrograms of streptomycin. Histologically little evidence of tuberculosis was found in any organ; in the three treated animals from which resistant strains of tubercle bacilli were isolated, the tuberculous process of recent origin was unrestricted.

M34. Tuberculostatic and Tuberculocidal Action of Streptomycin. DOROTHY G. SMITH and SELMAN A. WAKSMAN, New Jersey Agricultural Experiment Station, Department of Microbiology, New Brunswick, N. J.

By the use of the turbidimetric procedure for measuring diffuse growth of Mycobacterium tuberculosis in Dubos' medium, it is possible to study accurately the bacteriostatic activity of various antibiotic substances, especially streptomycin. The tuberculocidal action of this antibiotic was determined by plating procedures, using the above medium with agar. Streptomycin, in a concentration of 0.3 micrograms per ml, was completely bacteriostatic, although occasionally growth occurred even in concentrations of 0.5 µg per ml. The bactericidal effect was greatly influenced by the incubation period. Only 0.3 µg per ml of streptomycin was required for the killing of all cells of a given inoculum if incubation was continued for 48 hours, whereas 20 µg per ml were needed when the culture was incubated only 6 hours. The degree of activity of streptomycin upon the tubercle bacilli was found to be a function of the number of cells and the length of time of exposure of the cells to the antibiotic.
M35. Some Biological Properties of Proteins from Unheated Tubercle Bacillus Culture Filtrates. JANET R. McCARTER AND ELLEN B. BEVILACQUA, The University of Wisconsin, Department of Agricultural Bacteriology and Chemistry, Madison, Wis.

By means of quantitative precipitin and precipitin absorption tests and of physicochemical methods, a survey has been made of all fractions precipitable by (NH₄)₂SO₄ from unheated culture filtrates of the human tubercle bacillus. Two protein fractions were found to be distinct and homogeneous, both according to their sedimentation constants and to their behavior as precipitinogens. The one antigen, previously found by Seibert, came from a fraction precipitated by one-fourth saturation with (NH₄)₂SO₄ and had a sedimentation constant of about 3.5S. The other was precipitable by saturation with (NH₄)₂SO₄ and had a constant of about 2.0S. These two antigens were the only ones detected in culture filtrates of one strain of avirulent tubercle bacillus. A third antigen was found associated with the other two in certain fractions from a virulent strain. This "virulence" antigen was by far the best precipitinogen of the three antigens. All were good precipitants in comparison with other described bacterial proteins. Both the "one-fourth" and the "saturated" antigens were potent in eliciting skin reactions in tuberculous human beings and guinea pigs. The potency tests were made on individual animals since the simultaneous injection of the two proteins into guinea pigs sensitized with tubercle bacillus cells resulted in partial inhibition. Either of these two antigens injected intracutaneously into the normal guinea pigs caused sensitization so that subsequent injections gave skin reactions. Skin sensitivity to the homologous protein could be transferred passively to normal guinea pigs by intracutaneous injections of the serum from the sensitized animals.

M36. Metabolic Changes in Certain Mycobacteria Associated with Development of Resistance to Streptomycin. R. J. FITZGERALD AND F. BERNHEIM, Duke University School of Medicine, Department of Physiology and Pharmacology, Durham, N. C.

The growth of an avirulent strain of *Mycobacterium tuberculosis* (ATC 607) is completely inhibited by streptomycin in concentrations of 60 to 75 units per cent. Streptomycin also inhibits the growth of a saprophytic soil *Mycobacterium*, but concentrations of 125 to 150 units per cent are required. Both these strains can oxidize benzoic acid. Streptomycin in a concentration of 5.0 units per ml can completely inhibit the oxidation of benzoic acid by the 607 strain of *M. tuberculosis*, whereas 20 to 30 times that amount is necessary to inhibit this oxidation by the soil *Mycobacterium*. Streptomycin resistance was induced in these strains by growing them in increasing concentrations of the drug until they grew readily in the presence of 10,000 units per cent of streptomycin. With development of increasing resistance to streptomycin the sensitivity of the benzoic-acid-oxidizing mechanism to streptomycin decreases progressively, until finally, in strains resistant to 10,000 units per cent, the oxidation of benzoic acid is unaffected by large amounts of streptomycin.
M37. A Quick Microtechnique for the Detection of Acetylmethylcarbinol Production by Bacteria. Angelina Fabrizio and R. H. Weaver, University of Kentucky, Department of Bacteriology, Lexington, Ky.

The principles of Weaver, Arnold, and Hannan (1940) have been used in the development of a quick microtechnique for the detection of acetylmethylcarbinol production by bacteria. An infusion medium containing 1 per cent trypticase, 0.7 per cent glucose, and 0.5 per cent sodium chloride is distributed in 0.5-ml amounts in 10 × 75 mm tubes and the tubes are preheated to 37 C. Each tube is inoculated with a loopful of growth from a 6- to 12-hour infusion agar slant culture. The tubes are placed in a water bath at 30 C for 90 minutes, after which time tests are performed for the detection of acetylmethylcarbinol by the addition of 0.15 ml of 5 per cent α-naphthol solution followed by 0.05 ml of 40 per cent potassium hydroxide containing 0.3 per cent creatine. The tubes are shaken for 5 seconds after the addition of each reagent and returned to the water bath for 30 minutes. Of 408 recently isolated coliform cultures that produced acetylmethylcarbinol according to the Dorner and Hellinger procedure, 394 (96.5 per cent) yielded positive results by the quick microtechnique. Of the 14 that yielded negative results, 10 were atypical Aerobacter aerogenes according to their IMViC reactions. None of 372 cultures that were negative by the Dorner and Hellinger procedure were positive by the quick microtechnique.

M38. Further Observations on the Occurrence of Streptococci Other Than Group A in Human Infection. George E. Foley, The Children’s Hospital and Infant’s Hospital, Department of Pathology, Boston, Mass.

During a 2-year period the streptococci isolated from 118 cases of suppurative or generalized streptococcal infection were classified as to serological group. These cases represent but a small percentage of the total streptococcal admissions to the institutions where these data were collected during this time, and were selected in that only infections thought likely to be nonrespiratory in origin were studied. Non-group-A streptococci were isolated from 95 (80.0 per cent) of these cases. Groups D, B, K, C-G, F, and E were encountered in that order of frequency. Seventy-two (75.0 per cent) of these non-group-A strains occurred as pure cultures on primary isolation. The predominance of group D streptococci could be accounted for in part by the number of cases of urinary infections and subacute bacterial endocarditis in the series. However, even if these cases (50) were omitted, group D was second only to group A in frequency of occurrence. Of 34 cases of subacute bacterial endocarditis, group D streptococci were isolated from 19 (55.9 per cent), group K from 1 (2.9 per cent), and serologically unclassified alpha strains from 14 (41.2 per cent). Of the latter group, 6 were Streptococcus s.b.e., 5 were Streptococcus bovis, and 3 were Streptococcus mitis. Alpha and gamma as well as beta strains were encountered among these non-group-A streptococci. Consideration of the epidemiology of non-group-A streptococci suggests endogenous rather than exogenous events precipitating infection.
MS9. The Action of Pasteurella pestis Bacteriophage on Pasteurella, Salmonella, and Shigella. A. S. Lazarus and J. B. Gunnison, University of California Medical School, Department of Bacteriology, San Francisco 22, Calif.

A strain of Pasteurella pestis phage, which lysed all of 12 strains of P. pestis studied, has been investigated to determine its ability to lyse other bacteria. Solid and liquid media were used. Nineteen out of 27 strains of Pasteurella pseudotuberculosis were lysed by the phage. After adaptation to P. pseudotuberculosis, the phage lysed all strains of that organism, most of them in higher dilution than originally. Three of 42 Salmonella strains and six out of 37 Shigella cultures were susceptible to P. pestis phage. Seventy-seven cultures from 17 other genera were not affected by the same phage. After adaptation to Shigella species, the phage showed an increased potency toward the six susceptible Shigella strains. After adaptation to Shigella or P. pseudotuberculosis, the phage still retained completely its ability to lyse P. pestis. Minor serological relationships were shown to exist between P. pestis and certain strains of Salmonella and Shigella, using macroscopic agglutination tests. These relationships were not clearly correlated with susceptibility to phage. Either phage action in this case is not based on surface antigens held in common, or the minor antigenic relationship shown to exist between P. pestis and some salmonellas and shigellas is an unstable one. The use of P. pestis lysates as vaccines and the study of the lethal properties of some P. pseudotuberculosis lysates are suggested as worthy of further investigation.

MS40. The Effect of Yeast Concentrate on the Growth and Survival of Hemophilus influenzae in Infusion Broth. Erwin Neter, University of Buffalo and Children's Hospital, Department of Bacteriology and Immunology, Buffalo, N. Y.

