

Crystal violet, methylene blue, gentian violet and brilliant green were shown to be the growth inhibiting substances in the media. Bile was found to decrease the toxic action of the dyes used and to accelerate the growth of "false test organisms" in media in which oxgall was used.

Small numbers of colon organisms were able to initiate growth and produce gas in brilliant green bile broth. Small numbers of these organisms did not grow and produce gas in Salle's crystal violet broth.

Many bacteria responsible for "false tests" were able to grow in these media. The addition of 1 cc. of sterile milk to tubes of the media materially increased the number of "false test organisms" which were able to grow. Failure to inhibit the growth of these gas-producing bacteria, not known to be of any public health significance, is a major weakness in the media.

*G34. Eosin Methylene Blue Smear Agar for Rapid Direct Count of E. coli.*

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The standard eosine methylene blue agar for the confirmation of *E. coli* has been used for the direct count.

The obvious difficulties involved in using this medium for direct count by the pour method have led us to employ a smear method which gives more characteristic surface growths. The various dilutions are smeared on the hardened surface of this medium, allowed to dry and incubated for 24 hours.

After 24 hours incubation characteristic colonies are counted. Good distribution was observed in all cases, and very few other organisms developed. The counts obtained in 24 hours were consistently higher than those by the 48-hour brilliant green tube method but proportional to them. Incubation for an additional 24 hours gave few additional *E. coli* colonies on the plates.

MEDICAL BACTERIOLOGY, IMMUNOLOGY AND COMPARATIVE PATHOLOGY

*M1. The Place of Bacterial Allergy in the Immunization Process.* L. DIENES, Massachusetts General Hospital, Boston.

The immunization process does not start with the production of antibodies and their diffusion in the tissues and body fluids, but with a period of pure tissue hypersensitiveness the development of which precedes the production of circulating antibodies a few to many days. Guinea pigs after injection of a few milligrams of egg white give a slight

but definitely positive skin reaction as soon as the fourth day after injection, while the anaphylactic sensitiveness and antibodies do not appear usually before the eighth day. Similar observations were made on rabbits and on man. These slight early reactions differ considerably from the later reactions (a few weeks after treatment) and from the reactions of passively sensitized animals. The early reactions are delayed and permanent; they are similar in every respect to slight tuberculin reactions, in contrast to the quickly developing wheal characteristic of passively sensitized animals. If tuberculous guinea pigs are sensitized with egg white, under appropriate conditions, at the end of the first week the slight early reactions are replaced by strong necrotic reactions corresponding in every respect to strong tuberculin skin reactions. Histological studies furnish further evidence for the connection between the early slight reactions and bacterial allergy. For the slight tuberculin reaction and the early reactions, a slowly developing but eventually intensive cellular infiltration is characteristic, in which mononuclear cells predominate. The skin reactions of passively sensitized animals are markedly different, being characterized by quick exudation of serum and polymorphonuclear cells.

Bacterial allergy is the strong development of the early phase of the specific response to antigen preceding the production of circulating antibodies. Every active immunization process passes through this phase, but its development is strongly influenced by certain infectious diseases. The influence of these diseases was proven by treating animals with indifferent antigens such as egg white or horse serum during the disease, and it was also observed that the anatomical lesions play an important rôle in this effect. In a tuberculous guinea pig the most effective method to produce a strong tuberculin type of sensitiveness is to inject the egg white into the tuberculous lesions.

To form an opinion of the rôle which allergy plays in the immunity and the healing of infectious diseases, it is important to keep in mind that the allergy produces mainly a cellular reaction in the tissues which come in contact with the antigen, consisting mostly of mononuclear cells. In those diseases in which allergy persists, as in tuberculosis, the defensive reaction is mainly a tissue reaction. Once antibodies are formed, the reaction in the tissues to the antigen is a quick exudation. The influence of allergy on the tissue reaction is probably present also in acute diseases. But the allergy probably serves a more general purpose. The inefficacy of serum therapy in most diseases makes it very probable that the active immunization of the tissues, and not the diffusion of

antibodies, is the most powerful factor in the healing, and the bacterial allergy is a manifestation of the early phase of the active immunization process.