Since it was shown that yeast concentrate supported the growth of Neisseria gonorrhoeae, experiments were carried out to determine whether it also enhanced the growth and survival of Hemophilus influenzae. Several strains of H. influenzae type b, freshly isolated from patients with meningitis, and several stock culture strains of types a, b, and c (maintained on chocolate agar) were seeded into brain heart infusion broth containing various concentrations of yeast concentrate (bacto-supplement B, Difco Laboratories) as well as into broth without concentrate. The culture media were incubated at 37 C. The resulting visible growth was noted and subcultures were made, after various periods of incubation, to chocolate agar. In contrast to the control broth, visible growth may occur within 24 to 48 hours in broth containing yeast concentrate in concentrations ranging from 1 per cent to 20 per cent. Higher concentrations (5 per cent to 20 per cent) were found to be more effective than lower concentration (1 per cent). In infusion broth containing the yeast concentrate, H. influenzae type b produces SSS, as demonstrated by precipitation tests with type b-specific anti-H. influenzae serum. In the absence of grossly visible growth
yeast concentrate definitely prolonged the survival of *H. influenzae* in infusion broth. It is concluded that an autolysate rich in coenzyme, glutamine, co-carboxylase, and other B-vitamin factors favorably influences the growth of *H. influenzae* in infusion broth. The possible practical applications of this finding will be discussed.


The liquid culture medium described by Hornibrook has been simplified and modified by the removal of calcium chloride, sodium chloride, and sodium carbonate, the rebalancing of the quantities of casein hydrolyzate, potassium phosphate, and nicotinic acid, and the substitution of cystine for cystine hydrochloride. All ingredients are stable and readily available and may be added prior to sterilization. Fourteen strains of freshly isolated *Hemophilus pertussis* have been found to grow readily in this medium, and two have been carried through 19 consecutive subcultures without loss of virulence or evidences of phase changes. Cultures grown in this medium are not granular and develop to a density of 30 to 40 billion cells per ml in 48 hours. Vaccines prepared from liquid cultures have been found to be equal or superior to those prepared from organisms grown on Bordet-Gengou agar on the basis of mouse protection tests using the intracerebral route of infection. The liquid medium can be prepared as an agar containing 5 per cent human blood cells and this has been found to produce more colonies from suspensions of *H. pertussis* than does Bordet-Gengou agar. Because of the simplicity and growth-promoting qualities of this agar medium, its use as a diagnostic medium is suggested.

**M42. An Analysis of Weckstein’s Rapid Method for the Primary Identification of the Gonococcus.** J. D. THAYER, MATTHEW A. BUCCA, AND RUTH A. KIRTY, Research Laboratory, U. S. Public Health Service, New York (Staten Island 4), N. Y.

In a study of Weckstein’s method for isolation and identification of gonococci a variety of irregular, atypical, and misleading color reactions were found on the glucose and maltose plates. Forty-three per cent of the cultures showed typical fermentative color characteristics of the gonococcus. Twenty-three per cent of the cultures produced red-colored gonococcus colonies on both glucose and maltose plates and would have falsely indicated the meningococcus or *Neisseria sicca*, *Neisseria flava*, *Neisseria perflava*, or *Neisseria subflava*. Twenty-three per cent of the specimens did not grow on either the glucose or maltose plate and would have resulted in false negative reports. The highest concentration of the dye that can be added to the medium so that typical gonococcic fermentation color reactions are observed, varies considerably. The presence of contaminating bacteria can adversely influence the fermentative color reaction of the gonococcus colonies.
M43. The Effect of the Cyclic Changes of the Cervical Mucus upon the Isolation of the Gonococcus from Cervical Cultures. Marie L. Koch, Johns Hopkins Medical School, Department of Bacteriology, Baltimore, Md.

The use of pancreatic digest agar containing 5 per cent human chocolate blood should have eliminated the cultural difficulties encountered in isolating gonococci from cervical cultures. This, however, was not the case, so further studies were made to determine what other factor, or factors, were responsible for obtaining negative cervical cultures in cases of clinical gonorrheal cervicitis. In 1940 Lamar, Shettles, and Delfs showed that there was a relative change in the alkalinity and acidity of the cervical mucus during the menstrual cycle, and that there was a positive correlation between these cyclic changes with the penetrability of spermatozoa. Investigations were made to determine whether or not these cyclic changes had any effect upon the viability of the gonococci in vivo. A study of 50 untreated dispensary patients and 5 hospitalized patients with a clinical diagnosis of gonorrheal cervicitis showed that negative cervical cultures are associated with acid mucus and that only negative cultures are obtained during the latter part of the luteal phase of the cycle when the pH range of the cervical mucus is 6.6 to 5.2. Positive cultures are associated with the estrogenic phases of the cycle when the pH range of the cervical mucus is 7.6 to 6.8. There is a positive correlation between the cyclic changes in the pH of the cervical mucus and the ability to isolate gonococcus from cervical cultures.

M44. The Selection of a Suitable Medium for Culturing Root Canals. Donald E. Shay, University of Maryland Dental School, Department of Bacteriology, Baltimore, Md.

It is possible that some of the fermentative changes involved in the production of acid on the enamel surface of the tooth occur under both anaerobic and aerobic conditions. Thus, a medium most suitable for culturing root canals should promote growth of both types of organisms. Trypticase glucose broth and a modified thioglycolate medium were selected as the experimental media. As controls, four of the most frequently used media were chosen: brain heart infusion, Blayney's brain agar, Brewer's thioglycolate, and serum broth. Sterile tubes containing 2 ml of nutrient broth and alundum were inoculated with a paper point which had been in the root canal from 1 to 2 minutes. To obtain a suspension of the organisms present, the paper point was ground against the alundum with a sterile 1-ml pipette. Aseptically, 0.1 ml of the nutrient broth was added to each medium and incubated for 2 weeks. Slides were prepared from all negative tubes. If positive growth resulted, it was streaked to blood agar plates and incubated for 24 hours. Slides were then prepared from both the plate and the tube. Of the 709 root canals cultured, 183 were positive on one or more of the various media. Using trypticase glucose broth (pH 7.2), 166 were positive; 158 using brain heart infusion; 144 using serum broth; 137 using Blayney's brain agar; 131 using Brewer's thioglycolate; and 95 using trypti-
case glucose broth (pH 5.5). Modified thioglycolate was discontinued after checking 203 root canals. Of the 183 positive cultures, 84 were caused by the presence of gamma streptococci, 44 by staphylococci, and 20 by a combination of organisms. The tryptase glucose (pH 7.2) proved to be the most satisfactory medium, yielding a higher percentage of positives in the shortest period of time.


It was observed that the distribution of plates streaked with 2 to 7 shigellae or salmonellae follows the Poisson series. To utilize this observation in the evaluation of diagnostic media, series of 100 plates each of 20 media were inoculated with suspensions of fastidious Shigella dysenteriae or Salmonella sendai and a “contaminating mixture.” The true number of organisms in the suspension was determined by plating to tryptose agar. The means, the class frequencies, and the goodness of fit of the examined and of the control plates were compared. A satisfactory probability of detecting S. dysenteriae could be expected only by streaking series of 5 to 6 reliable plates (MacConkey, Levine, Panja and Ghosh, desoxycholate, D. C. L. S.). The use of tetrathionate broth or selenite enrichment with consecutive streaking of two media (S. S., D. C. L. S., or Panja and Ghosh) increased the probability of the isolation of S. sendai to 0.99. When similar series of plates were tested with less fastidious, but in America more frequent, organisms (Shigella paradysenteriae III and Eberthella typhosa) with a combination of one little selective plate (MacConkey, Levine, desoxycholate, D. C. L. S.), two medium selective plates (S. S. or D. C.), and one enrichment tube (tetrathionate or selenite) streaked to a bismuth sulfite plate (Wilson and Blair, Hajna and Perry, or Difco), the probability of a positive result was higher than 0.97.


The newer media for the detection of bacteria of the coliform group in water, which are reported to have given promising results, are lauryl sulfate tryptone broth (L.S.T.) by Mallman and Escherichia coli broth (E.C.) by Perry. These two media have been compared with standard lactose using water samples of widely varying quality. Presumptive tubes of all three media were incubated at 37 C. In addition duplicate sets of tubes of the E.C. medium were incubated at 44 C and 45.5 C in both air and water immersion incubators. All tubes showing gas formation in any amount were confirmed in brilliant green bile broth and on eosin methylene blue agar plates. Cultures which confirmed were purified and subjected to the completed tests. Detailed data on the productivity of these media will be presented. In general it may be said: (1) Standard
lactose broth produced a considerable number of false presumptives. (2) L.S.T. broth was almost as productive as standard lactose broth, the number of false positives were reduced, but results were unreliable without confirmation. (3) E.C. medium at 37 C was less productive than the other media, with fewer false positives. (4) The productivity of E.C. medium at 45.5 C in both air and water immersion was less than 50 per cent of that of the other two media or of E.C. at 37 C. (5) E.C. medium at 44 C was slightly more productive than at 45.5 C.

M47. Studies on Rabies Infection in Developing Chick Embryos. HILARY KOPROWSKI and HERALD R. COX, Lederle Laboratories Division, American Cyanamid Company, Section of Viral and Rickettsial Research, Pearl River, N. Y.

The chick-brain-adapted Flurry strain of rabies was used. Egg passages were initiated with a 138th-chick-passage brain suspension. The virus was carried through 40 passages by inoculation into the yolk of fertile hens' eggs. Subsequent experiments showed that inoculation into the allantoic sac gave equally good results. The LD₅₀ titers of embryos infected by the yolk sac route ranged from 10⁻⁴ to 10⁻⁸. The virus content of the embryo was highest between the 7th and 12th days after inoculation, the incubation period being inversely proportional to the concentration of virus in the inoculum. The virus was present in all tissues. The virus content was greatest in the embryo itself, the concentration in the CNS being only slightly higher than in the remainder of the embryo. Embryonic blood is infectious from the 3rd to 15th day after inoculation. All embryos inoculated on 7, 9, 11, and 13 days of incubation died at time of hatching. Embryos inoculated on 15, 17, and 19 days of incubation ordinarily hatched out, but some died soon after hatching with virus recoverable from their brains. Surviving chicks possessed high titer neutralizing antibodies in their sera obtained 14 days after hatching. The chick-embryo-adapted virus is pathogenic for mice, guinea pigs, hamsters, and cotton rats inoculated intracerebrally. It was of low virulence for rabbits intracerebrally and avirulent parenterally. The living virus protected mice against intracerebral challenge with Pasteur strain rabbit virus and protected rabbits against parenteral challenge with Pasteur strain guinea pig virus.

M48. Transmission of Human Poliomyelitis Virus to Mice. ALBERT MILSER, CHESTER L. BYRD, and SIDNEY O. LEVINSON, Michael Reese Research Foundation, Michael Reese Hospital, Department of Bacteriology, Chicago, Ill.

We have previously reported that autolyzed brain diluent shortens the incubation period and facilitates the transfer of poliomyelitis virus to CFW Swiss mice, hamsters, and rhesus monkeys. The Leon monkey passage strain of poliomyelitis virus has been successfully adapted to CFW mice by means of this technique. In the present studies poliomyelitis virus was isolated in CFW Swiss mice from 11 to 12 stools obtained during the acute stage from patients
with paralytic poliomyelitis involving two or more extremities. Poliomyelitis virus was also isolated in mice from spinal cord from a fatal case of bulbar poliomyelitis. Autolyzed brain tissue diluent was prepared as described previously. The proper preparation of the autolyzed brain is of critical importance for successful transmission. Stool suspensions were prepared by grinding feces in approximately 10 volumes of sterile distilled water, centrifuging at 2,000 rpm for 10 minutes, and treating the supernatant after chilling with ether (20 per cent by volume) overnight in the refrigerator. On the following day, the ether was evaporated by negative pressure, and the suspension was centrifuged at 5,000 rpm for 1 hour. The supernatant was next removed, mixed with equal volumes of autolyzed brain, and inoculated intracerebrally and intraperitoneally into CFW mice. Proof of adaptation was shown by successful transfer to rhesus monkeys and neutralization by 1:50 dilution of human serum immune globulin.

M49. Studies of the Distribution of Poliomyelitis Virus. IV. In Rural Schools Following an Epidemic. Thomas Francis, Jr., and Gordon C. Brown, School of Public Health, University of Michigan, Department of Epidemiology, Ann Arbor, Mich.

In August, 1945, the rural schools in Henderson County, Tennessee, were opened toward the end of an epidemic of poliomyelitis in that area. In view of the much debated question of opening schools following epidemics a study was undertaken to reveal evidence of the occurrence and possible spread of the virus under such circumstances. Stools were collected from all 99 pupils of two rural schools on the opening day and again 2 weeks later. Collections were also made at the same times from 37 children in the small town of Lexington, whose school was not to open for a month. The stools were pooled in groups of three, rendered bacteriologically sterile, and inoculated into monkeys. If positive, the pool was broken down and the specimens tested separately. All the specimens from one school were negative on the opening day, but 2 weeks later one was positive. Two children in the second rural school were carrying the virus on the opening day but only one of these was positive 2 weeks later. All other tests were negative at both collections. In the control group, one was positive at the first collection but all were negative two weeks later. The results indicate that although these children may have undergone a previous subclinical infection, few were found to be carriers of poliomyelitis at the time of the study. Though virus was present on the opening day, there was no greater spread among the pupils than in children not yet returned to school.

M50. Laboratory Studies on the Epidemiology of Poliomyelitis. F. B. Gordon, F. M. Schabel, Jr., Albert E. Casey, and William I. Fishbein, University of Chicago, Department of Bacteriology and Parasitology, and the Chicago Health Department, Chicago, Ill.

During an epidemiologic study of poliomyelitis in Chicago in 1945, stool specimens were obtained from children classified as household contacts, non-
household contacts, and noncontacts, residing in the neighborhood of paralytic cases. Control specimens were also obtained in nonpoliomyelitis neighborhoods. Tests of stool specimens of 71 children by combined intracerebral, intraperitoneal, and intranasal inoculation of monkeys revealed virus in the following incidences: of 20 household contacts, 16 (80 per cent) were positive; of 28 non-household contacts, 10 (36 per cent) were positive; of 18 noncontacts, 2 (11 per cent) were positive; of 5 controls, none was positive. The average age of the children in the four groups was similar in each case. Of the total of 28 children with virus in their stools, only 3 had clinically recognizable poliomyelitis. These results indicate a high incidence of subclinical infection in the immediate contacts of poliomyelitis cases, and provide added evidence for transmission of the virus by direct contact.

M51. Active Immunity in Monkeys to Poliomyelitis Virus. Isabel M. Morgan, Poliomyelitis Research Center, Johns Hopkins University, Department of Epidemiology, Baltimore 5, Md.

Immunity to intracerebral or intranasal inoculation of Lansing poliomyelitis virus has been induced in M. rhesus monkeys by intramuscular injection of active Lansing virus. This immunity is associated with high titers of serum antibody. Monkeys were vaccinated intramuscularly with 20 per cent Lansing infected monkey spinal cord. Six weeks after receiving total doses of virus suspension ranging from 0.8 g to 3.2 g infected spinal cord given in 4 injections, all of 17 monkeys resisted intracerebral injection of 10 per cent Lansing monkey cord suspension. This represented 10,000 PD<sub>60</sub>. The group was then divided. One-half proved immune to subsequent intranasal instillation of active Lansing virus, whereas the other half became paralyzed after a similar instillation of Brunhilde virus. This heterologous strain has been shown to have only slight immunological relationship with Lansing virus. Serum neutralization tests were carried out with Lansing virus in mice. The 50 per cent end points of the sera of immunized monkeys just prior to intracerebral challenge ranged from 1/1,000 to 1/8,000. Thus by intramuscular vaccination with active virus monkeys have been rendered solidly immune to intracerebral or intranasal inoculation of homologous poliomyelitis virus. This immunity is associated with high titers of circulating antibody. These monkeys failed to resist intranasal instillation of a heterologous strain of poliomyelitis virus.

M52. The Antibody Response in Human Beings Inoculated with Japanese Encephalitis Vaccine. Joel Warren, Joseph E. SmaDEL, and A. F. Rasmussen, Jr., Army Medical Department Research and Graduate School, Department of Virus and Rickettsial Diseases, Washington 12, D. C.

Previous experiments have shown that Japanese encephalitis vaccine prepared from infected chick embryos immunized mice against infection as well as did mouse brain vaccine. The serological response of human beings to chick
embryo and mouse brain types of Japanese encephalitis vaccine was studied. In addition, the response following different methods of administration was tested. Administration of two doses of 2 ml at a 4-day interval elicited demonstrable neutralizing antibody in about 30 per cent of the persons who received either chick embryo or mouse brain type vaccine. When chick embryo type vaccine was given in 1-ml amounts on 1, 7, and 30 days, approximately 60 per cent developed neutralizing substances. In contrast, complement-fixing antibodies of Japanese encephalitis appeared rarely and in low concentration. Six persons who had neutralizing antibodies following immunization according to the second schedule were bled at 5 months. Only two still possessed immune substances, and these maintained them for a year. Reinjection of four individuals with 1 ml of chick embryo vaccine 1 year after the basic immunization elicited neutralizing antibodies in those who had shown antibody following the basic immunization.

M63. Complement-fixing Antibodies Reacting with Normal Chick Embryo Antigens in Sera of Persons Repeatedly Immunized with Chick Embryo Type Vaccines. JOSEPH E. SMADEL, JOEL WARREN, AND MERRILL J. SNYDER, Army Medical Department Research and Graduate School, Department of Virus and Rickettsial Diseases, Washington 12, D. C.