The immunity response during the disease differs in many respects from the response observed in normal animals after the introduction of simple antigens.

*M2. Duration of Local Skin Reactivity Induced by Bacterial Filtrates.*

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It was reported in early publications that the state of local skin reactivity to bacterial filtrates disappears within 48 hours following the intradermal injection of preparatory bacterial factors. Subsequent experiments demonstrate the following:

The duration of reactivity depends upon the mode of preparation of filtrates and the microorganisms employed. Thus, it lasts for 96 hours with meningococcus "agar washings" filtrates; for 72 hours with *B. typhosus* "agar washings" filtrates; for 48 hours with *B. typhosus* broth culture filtrates, and only for 24 hours with *B. typhosus* "agar washings" filtrates previously heated in the Arnold sterilizer for 20 minutes. Comparative titrations of these preparations show that the duration of vulnerability is in direct relationship to the strengths of the preparations employed.

It was also previously reported that the skin sites prepared by bacterial filtrates undergo severe hemorrhagic necrosis when acted upon by toxic principles resulting from intravascular interaction of non-bacterial antigens with homologous antibodies. This interaction can be obtained either by separate intravenous injections of the antigen and the antibody, or by intravenous injections of an antigen into rabbits possessing active acquired homologous antibodies. It was of interest to study the duration of local skin reactivity induced by bacterial filtrates to these antigen + antibody complexes. The duration of vulnerability was studied weekly in rabbits sensitized by one to 4 repeated weekly injections of horse serum. The state of reactivity disappeared uniformly in sensitized rabbits, 96 hours after the intradermal injections of a meningococcus "agar washings" filtrate.

In dilution, the skin-preparatory potency of *B. typhosus* "agar washings" was titrated in normal and horse serum-sensitized rabbits. Rabbits received intradermally 0.25 cc. of dilutions ranging between 1:50 to 1:300 and 24 hours later intravenously 1 cc. per kilo of body weight

of dilution 1:100. The skin-preparatory titer was the same in normal and sensitized rabbits (800 to 1000 units per 1 cc.).

It is definitely shown, therefore, that the duration of the local skin reactivity elicited by bacterial filtrates is, first, in direct relationship to the potency of these filtrates and that second, it has no apparent bearing upon the anaphylactic sensitization (i.e. Arthus phenomenon) to animal proteins.

*M3. The Relationship of the Potency and the Antigenicity of the Tuberculin Protein to its Molecular Size.* FLORENCE B. SEIBERT, The Henry Phipps Institute of the University of Pennsylvania, Philadelphia.

Various protein fractions obtained from tuberculin differing widely in their physico-chemical properties, such as precipitability, solubility, filtrability, heat coagulability, etc., are nevertheless equally potent in their ability to elicit the specific tuberculin skin reaction. On the other hand, the antigenicities, i.e., the ability to elicit precipitins and the Arthus reaction in normal animals by repeated injections of these antigens, are quite different. For example, the fraction made by ammonium sulfate precipitation is an excellent antigen, better, in fact, than the well-known crystalline egg albumin, serum proteins, etc., while fractions isolated from OT or made by heating an antigenic fraction of tuberculo-protein with weak alkali are completely lacking in antigenicity. Molecular weight determinations by means of osmotic pressure measurements show in these fractions variations as great as 25,000 to 2,000. Moreover, there is a close parallelism between the size of molecule and its antigenicity. In other words, the antigenicity depends upon the size of the molecule, whereas the specific tuberculin skin potency is independent of the size of the molecule, and appears to reside in a small unit of the whole molecular complex.

Just as it has been possible to abolish antigenicity by disaggregating the large into the small molecules without loss in specific skin potency, so it should be possible again to build up the large antigenic molecule from the small non-antigenic pieces. In view of the fact that antigenic properties of toxins can be improved by adsorption to inert colloids, as shown by Glenny and coworkers and many other investigators, and that the precipitinogen properties of antigens in general were increased by Hektoen and Welker through adsorption to aluminium hydroxide, a study of the effect of adsorbing the small molecular tuberculin molecule to aluminium hydroxide was made. It was found that such adsorption

(i.e., to aluminium hydroxide or to charcoal) rendered the previously non-antigenic fraction of molecular weight of about 4,000 capable of eliciting in normal rabbits as high a precipitin titer as the large antigenic molecule of molecular weight 25,000 and also increased its ability to provoke the Arthus reaction. Moreover, some degree of inhibition in the *in vitro* precipitin reaction could be obtained by means of the small non-antigenic molecule. These results strongly suggest a similarity between the small molecular specifically potent molecule (as probably exists in OT) and haptens in the sense of the word as used by Landsteiner. That is, it is practically unable by itself to elicit antibodies, but is capable of acting specifically upon antibodies when they are present, as in the normal sensitized or in the tuberculous animal.

*M4. The Relation of Antibody Content to Allergy and Resistance in Animals Vaccinated with B.C.G.* B. J. CLAWSON, Department of Pathology, University of Minnesota.

Rabbits were vaccinated by injecting them with from 1 to 2 mgm. of B.C.G. four times at weekly intervals. Some animals were injected with living organisms and others with heat-killed organisms. Injections were given subcutaneously in some animals and intravenously in others with living and heat-killed organisms, respectively.

The degree of allergy, if present, was determined by the Mantoux test. The amount of resistance developed was observed by comparing the length of life and the degree of tuberculosis, if present, in vaccinated and normal animals infected with a virulent bovine strain of the tubercle bacillus.

The concentration of the antibodies in the blood was estimated by the complement fixation, agglutination and opsonic index tests.

The experiments showed a correlation between the antibody content and resistance, but there was no definite proportionate or necessary relation between the presence of allergy and antibodies.

*M5. Tissue Extracts and Anaphylactic Shock.* L. GERBER AND E. GERWE, The Wm. S. Merrell Co., Cincinnati, Ohio.

When saline extracts of lung, muscle, kidney or other tissues are injected into the veins of a rabbit or guinea pig, a series of symptoms arises that so markedly simulates true anaphylactic shock that some confusion still persists concerning the two phenomena. Differentiation of death from thrombosis produced by tissue extracts and that from acute anaphylactic shock may be effected by (1) the use of a zoologically

unrelated species for sensitization; tissue extracts do not exert a coagulant effect *in vivo* on unrelated species, (2) the presence of dark red, organized blood clots in the right heart of all animals that have been killed by tissue extracts, (3) the constancy of the characteristic lesion of acute anaphylactic shock in the guinea pig; completely emphysematous lungs that appear rarely with thrombosis produced by tissue extracts, and (4) a dissimilarity in the syndrome when pigeons are employed as experimental subjects.

*M6. Loewenstein's Method of Culturing B. tuberculosis.* L. M. KOPELOFF, N. KOPELOFF, L. E. HINSIE, AND J. L. ETCHELLS, Department of Bacteriology, Psychiatric Institute and Hospital, New York.

Because Loewenstein has claimed that *B. tuberculosis* may be cultured by his methods from 40 per cent of dementia praecox patients we arranged to send him samples from such patients while attempting to culture duplicate samples in our own laboratory.

In most instances triplicate samples of blood from patients with mental and physical disease as well as from control subjects were sent to Loewenstein as individual specimens of unknown origin. Among 60 controls 5 positive macroscopic cultures of *B. tuberculosis* were reported as coming from healthy young males in whom there had been no demonstrable tuberculous infection. No macroscopic cultures of *B. tuberculosis* occurred in 25 cases of active pulmonary tuberculosis or 24 cases of eye infection (2 of which were diagnosed tuberculous retinitis). Twenty-seven positives were reported in 96 cases of dementia praecox and 7 positives in 19 miscellaneous psychoses and neuroses.

The above data contradict Loewenstein's previous claims of: (a) a high incidence of positive blood cultures of *B. tuberculosis* in febrile pulmonary tuberculosis and (b) in dementia praecox; and (c) a complete absence of positive findings in controls.