In measuring the response of human beings to Japanese encephalitis vaccine, chick embryo type, 72 soldiers were selected who had previously been immunized with vaccines prepared in embryonated eggs (yellow fever, typhus, influenza). Thirty-eight volunteers received a course of two injections of chick vaccine and 34 received three injections. They were bled before vaccination and after the second and third injections. Sera were tested for complement-fixing antibodies against normal and infected chick embryo antigens, which were clarified by centrifugation at 12,000 rpm. Sera from 21 of the 72 persons reacted with normal embryo antigen prior to the first injection of encephalitis vaccine. Fifty-five of the 72 soldiers gave positive reactions after two injections, and of 34 given a third dose 31 were positive. The complement-fixation titers ranged from 1/4 to 1/8 in prevaccination sera and covered the same range in the postvaccination specimens. Four of twelve positive reactors still had antibodies, titers 1/8 to 1/32, 5 months after vaccination. Fourteen of the higher titered sera were tested for Wassermann-reacting substance by the Kolmer technique; all were negative. The substance which reacted with the sera of vaccinated individuals occurred in greater amounts in antigens made from 13-day embryos than from 6-day embryos. Ten soldiers who possessed antibody prior to vaccination were devoid of cutaneous sensitivity to chick embryo antigen. No untoward allergic reactions occurred in this group of 72 individuals as a result of injection of vaccine.

M64. Vaccination Against Q Fever. MERRILL J. SNYDER, JOSEPH E. SMADEL, and FREDERICK C. ROBBINS, Army Medical Department Research and
Formalinized vaccines prepared from rodent or yolk sac tissues infected with *Rickettsia burnetii* have been shown by others to induce resistance in animals to infection with this agent. Ten per cent suspensions of yolk sacs infected with the Henzerling (Italian) and Dyer (American) strains were inactivated with formalin and extracted with ether. Such vaccines injected subcutaneously or intraperitoneally into guinea pigs in 1 to 3 doses of 1 ml each elicited appreciable amounts of complement-fixing antibody which reacted with Henzerling antigen but not with Dyer antigen. Beginning about 30 days after vaccination, antibodies against Dyer antigen appeared and increased in titer until they approached the Henzerling level at 60 days. Guinea pigs immunized with either type vaccine displayed resistance to infection with both strains. The vaccines protected guinea pigs against death from Q fever and mitigated the febrile response, but did not induce complete resistance to very large doses of *R. burnetii*. Thirty-two persons were immunized with 3 subcutaneous injections of 1 ml of vaccine. Twenty-one of the 25 receiving Henzerling vaccine developed complement-fixing antibodies with titers ranging from 1 to 10, average 6. Only 1 of the 7 receiving Dyer vaccine developed antibodies. Sera of all 22 persons who developed antibodies reacted with Henzerling antigen but only 1 of these fixed with Dyer antigen. No data became available on the resistance of vaccinated persons to infection. The variations in serological response will be discussed.

**M55. Metabolic Differences Between Phage-susceptible and Phage-resistant Variants of a Strain of Escherichia coli.** Henry W. Scherp, S. Farnum Coffin, and John F. Waldo, University of Rochester School of Medicine and Dentistry, Department of Bacteriology, Rochester 7, N. Y.

The dependence of bacteriophage upon the bacterial host for reproduction suggested that the acquisition of resistance to bacteriophage by the host organism might be accompanied by changes in metabolic activities. Resistant variants were isolated from lysed cultures of a strain of *Escherichia coli* susceptible to Burnet's bacteriophage C13. By the usual cultural tests, these variants were identical with the parent strain, except that indole was not produced and they grew more slowly. Methylene blue reduction times were determined simultaneously for the resistant and susceptible organisms at 37 C under vaseline seal in mixtures comprising washed organisms (0.015 mg bacterial nitrogen per ml), 1:250,000 methylene blue, 0.40 phosphate buffer at pH 7.4, and 0.0001 M substrate. The average ratios, reduction time for resistant : reduction time for susceptible, were: glucose, 1.2; glycerol, 2.6; lactate, 4.1; succinate, 3.8; pyruvate, 1.4; alanine, 2.6; galactose, 1.9; maltose, 1.4. Under the conditions of these experiments, methylene blue was not reduced in the presence of acetate, citrate, α-ketoglutarate, glutamate, tyrosine, xylose, lactose, and dulcitol. Infection of susceptible cells with the phage produced no significant change in the
methylen blue reduction times unless the reaction was allowed to progress to the verge of lysis, whereupon all dehydrogenase activity disappeared. The results showed that the acquisition of resistance to the bacteriophage was paralleled by a diminution of various metabolic activities of the host organism. No evidence regarding the specificity of these findings was provided.

M66. Reactivation of Ultraviolet-inactivated Bacteriophage Particles Inside Double-infected Host Cells. S. E. Luria, Indiana University, Bacteriological Laboratories, Bloomington, Ind.

Inactivation of a bacteriophage particle by ultraviolet radiation can be detected by the inability of the particle to produce more phage when absorbed by a sensitive bacterial cell. A large amount of reactivation takes place, however, if concentrated suspensions of inactivated phage are put in contact with sensitive bacteria. Statistical analysis proves that reactivation occurs in a fixed proportion of those bacterial cells that absorb two inactive phage particles. The probability of reactivation is a function of the dose of radiation received by the bacteriophage. Reactivation only occurs for "large particle" phages. Cross reactivation can also take place between particles of serologically related phages, not between unrelated strains. No reactivation was found after X-ray inactivation. By analogy with the phenomenon of transfer of genetic characters between phage particles growing in the same host cell, the reactivation experiments are tentatively interpreted as indicating genetic transfers at loci that have undergone "lethal" mutations. Statistical analysis of the experimental data proves them compatible with this hypothesis. The results are analyzed in their bearing on the genetic constitution of the phage particle and its growth mechanism.


The present study was done to test the effect of streptomycin, penicillin, and sulfa drugs on five strains of Actinomyces bovis, three of human and two of bovine origin. Strain susceptibility has been ascertained and the relative amounts of tested agents required for inhibition determined. Chick embryos were infected with this organism but the results, after using chemotherapeutic agents, were not conclusive because of inherent difficulties in this method. The experimental work utilized thioglycolate media, into which was incorporated varying concentrations of the test drugs. The antagonistic activity of this medium against the drugs is taken into consideration, as are also the difficulties in interpretation of the results caused by the cultural idiosyncrasies of the organism. Under the conditions of these experiments it was found that for three human and two bovine strains sulfathiazole and sulfadiazine were not inhibitory in concentrations of 50 mg per cent; penicillin was inhibitory in concentrations varying from 0.1 to 0.5 units per ml; streptomycin inhibited all but one strain
in concentrations of 100 units per ml. From these results penicillin and streptomycin appear to be more effective against Actinomyces bovis than the sulfonamides in clinically practical concentrations.


The purpose of this investigation was to determine the influence of BAL on the therapeutic activity of arsenicals in relation to its detoxifying effect. Mice infected with Trypanosoma equiperdum were treated subcutaneously with various amounts of BAL in oil and with mapharsen, which were injected at separate sites. BAL interfered with the therapeutic effect of mapharsen in doses much lower than those required to antagonize toxic mapharsen doses. (Lethal doses of mapharsen required 2 treatments with 60 to 100 mg per kg BAL in order to obtain survival.) Two treatments with BAL (5.0 mg per kg) completely abolished the trypanocidal activity of the curative dose of mapharsen by single injection (5.0 mg per kg). With lower doses of BAL (1.25 to 2.5 mg per kg) relapses followed and BAL doses still lower than these (0.6 mg per kg) did not influence noticeably the therapeutic activity. A higher dosage of mapharsen, such as 20 mg per kg, required for inhibition 15 to 10 mg per kg BAL. Correspondingly, the minimal dose of arsenical required for therapy increased in proportion to the amount of BAL administered. It is concluded that the loss of toxicity of the arsenical induced by BAL is accompanied by a corresponding, or even greater, loss in the therapeutic effectiveness.

M59. Streptomycin in the Treatment of Experimental Trypanosomiasis in White Mice and Chick Embryos. DONALD J. MERCHANT AND M. H. SOULE, University of Michigan, Department of Bacteriology, Ann Arbor, Mich.

White mice and chick embryos were infected with Trypanosoma brucei, Trypanosoma equiperdum, and Trypanosoma hippicum. After a lapse of 24 to 36 hours streptomycin hydrochloride was administered subcutaneously to the mice and was injected into the yolk sac of the chick embryos. Each mouse received a total of 16,000 units and each chick embryo a total of 40,000 units of streptomycin. The course of the disease was followed by microscopic examination of blood specimens taken at regular intervals. As far as could be determined, this antibiotic agent did not alter the course of the infections or prolong the life of the treated mice or embryos.

M60. Streptomycin in Experimental Brucellosis. E. H. KELLY AND THOMAS F. HENLEY, Jr., Department of Research and Development, Camp Detrick, Frederick, Md.

The effect of streptomycin on experimental brucellosis was investigated in mice and guinea pigs. Mice infected with Brucella suis and treated with strep-
tomycin from the time of infection were sacrificed in two groups, half at the end of therapy and the remainder 2 weeks later. The first group showed a lower incidence of infection than the controls but in the latter group the number showing infection was higher than in the untreated control group. Guinea pigs infected with Brucella suis in a dose insufficient to infect all of the controls responded to streptomycin therapy. In animals receiving a heavier infecting dose (approximately 24 ID₉₀) no significant effect of the antibiotic could be detected. It appears, therefore, that streptomycin, in the form in which it was used, is of little value for the therapy of brucellosis.