In our own laboratory by rigidly following Loewenstein's method on 375 blood samples from the same patients we were unable in any instance to culture *B. tuberculosis*.

Of 8 spinal fluid specimens from suspected cases of tuberculous meningitis 6 yielded positive cultures of *B. tuberculosis* on Loewenstein medium in 16, 23, 27, 45, 52 and 125 days, respectively.

Loewenstein's results reported here and our failure to obtain any positive blood cultures indicate that there is no justification for our further study of patients with dementia praecox by his methods.

*M7. The Rôle of Mucin in the Production of Experimental Lobar Pneumonia in the Rat.* W. J. NUNGESTER AND L. F. JOURDONAIS, Department of Bacteriology, Northwestern University Medical School.

A chance observation led to the finding that the virulence of bacteria could be greatly increased if the organisms were suspended in sterilized gastric mucin for inoculation. The possibility that mucin might be a factor in spontaneous disease was suggested by this finding, and the rôle of mucin in experimental pneumonia has been investigated with the following results. Forty-nine white rats were inoculated intratracheally with 0.00005 or 0.000005 cc. of a 24-hour glucose broth culture of a type III pneumococcus suspended in 0.5 cc. of mucin. Forty-three of these died, of which 32 had an entire lobe consolidated, 9 had over 50 per cent of a lobe consolidated and two had less than 50 per cent of a lobe consolidated. The lesions resembled grossly and histologically the various stages of lobar pneumonia except the stage of resolution. Fibrinous pleurisy and pericarditis were commonly seen.

Similar doses of the organism suspended in saline were inoculated intratracheally into 35 rats. Three of these died. Consolidation was noted in these and in 3 others that were sacrificed. Of 31 rats inoculated with mucin alone 1 died. A mild reaction to the mucin inoculation was noted in 13 of the 30 animals sacrificed.

The foregoing results indicate that mucin in the respiratory tract may play an important rôle in the pathogenesis of lobar pneumonia. It is believed that the mucin acts in the same way as the starch used by Terrell and Robertson in their study of experimental pneumonia in the dog and that in both instances there is mechanical interference with the normal defense agents of the body, probably the phagocytes.

*M8. The Virulence of H. pertussis.* JOHN A. TOOMEY, City Hospital and Department of Pediatrics, Western Reserve University, Cleveland, Ohio.

When the minimal lethal dose of freshly isolated hemolytic *H. pertussis* organism is ascertained for the guinea pig and then comparative weights of wet, freshly isolated, non-hemolytic organisms are injected intraperitoneally in other animals, it is found that the non-hemolytic organisms are as virulent as the hemolytic ones. *H. pertussis* cultures decrease in virulence after they become acclimated to veal brain, plain agar or any other bloodless medium, but there is a point reached after which there is no further loss of virulence no matter how much sub-

transplanting is done. In fact, *H. pertussis* never becomes totally avirulent even though the organism may lose its power to produce massive agglutinins in the rabbit's serum and may change in morphology to 5 to 10 times the size of the originally isolated organism (55 strains studied). From a virulence standpoint, this final stage of lessened virulence could be termed the fixed or R stage of the organism's existence. Since the changed organisms are approximately 5 times the size of freshly isolated ones and one-fifth as virulent there is a possibility that equal numbers of organisms, irrespective of weight, may be equally virulent. Based on virulence tests, *H. pertussis* organisms retain their original characteristics when grown for over 2 years on potato blood agar. There is a modification of this virulence after as few as 10 sub-transplants on veal brain or plain agar, but not so soon on chocolate brown agar medium.

*M9. A Comparison of the Incidence and Biological Characteristics of the Hemolytic Bacillus coli Recovered from the Intestinal Tract of Healthy Individuals and Patients with Ulcerative Colitis.* EDITH E. NICHOLLS, New York Hospital-Cornell University Medical College.