M61. Comparative Effectiveness of Penicillins F, G, K, and X in Spirochetal Infections as Determined by Short in Vivo Methods. Thomas B. Turner, Mary C. Cumberland, and Huan-Ying Li, Johns Hopkins School of Hygiene and Public Health, Department of Bacteriology, Baltimore, Md.

A method has been developed for the in vivo assay of various penicillins against Treponema pallidum. The assay requires 3 weeks and results are qualitatively similar to those obtained by the only other in vivo method yet reported, one which requires large numbers of rabbits and 9 months for completion. Multiple syphilomas are produced on rabbits’ backs by intracutaneous inoculation. Spirochete counts are made on representative lesions before, and 24 hours after, treatment with penicillin, which is given in three equal doses at 2-hour intervals. Pretreatment counts averaged about 1,000 T. pallida per 200 dark fields. To reduce the count to 10 or fewer spirochetes in 50 per cent of the animals required 0.1 mg per kg of penicillin G, 0.6 mg per kg of F, 1.1 mg per kg of X, and more than 2.0 mg per kg of K. Assays have also been made against the spirochete of relapsing fever, Borrelia novyi. Mice are treated 24 hours after infection and counts made 24 hours later. Controls show about 27 spirochetes per 3-minute count. To reduce the count to two or fewer spirochetes in 50 per cent of the mice requires 8.3 mg per kg of penicillin G, 15 mg per kg of F, 24 mg per kg of X, and 37 mg per kg of K. While this test seems less sensitive than that for T. pallidum, the results show the same qualitative differences between the penicillins. Results obtained by these short methods are consistent and apparently reflect within limits the comparative therapeutic activity of these four penicillins against experimental syphilis and relapsing fever.


During a study of acquired resistance of bacteria to streptomycin an atypical form of Escherichia coli, possibly identical to Kuhn’s A forms, was encountered. This organism when transferred from streptomycin agar to plain agar grew only as globular cells varying in diameter from 1 to 7 microns. On streptomycin agar it grew as a mixture of bizarre rods with branching and curved forms and a
few round cells. Repeated transfers of the variant on plain agar brought about a gradual reversion to the typical rod form of *E. coli*. Alternate transfers on plain agar and streptomycin agar maintained the culture in the round form. This atypical form of *E. coli* became resistant to 10,000 micrograms of streptomycin per ml of agar much more slowly than did 11 other gram-negative rods and 8 colony subcultures of the same strain of *E. coli*. When a resistant culture of the globular organism was transferred on plain agar until the culture consisted of predominantly typical rod forms, the only cells which retained resistance to the high level of streptomycin were the few atypical forms remaining. Cultural and antigenic studies were made on the globular culture in comparison with the parent strain of *E. coli*.

**M63. Anaphylaxis in the Fish.** N. B. Dreyer and J. W. King, University of Vermont, Medical College, Departments of Pharmacology and Bacteriology, Burlington, Vt.

A series of teleost fish was sensitized to various antigens in an attempt to demonstrate the production of anaphylaxis in these cold-blooded vertebrates. The antigen was given intraperitoneally both for the sensitizing and for the subsequent shocking dose. In all instances sensitivity could be demonstrated after 11 days and after periods as long as 6 weeks. An injection of antigens at intervals after the first shocking reaction demonstrated a repetition of anaphylaxis-like symptoms previously observed. The use of antigens on normal fish and saline on sensitized fish showed the reaction to be a specific one. A similar but more violent and transitory phenomenon could be produced using relatively large doses of histamine. Following injection of the shocking material the fish showed moderate uneasiness as manifest by swimming agitatedly about the jar and bumping its nose on the sides of the container. Subsequent to this one could observe a fanning of the dorsal fin, most marked in the anterior part. There was also an associated curling and closing of the caudal fin and a temporary loss of equilibrium. As these symptoms subsided the fish fell to the bottom of the jar and lay quietly for some time. There was marked increase in the excursions of the gill clefts, which may or may not have been accompanied by an increase in respiratory rate. The reaction has never been lethal although only minute doses of shocking antigen have been used.

**M64. Effects of Antihistaminic Substances on the Tuberculin Reaction.** Jorgen M. Birkeland and Lottie Kornfeld, Ohio State University, Department of Bacteriology, Columbus, Ohio.

There is no general agreement as to the role of hypersensitivity in the development of resistance to infection in tuberculosis, nor is there any agreement as to the nature of the tuberculin reaction. It is generally assumed that histamine or histaminelike substances are involved in the hypersensitive state. In an attempt to determine whether these agents play a part in the immune response and in the tuberculin reaction, infected animals were treated with antihistaminic agents, and the effect on the tuberculin reaction as well as on the course of the
infection as shown by survival time was observed. Rabbits and guinea pigs were immunized to histamine azo-protein ("hapamine") and then infected. Other groups of animals were infected and treated with benadryl and pyribenazine. Tuberculin sensitivity and survival time were determined. The results indicate that the antihistaminic agents employed did not delay the appearance of the tuberculin reaction, lessen its severity, nor affect the longevity of the animals. The question is raised as to whether the hypersensitive manifestations of tuberculosis involved histamine or histaminelike substances. Since antihistaminic agents ordinarily successful in combating anaphylactic and allergic phenomena failed to show a significant effect on the tuberculin reaction, these data cast doubt on the assumption that histamine or histaminelike substances play a predominant role in the reaction in rabbits and guinea pigs.

M65. The Application of the Ascoli Test in Tularemia. Carl L. Larson, National Institute of Health, Division of Infectious Diseases, Bethesda, Md.

The Ascoli test was studied employing suspensions in 0.85 per cent salt solution of cultures of Pasturella tularensis and of spleen and liver tissue from animals dying of tularemic infections. The suspensions were heated for 30 minutes in flowing steam in the autoclave, cooled, centrifuged, and the clear supernatant fluid was employed as antigen. Immune serums from humans convalescent from tularemia and certain other diseases and serums from immunized animals were employed in the tests. Precipitin tests were done by the capillary tube and macroscopic methods. The antigen reacted with serums from cases of tularemia but failed to react with serums from cases of brucellosis, typhus fever, Rocky Mountain spotted fever, typhoid fever, and shigellosis. Tularemia serums reacted with antigens prepared from P. tularensis but not with antigens prepared from Brucella sp., Pasteurella septica, Shigella dysenteriae, and psittacosis virus. No precipitation occurred with normal serums. White mice, white rats, and guinea pigs were infected with P. tularensis. The spleens and livers were removed 24 hours after death and portions were converted to Ascoli antigen and tested. The remainder was placed in the incubator at 37 C and at intervals of 7 and 14 days further tests were performed. There was no significant loss of antigenicity during this period. The antigen was precipitated by acetone. The soluble antigen prepared by ether extraction of organisms yielded Ascoli antigen when heated. Adaptation of the Ascoli test to tularemia has been of value in establishing early diagnosis and may be of practical value in ecological studies of tularemia.

M66. Studies on the Pathogenesis and Immunity in Tularemia. I. The Course of Infection with Bacterium tularensis as Seen in the Normal, Vaccinated, and Recovered White Rat. Cora M. Downs, Luther Buchele, and Barbara J. Owen, University of Kansas, Department of Bacteriology, Lawrence, Kans.

The first step in the study of the pathogenesis of tularemia was accomplished
by the following experiments. Normal, vaccinated, and recovered rats were used in groups of 40 each. The rats were injected with multiple infective doses of a virulent strain of *Bacterium tularenses* by the intraperitoneal, subcutaneous, or intradermal routes. Two animals from the normal, vaccinated, and recovered groups were bled for antibody determination, killed, and quantitative plate counts were made on the blood and weighed pieces of tissue. Animals were killed immediately after inoculation, at 12 hours, daily for 4 to 5 days, then at weekly intervals. Half of the animals were used for statistical results on mortality. It is apparent that in the normal animals the organisms invaded the blood stream within a few hours and persisted in large numbers until death. In other tissues there was multiplication of the organisms within 24 hours, increasing to the 3rd or 4th days, the days of greatest mortality in the normal infected animals. By the 5th day any normal surviving rats were convalescent and the organisms were decreasing in number. In the vaccinated and recovered animals the organisms were first found in the regional lymph nodes, in the spleen, and in other tissues, but in fewer numbers than in normal animals. In the convalescent animal the organisms disappear from the tissues in the following order, blood, liver, lymph nodes, and spleen. They may persist in the spleen for 50 days after infection.

**M67. Quantitative Aspects of Streptomycin Therapy in Experimental Tularemia.**

Joseph T. Tamura and William Suyemoto, University of Cincinnati, College of Medicine, Department of Bacteriology, Cincinnati, Ohio.