In a previous article the author reported the incidence and biological characteristics of the hemolytic *Bacillus coli* in the stools of healthy individuals (J. Clin. Invest. 13: 479, 1934). The present report consists of a similar study of samples of feces removed from ulcers by swabs introduced through the proctoscope in cases of ulcerative colitis and a comparison of the results with those found in healthy people.

Fifty smears from 27 patients with ulcerative colitis were cultured for the presence of *Bacillus coli* and the hemolytic type was found in 37 or 74 per cent of the samples. This is only slightly higher than the 64 per cent found for healthy individuals. In the first or single specimens cultured, the incidence of hemolytic *Bacillus coli* was 66 per cent for the colitis cases and 56.2 per cent for the normals. Where two or more specimens from the same individual were studied, the figure rose to 100 per cent for the former and 88 per cent for the latter.

The hemolytic *Bacillus coli* recovered from patients with ulcerative colitis showed no greater virulence for white mice than those recovered from controls.

The *Bacillus coli* from both groups appeared to be heterologous strains having agglutinins more or less in common.

In cases of ulcerative colitis the incidence of hemolytic *Bacillus coli* is



similar to that found in stools of healthy people and the organisms have the same biological characteristics.

*M10. The Isolation of Neisseria gonorrhoeae.* ALICE D. LEAHY AND CHARLES M. CARPENTER, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.

The isolation of *Neisseria gonorrhoeae* is attempted in all patients suspected of having gonococcal infections. Observations have been made on the suitability of different media, on various atmospheric requirements and temperature of incubation, and on the value of the "oxydase reaction," described McLeod and his associates (McLeod, J. W., Coates, J. C., Happold, F. C., Priestly, D. P., and Wheatley, B., J. Path. and Bact., 39: 221, 1934) for identifying gonococcus colonies in mixed cultures.

Chocolate blood agar plates incubated at 37°C. in an atmosphere containing 10 per cent CO<sub>2</sub> yielded the greatest number of positive cultures. Because a few strains failed to grow under these conditions, it was necessary to incubate duplicate cultures at 34°C. in a normal atmosphere.

Prior to the use of this method, 40 cultures of *N. gonorrhoeae* were isolated from 155 patients with gonococcal infections (25+ per cent). By employing all of the above factors, 42 strains were isolated from 74 patients (56+ per cent).

The "oxydase reaction" alone demonstrated 13 of the 42 positive cultures, which could not be identified by direct macroscopic examination of suspected colonies followed by microscopic examination of smears. The use of these procedures resulted in only one failure to isolate the gonococcus from 30 patients when smears from the accompanying inoculum showed the presence of Gram negative diplococci.

*M11. Endogenous Infections by Clostridium welchii.* LUCILLE C. LYNCH AND PAUL F. CLARK, Department of Pathology and Medical Bacteriology, University of Wisconsin Medical School, Madison

The uncertainty as to the source of the etiologic agent of certain post operative cases of gas gangrene developing at the Wisconsin General Hospital has brought into question the pathogenesis of this type of the disease. The possibility of an endogenous origin of the organism suggests that physical or chemical factors introduced at operation may alter the environment so that the growth of *Clostridium welchii* is stimulated.

Our work has been directed along the following lines:

1. Distribution of *Clostridium welchii* in the animal body.

a. *Clostridium welchii* was isolated from 12 of 14 human livers obtained at autopsy; 8 of the specimens were removed within 6 hours post mortem. In only one case was liver pathology present.

b. *Clostridium welchii* was isolated from 8 of 15 livers removed during life from dogs during operative procedure.

c. *Clostridium welchii* was isolated from the meconium of 10 infants during their second day of life at the Wisconsin General Hospital.

2. Experimental endogenous infections with *Clostridium welchii*.

a. *Clostridium welchii* was isolated from peritonitis produced in laboratory animals by the intraperitoneal injection of sterile bile.

*M12. Effect of Dissociation on Specificity of Hemolytic Streptococci.*

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Earlier papers have shown that hemolytic streptococci isolated during the acute stage and from complicating lesions of scarlet fever and those from erysipelas form distinct immunologic groups, to neither of which belong those from septic sore throat. The streptococci from each of these three diseases were observed to produce distinctive types of colonies on chocolate agar which is not changed in color by typical scarlatinal streptococci, but is turned a vivid green by those peculiar to erysipelas and septic sore throat.