Preliminary *in vitro* tests in a gelatin hydrolyzate liquid medium, inoculated to give 2 million cells per ml, gave bacteriostatic levels of 0.2 to 0.4 microgram per ml. Visible turbidity during 7 days of incubation was the criterion for growth. When viability was judged similarly after periodic subcultivation to fresh medium, the bactericidal values per exposure periods were 1.0 microgram per ml for 30 minutes, 2 micrograms per ml for 8 minutes, 4 micrograms per ml for 2 minutes, and 6 micrograms per ml for less than 1 minute. The median effective dose of streptomycin was determined for white mice after subcutaneous challenge with 30 to 50 LD₅₀ doses of a strain of maximal virulence. One-quarter of the total streptomycin, calculated for mice in each group of a twofold geometric dosage series, was injected intraperitoneally immediately after challenge, and the remainder in 3 equal doses at intervals of 2 hours. Eight tests and titrations gave ED₅₀ doses from 352 to 358 micrograms. Hence the average ED₅₀ dose for these arbitrary and convenient conditions of challenge and treatment was 350 micrograms of streptomycin per 20-gram mouse, or 17.5 micrograms per gram.

**M68. Universal Serological Reactions with Lipid Antigen in Leprosy.**

Reuben L. Kahn, Flora T. Villalon, and Betty J. Barideau, University Hospital, Serology Laboratory, Ann Arbor, Mich.

It was observed that quantitative precipitation systems with sera and lipid antigens employing salt concentrations lower than physiological (0, 0.15, 0.3,
0.0 per cent) and higher than physiological (1.2, 1.5, 1.8, 2.5 per cent) will give positive reactions after icebox incubation in practically all persons and in animals. In some instances, universal reactions are also obtained on immediate readings of the tests, without incubation. The term "universal" has been applied to these reactions because they apparently represent a common characteristic of all sera. The strength, and serological patterns, of these reactions vary to some extent in different persons and in different animals. The present report deals with results of universal reactions in cases of leprosy. It was found that in tuberculoid leprosy, in which the host's immunity to the disease is presumably high, the precipitation results, without incubation, are practically negative. In lepromatous leprosy, in which the host's immunity to the disease is presumably low, the precipitation results, without incubation, are markedly high. The precipitation results in transitional cases of leprosy are intermediate between these two extremes. It is believed that these findings are of practical value in the diagnosis of the various types of leprosy and in the prognosis of the disease.

M69. A Practical Method of Pertussis Vaccine Assay by Mouse Protective Test. August Holm, E. R. Squibb and Sons, Biological Laboratories, New Brunswick, N. J.

There is no uniformly accepted method of determining the antigenicity of *Hemophilus pertussis* vaccine. The method described here uses Swiss mice, which are given four subcutaneous injections of decreasing vaccine dilutions at an interval of respectively 2, 3, and 4 days, followed by an intraperitoneal challenge dose in mucin suspension 10 days after the last immunizing injection. By using a series of decreasing vaccine amounts for the immunization of the mice, it is possible to determine the LD$_{50}$ point of a certain vaccine, and consequently to assay its antigenicity quantitatively. The test is easy to perform and has given satisfactory results in our hands for several years.

M70. Specific Aggregation of Streptococcal Proteins Adsorbed on Oil Globules. II. Behavior of Acid-precipitable Fraction. D. A. Boroff and L. M. Tripp, Jr., Camp Detrick, Frederick, Md.

A bacterial antigen in solution may possess several specificities, some of which become apparent only under special conditions. Adsorption of a group-specific protein (NPA) derived from streptococci, Lancefield group A, on olive oil changes the reactivity in an agglutination reaction from predominantly group-specific to predominantly type-specific. When this protein is adsorbed on olive oil globules, it behaves serologically and immunologically more like intact streptococci than when it is in solution. NPA adsorbed on olive oil globules induces the production of type-specific antibodies when injected into rabbits, shows a prozone in agglutination reactions in the region of antibody excess, and exhibits cross reactivity in low dilutions of antisera. Adsorption of NPA on a non-streptococcal bacterial surface does not change the specificity of the protein from
that exhibited by NPA in solution. It is suggested that the type-specific activity of NPA complex may be due to Lancefield's substance "T."

M71. Persistence of Antigen at the Site of Inoculation of Vaccine Emulsified in Oil. MIRIAM HERDEGEN, SEYMOUR P. HALBERT, AND STUART MUDD, University of Pennsylvania, Department of Bacteriology, Philadelphia, Pa.

It has been postulated that persistence of antigen in mineral oil emulsion at the site of inoculation permits opportunity for hyperimmunization by the slow, continuous absorption of antigen from the localized mass of vaccine, accounting in part for the adjuvant effect of mineral oil on vaccines. The present work was undertaken to study the persistence of antigen quantitatively, as determined by the ability of the residual antigen removed from the original test animal to stimulate antibody production in other mice to which the antigen was transferred. The correlation between the residual antigen and antibody titer in original test mice was also determined. The results indicate that active antigen persisted at the site of inoculation up to 18 and 24 weeks, depending on the original dose of antigen, but there was a gradual diminution with time in the amount recovered. Only a relatively small amount of this deterioration of antigen at the site of inoculation was paralleled by a fall in the agglutinin titers of the mice receiving the vaccine. The data presented afford quantitative evidence that, for the system studied, prolonged absorption of active antigen is a prime factor involved in the adjuvant effect of the mineral oil menstruum.


An attempt was made to determine precipitin production by alkali-soluble protein extract of Corynebacterium diphtheriae, several derived variants, and Corynebacterium hofmanni. The alkali-soluble protein extracts were prepared according to the method of Wong and T'ung (1939). Significant amounts of nitrogen were obtained from the virulent parent strain, approximately 10 times that of the nonvirulent parent type variants and about 50 times that of the nonvirulent small colony variants. Superimmunization of rabbits was necessary in order to produce high titers. The antiprotein serum from the parent strain showed precipitation not only with the homologous protein extract but with extracts of parent type variants. It exhibits no precipitation with the small nonvirulent variants. The parent type variants showed a low degree of cross reaction with the parent antiserum suggesting alteration in composition or reduction of protein. In the small variants the protein appeared altered beyond detection or destroyed, there being no cross reaction with the parent antiserum. The quantity of protein in the extract was directly correlated with the strain of C. diphtheriae that maintained virulence, there being considerably less in the nonvirulent variants and these also being less capable of producing antibody formation when injected into rabbits.
**M73. Immunization of Mice Against Pneumococcal Pneumonia by Inhaled Polysaccharide.** **Vernon Bryson and Maryda Swanstrom**, Biological Laboratory, Cold Spring Harbor, N. Y.

As part of a research program conducted for the Medical Division, Chemical Warfare Service, an attempt was made to immunize mice via the respiratory route against experimental pneumonia. Eighty CFI female mice weighing about 20 grams were divided in four lots of 20 animals. Twenty of these animals were set aside as controls. The remaining 60 mice were exposed in glass chambers to aerosolized polysaccharide in three equal groups, receiving respectively 1, 5, and 25 milligrams of type I polysaccharide generated as aerosol by nebulization of 3 ml of antigen solution in 60 liters of air during a 15-minute period. The 15-minute exposure to polysaccharide aerosol was repeated on the third and sixth days following initial exposure to antigen. Six days after the final exposure to polysaccharide all animals, including controls, were injected intraperitoneally with approximately $4 \times 10^4$ type I pneumococci suspended in 0.1 ml of sterile broth. On the basis of experiments conducted with radioactive tracers it may be estimated that 0.3 per cent of the dispensed polysaccharide was retained in each animal under conditions of the experiment. Total polysaccharide dispensed and estimated individual dose inhaled may then be correlated with mortality: Group I, untreated; no survivors in twenty. Group II, estimated polysaccharide inhaled per animal, 9 μg; one survivor in twenty. Group III, estimated polysaccharide inhaled per animal, 45 μg; two survivors in twenty. Group IV, estimated polysaccharide inhaled per animal, 225 μg; thirteen survivors in twenty. Immunity against pneumococcal infection by exposure to polysaccharide aerosol has almost certainly been produced in some animals of group IV ($P = <.01$).

**M74. Antigens of Vegetable Origin Active in Pneumococcus Infections.** **Lloyd D. Felton, Benjamin Prescott, Gladys Kauffmann, and Barbara Ottinger**, National Institute of Health, Division of Infectious Diseases, Bethesda, Md.

This investigation was motivated by the fact that bacteria are plantlike organisms, and consequently have certain biological characteristics in common with members of the vegetable kingdom. Both bacteria and plants contain polysaccharides, water-soluble at pH 7.0, and classified in the latter as hemi-, or pseudo-, or reserve cellulose. The immediate objective was to determine whether these plant polysaccharides have antigenic properties similar to those of bacteria, especially pneumococci. Thus far 59 members of the vegetable kingdom have been studied. Soluble products were obtained with definite immunological activity as measured by both precipitin tests and active immunity in mice against virulent pneumococci. Analysis of the active fractions, with two exceptions, showed the presence of hydrolyzable polysaccharides with sugar content ranging from 5 to 49 per cent. Both viscosity and optical rotation were measured. However, the degree of correlation was low between these results and the degree of antigenicity. Although of relatively low titer as measured by precipitin reactions, most of these samples stimulated active immunity in mice.
against from 100 to 1,000,000 lethal doses of virulent pneumococci. Fractions from collard, Irish moss, sunflower seed, tomato, and wheat germ were of this maximum titer. In some cases a single preparation produced active immunity against three types of pneumococci, types I, II, and III, the only types thus far studied. Preliminary tests indicate that this polyvalent characteristic may be due to a mixture of type-specific components. In no instance were precipitins or active immunity of as high titer as those of the antigenic polysaccharide of the pneumococcus secured. It has been observed that 0.01 µg increases resistance of mice to the degree that they survive at least 1,000 lethal doses of virulent pneumococci.


For economical and efficient large volume production of anti-Hemophilus influenzae type B rabbit serum, it is important to be able to predict the mortality and expected yield from a given number of animals. These predictions are aided by collection and analysis of data from a large number of hyperimmunized rabbits which were subject to specified production procedures. Rabbits were started on a schedule of hyperimmunization in groups of 200, and the group mortalities were recorded from day to day for more than 10,000 rabbits. This procedure permitted construction of three mortality curves from which it is possible to predict the volumes of blood obtainable at various periods of immunization, depending upon the time at which the first production bleedings are taken. Since the greatest mortality occurs during the weeks that production bleedings are taken, it is important to correlate mortality and potency in order to determine the most economical time at which to take the first production bleedings. A tabulation has been prepared which correlates potency and mortality studies, and the data indicate that, within the specifications of the production methods employed, it is most economical to take first production bleedings during the eleventh week of hyperimmunization instead of the fifth or eighth week, and the total yield of antibody units is relatively the same whether first production bleedings are taken on the fifth, eighth, or eleventh week.

M76. Studies on the Effect of Immune Reactions on the Respiration of Bacteria. I. Methods and Results with Eberthella typhosa. M. G. Sevag and Ruth E. Miller, University of Pennsylvania, Department of Bacteriology, and Woman's Medical College of Pennsylvania, Philadelphia, Pa.

The effect of homologous immune serum with and without complement on the oxygen consumption by Eberthella typhosa (strains O-901 and H-901) and pneumococcus has been studied. A method has been worked out which makes it possible to calculate QO₂ values (mm³ O₂ per mg bacteria per hour) for intact, ag-
glutinated, and lysed fractions of bacteria. Agglutinated E. typhosa and pneumococci consume volumes of oxygen equal to those of the respective controls. Apparently in intact sensitized bacterial cells, the activity of the intracellular enzymes is not affected by this reaction. Sensitized E. typhosa (O-901) cells acted upon by complement undergo lysis, eliminating the cell wall barriers to the action of the immune factors on intracellular enzymes. Immediately after lysis the liberated enzymes use considerably more oxygen than the controls containing the intact cells. Subsequently, the oxygen consumption of the lysed system undergoes up to 88 per cent reduction. In systems containing unlysed bacteria, oxygen consumption in the presence of glycerol alone or yeast extract and glucose is markedly greater than when glucose alone is used. Under these conditions, the reduction in oxygen uptake of the lysed systems is likewise much greater and prompter. Potassium cyanide causes 90 per cent inhibition of oxygen consumption by the unlysed and lysed cells, showing that the oxygen consumption in lysed systems is mediated by intact and oxidative enzymes.

M77. Contribution to B.C.G.: Experiments on Bovines. ALBERTO A. ASCOLI, Cornell Medical College, Department of Public Health and Preventive Medicine, N. Y.

The Instituto Vaccinogeno Antitubercolare provided the first definite proof that B.C.G. protected the bovine species against natural infection through exposure and that a reduction of the disease to a half could be obtained by means of annual revaccinations. However, even when given its best chance, B.C.G. failed to provide complete protection to calves which were confronted with uninterrupted exposure for 10 months offered by facing cows affected with open tuberculosis. Better results can hardly be expected from the cross immunization one has to deal with, when B.C.G., an attenuated bovine strain, is being administered to human beings as a prophylactic against infection with the human type of Mycobacterium tuberculosis. Attention is called to the influence of other extrinsic factors, mainly age, on the development of the specific resistance. Under the circumstances you are bound to question whether, in their anxiety to ensure its harmlessness, Calmette and Guerin did not go too far in the attenuation of the virulent bovine strain; if so, it is understandable why the peak of immunity developed by the B.C.G. might not reach at all the level giving complete protection. In such a case a stronger vaccine, that is, a less attenuated strain, ought to be resorted to. Actually several less attenuated strains have been prepared by the IVA and tested on bovines for their harmlessness and efficiency.

M78. Streptomycin Dosage Schedules. ERNA ALTURE-WERBER AND LEO LOEWE, Jewish Hospital, Department of Laboratories, Brooklyn, N. Y.

Streptomycin blood levels were assayed following fractional, intramuscular administration of the antibiotic. The method of assay, previously described
by the authors, was made sensitive by utilizing *Klebsiella pneumoniae* as the test organism. With this method, detectable amounts of streptomycin were found for 24 to 36 hours following a single intramuscular injection of 250 to 1,000 micrograms. Optimum dosages were predicated on *in vitro* streptomycin sensitivity tests of the infective organisms. Although both bacteriostatic and minimal lethal dosages were estimated, clinical dosage schedules were based on the latter. The peak levels after 500 milligrams were suitable for many streptomycin inhibitable organisms, and endured for at least 6 hours. A rational method for planning the intermittent, intramuscular injection was thus established.

**M79. The in Vitro Streptomycin Sensitivity of Salmonella Isolated from Cases and Carriers in Massachusetts.** George E. Foley and A. Daniel Rubenstein, The Children's Hospital and Infants' Hospital, and Massachusetts Department of Public Health, Department of Pathology, Boston, Mass.

Seven strains of *Eberthella typhosa* and 57 strains of *Salmonella*, covering 15 species, all recently isolated from cases and carriers in Massachusetts, have been studied for *in vitro* sensitivity by titration with varying concentrations of streptomycin in tryptic digest broth. The species examined were *S. typhi-murium* (24), *S. thompson* (6), *S. paratyphi B* (6), *S. newport* (4), *S. enteritidis* (3), *S. montevideo* (3), *S. minnesota* (2), *S. st. paul* (2), *S. tennessie* (2), and one strain each of *S. derby*, *S. manhattan*, *S. morbilliformis-bovis*, *S. newington*, *S. oregen*, and *S. oranienberg*. All strains were inhibited by streptomycin concentrations ranging from 0.004 to 0.064 μg per ml. Although there seemed to be significant differences in the sensitivity of individual strains of the same or different species, no species was more susceptible or resistant than other species. In general, strains isolated from related cases or carriers in different areas of Massachusetts exhibited similar *in vitro* sensitivity. The range of *in vitro* streptomycin sensitivity of freshly isolated salmonellas is essentially similar to that previously observed in a survey of a collection of stock strains.

**M80. Prolongation of Penicillin Activity in Animals.** Roger D. Reid, Hyndson, Westcott & Dunning, Inc., Biological Research Division, Baltimore 1, Md.

*In vitro* studies have disclosed a few substances that have the ability to protect penicillin from the action of penicillinase and penicillinaselike enzymes. These studies have been continued with special reference to prolongation of penicillin levels in serum and enhanced protection of infected animals given penicillin in conjunction with such compounds. This report will summarize these investigations and will survey the possible therapeutic application of the compounds studied.

**M81. A Preliminary Report on a Selective Medium for the Isolation of Pathogenic Fungi.** Paul J. Boening and Norman C. Laffer, Bowey's, Inc.,
The mycology section of this laboratory was confronted with a suspected case of maduromycosis, in which much secondary infection was evidenced. It was decided to try the medium suggested by Thompson (1945), which utilizes 10 units of streptomycin per ml and 2 units per ml of penicillin in an attempt to isolate the causative organism. Thompson's medium was tried in the manner suggested in his publication and the infectious material, curetted from the lesions of the suspected maduromycosis, streaked on the plates. The secondary bacterial invader grew in abundance, so it was decided that higher concentrations of the antibiotics mentioned should be utilized. To determine the optimum antibiotic concentrations necessary, several series of media were prepared using increasing amounts of streptomycin and penicillin. The infectious material from the suspected maduromycosis, Blastomyces dermatitidis, Candida albicans, Sporotrichum schenkii, Cryptococcus neoformans, Histoplasma capsulatum, Coccidioides immitis, Monosporium apiospermum, Blastomyces brasiliensis, and several strains of gram-negative and gram-positive bacteria were inoculated onto these plates. One lot of media was prepared using no antibiotics at all as a control. The information presented in this paper indicated that a medium containing heart infusion agar base with 6 per cent human blood, 25 units of streptomycin, and 6 units of penicillin per ml should be satisfactory for the isolation of pathogenic fungi from infectious material.

**M82. Studies on the Causal Agent of Granuloma Inguinale.** R. B. Dienst, C. R. Reinstein, H. S. Kupperman, and R. B. Greenblatt, University of Georgia School of Medicine, Department of Medical Microbiology and Public Health and Endocrinology, Augusta, Ga.

The present investigation presents method used to isolate and cultivate Donovan bodies from patients with granuloma inguinale. Preliminary report is given on reproducing clinical symptoms in human volunteers using pure cultures of organisms grown in yolk sac of developing chick embryo.

**M83. Morning Versus Evening Rectal Temperature Response in Rabbits.** H. E. Wright and I. B. Dorrell, Schenley Laboratories, Inc., Physiological Control Laboratory, Lawrenceburg, Ind.

This investigation was undertaken to determine whether rabbits could be used for pyrogen testing at night as well as during the day. Control rectal temperatures of ten male New Zealand rabbits in the weight range of 2,200 to 2,700 grams were taken and recorded in the morning and again in the evening, 5 days a week for 6 weeks. The animals were housed in an air-conditioned and fluorescent-lighted environment throughout the test. Pyrogen tests were run on the same rabbits both in the morning and at night 5 days a week for 2 weeks. There was only a slight increase in evening rectal temperature response over morning temperature response in both the pretest and pyrogen-testing periods. All
temperatures remained within the normal range as prescribed by the U. S. P. XII. There was no significant change in the weight of the animals during the 6-week control period nor during the 2-week pyrogen-testing period. These data seem to indicate that rabbits can be used for pyrogen testing at night as well as during the day. Further studies with actual pyrogenic stock are contemplated using rabbits as test animals both in the morning and at night.

**M84. Cutaneous Reactions in Persons Suffering from Diverse Diseases Following Intradermal Injection of Streptococcal Antibody and Antigen.** Edward C. Rosenow, Longview Hospital, Bacteriologic Research Laboratory, Cincinnati, Ohio.

The study concerns the isolation of specific types of alpha streptococci, and production and intradermal use of natural and artificial antibody and of antigen to determine the presence of respective specific streptococcal antigen and antibody in the skin or blood of persons ill. Pure cultures of specific types of streptococci were obtained from the end point of growth of serial dilution cultures in glucose brain broth of material obtained from nasopharynx, tonsils, or infected teeth. The centrifugated organisms from large volumes of glucose broth were preserved in dense suspensions in two parts glycerol and one part saturated NaCl solution.

Horses were immunized and artificial antibody obtained, using antigen prepared from appropriate dilutions of these dense suspensions. Ten per cent solutions of the euglobulin fraction of the serum of immunized horses and the supernatant of suspensions of 10 billion streptococci per ml that had been autoclaved for 96 hours were used as natural and as artificial antibody in cutaneous tests for the detection of antigen and the supernatant of corresponding suspensions heated to 70° C for one hour was used for the detection of antibody in skin or blood. Cutaneous reactions indicating the presence of specific antigen were consistently obtained with natural and artificial antibody at all stages of the disease, often proportional to its severity, and specific antibody during convalescence in persons suffering from influenza, other respiratory infections, “virus” pneumonia, encephalitis, poliomyelitis, arthritis, epilepsy, and schizophrenia. Reactions in well persons tested as controls did not occur or were relatively slight.

**M85. Differences in Strains of Rickettsia orientalis as Demonstrated by Cross-Vaccination Studies.** Fred L. Rights, Joseph E. Smadel, and Elizabeth B. Jackson, Army Medical Department Research and Graduate School, Department of Virus and Rickettsial Diseases, Washington 12, D. C.

Scrub typhus vaccines were prepared from lungs and spleens of white rats infected with Imphal, Karp, Kostival, or Mite 21 strain of *Rickettsia orientalis* and assayed by methods previously described. Groups of mice were immunized with these four vaccines and tested for resistance to infection, with the
homologous strain. In addition, groups of mice immunized with each vaccine were challenged with one of seven heterologous strains of the organism. Most of the vaccines protected against 1,000 to 10,000 MLD of the homologous strain. In practically all instances greater protection was elicited against the homologous than against the heterologous organisms. All four types induced immunity to Imphal, Karp, and Kostival rickettsiae. Mite 21 vaccine provided protection against these three strains, but vaccines prepared from the latter failed to protect against Mite 21. None of the four vaccines immunized mice against the Seerangayee strain. Some but not all of the vaccines protected against Wild Rat 235, Volner, and Pescadores strains. Thus, strain differences in \textit{R. orientalis} are demonstrated by cross-vaccination studies as well as by studies employing neutralization, complement-fixation, and antitoxin tests. Despite these demonstrable differences, mice recovered from infection with any one of the strains studied are solidly immune to challenge with the heterologous strains. No correlation existed between the antigenic relationships of the strains and their geographical origins. The need for a broadly antigenic strain for use in preparing vaccine for immunization of human beings will be discussed.

\textbf{M86. Differences in Strains of \textit{Rickettsia orientalis} as Demonstrated by Cross-Neutralization Tests.} \textsc{Byron L. Bennett, Joseph E. Smadel, and Ross L. Gauld}, Army Medical Department Research and Graduate School, Department of Virus and Rickettsial Diseases, Washington 12, D. C.

Previous work performed in this laboratory indicated that differences in strains of \textit{Rickettsia orientalis} were demonstrable by means of cross-neutralization, cross-vaccination, and cross-antitoxin tests. Bengtson has also demonstrated differences in strains of this organism by complement-fixation tests. Ten strains of \textit{R. orientalis} recovered from man, mites, or rodents from widely scattered areas in the South Pacific and Orient were used to prepare antisera in rabbits and to perform neutralization tests with these sera in mice. Pooled immune rabbit sera protected against the homologous strain in each instance but provided variable protection when tested against the other nine heterologous strains. Antisera against certain of the strains, i.e., Seerangayee and Gilliam, protected little if any against infection with the heterologous strains. On the other hand, Volner and Buie antisera contained substances which provided at least some protection against practically all of the heterologous organisms. Present information is insufficient to arrange an immunological pattern but does indicate that certain strains occupy an intermediate position between those which are very broad and those which are very narrow. Furthermore, the data are consistent with findings obtained by other techniques which indicate that antigenic variations exist among strains now grouped under \textit{R. orientalis}.

\textbf{M87. The Practical Application of an Oiling Program in the Control of Respiratory Disease.} \textsc{I. L. Shechmeister and Francis S. Greenspan}, University
of California, Department of Bacteriology, Berkeley, Calif., and The New York Hospital, New York, N. Y.

This study, carried out under the auspices of the U. S. Navy, dealt with the role of oiled floors and blankets in control of certain respiratory diseases. The group chosen for the investigation consisted of approximately 2,400 men, who were divided equally into an experimental and a control group. The floors of the barracks housing the experimental group were treated with a 20 per cent germicidal oil-water-rococal emulsion, while the blankets were impregnated with 2 to 3 per cent (by weight) of a similar preparation. Periodic determinations were made of the bacterial content of dust, air, and blankets, as well as of the dispensary admission rates and the carrier rates for beta hemolytic streptococci. The results indicated that the above treatment of floors and blankets (1) reduced the number of organisms in the air 33 to 63 per cent; (2) sharply reduced the total amount of dust in the oiled environment, although it apparently did not change the beta hemolytic streptococcus count per gram of dust—isolated streptococci were mostly nonpathogenic, only 5 per cent being typed in group "A"—(3) caused a slight but significant reduction in the beta hemolytic streptococcus carrier rates in the group living in oiled environs; (4) seemed to cause a reduction in the number of cases of respiratory disease during periods of low respiratory disease incidence, but had no effect during a period of high respiratory disease incidence.


Preliminary laboratory procedures involving the use of relatively small groups of rabbits had indicated that the titer of the plasma of hyperimmunized rabbits reached a level of 0.4 to 0.6 mg of agglutinin nitrogen per ml by the fifth week. In order to determine some of the factors which contributed to yields of relatively low potency plasma when a large scale production schedule was followed, an analysis is made of data from over 1,000 rabbits used in production of anti-Hemophilus influenzae type B rabbit serum. From the production data a potency curve is drawn, from which it is possible to follow the average titer of production groups at various periods of immunization. This curve indicates that the average rise in titer is much slower in large groups of rabbits than is the case with individually selected animals which, by small groups, are the subjects of assiduous laboratory experiments. For the large groups of animals, it is profitable to omit the fifth and eighth week bleedings. The plasma volumes obtained from the groups at various periods of immunization are given, and by means of correlation of these volumes and the potency curve it is found that the average potency of combined plasma from all bleedings over a period of 20 weeks is 0.30 mg of antibody nitrogen per ml. When refined and concentrated, this plasma yields a final product which contains 3.0 mg of antibody nitrogen per ml.
M89. Localization of Radioactive Azoprotein in Tissues. HERBERT J. WELSH-IMER, GRANT L. STAHL, and WILLIAM G. MYERS, The Ohio State University, Department of Bacteriology and Medicine, Columbus 10, Ohio.

Para-aminobenzenestibonic acid labeled with radioactive antimony was coupled with purified egg albumin by diazotization. Following the injection of this substance the localization of a defined protein in the animal body was observed by sacrificing the animals, removing the various tissues, and determining their radioactivity by means of a Geiger-Müller counter.