Dissociation of some of these strains produces a variety of colonies including mucoid and rough, which immunologically differ from these in the original culture. Many of these dissociants can be reverted to the original type, colonially and immunologically, by subculturing in dextrose broth.

During the course of scarlet fever not only the specific type of conical colony has been isolated, but also the same nonspecific granular and smooth convex and other types of colonies found in dissociating scarlatinal cultures, with the exception of the extremely rough flat colony. The non-specific strains can in turn be reverted to the type specific for scarlet fever.

Besides opsonic tests used for differentiating typical strains of scarlatinal streptococci from their dissociants, a few toxin neutralization experiments were made on paramecia, with similar results. The toxins from typical scarlatinal streptococci are neutralized by specific scarlet fever antitoxin, while the toxins from the dissociants are not.

During a recent hospital epidemic of scarlet fever 105 cultures of hemolytic streptococci were isolated and studied. Streptococci apparently specific for scarlet fever were identified in cultures from carriers and patients without a rash but suffering from sore throat and infections of the nasal sinuses and the ears. These streptococci were recognized by the fact that they formed conical colonies which did not turn chocolate agar green and were specifically opsonified.

Eight of the 10 strains of opsonically nonspecific hemolytic streptococci from convex and conical colonies which greened chocolate agar, were shown to be dissociants of *S. scarlatinae* when they were reverted to cocci which formed conical non-greening colonies, and were now opsonified specifically by antiscarlatinal streptococcus horse serum.

It is obvious that to determine specificity of bacteria, it is necessary to use both a specific serum and a stable organism and that dissociating strains are unsuitable for either specific immunization or immunity tests. Over-immunization may also produce a nonspecific serum. The opsonic test has the advantage that suspensions for the test can be made from the growth on a chocolate agar plate on which changes in color and type of colony may readily be seen. Fluid cultures for agglutination and toxin-neutralization tests should therefore be subcultured on to chocolate agar just before the experiment to determine whether the culture has dissociated during the incubation period. A dissociating strain will show specific immunologic reactions only if the specific factor predominates.

*M13. The Cultural and Serological Characteristics of Minute Hemolytic Streptococci.* ELEANOR A. BLISS AND PERRIN H. LONG, The Johns Hopkins University Medical School, Baltimore, Md.

Minute beta hemolytic streptococci have been isolated primarily from the rhinopharynges of normal and diseased human beings. These organisms grow satisfactorily in blood broth and upon 5 per cent blood, neopeptone, beef infusion agar plates. The colonies are very small and the area of hemolysis is of the beta type. The organisms are smaller than the ordinary beta hemolytic streptococci; they tend to occur in short chains and masses. The majority of our strains have *Streptococcus equi*-like fermentation reactions in lactose, mannite and salicin, but certain strains have the pyogenes, infrequens or sub-acidus type of fermentation reactions. The majority of the strains ferment trehalose. None ferments sorbitol. Sodium hippurate is not hydrolyzed and methylene blue is not reduced. A moderate amount of acid is generally

produced in 1 per cent dextrose broth. Preliminary studies of the antigenic structure of these organisms show that, upon the basis of Lancefield's precipitin reaction they fall into three groups. When serological differentiation is attempted by means of agglutination reactions two distinct groups have been outlined. Human beings suffering from purulent infections caused by these minute organisms show agglutinins in their blood serum.

*M. 14. The Incidence and Significance of Minute Hemolytic Streptococci.*

PERRIN H. LONG AND ELEANOR A. BLISS, The Johns Hopkins University Medical School, Baltimore, Md.

Minute beta hemolytic streptococci have been isolated from the throats of 80 per cent of individuals ill with glomerular nephritis and 50 per cent of individuals ill with rheumatic infection. These organisms are rarely found in the throats of individuals ill with chronic disease or acute infections. Their incidence in normal human beings is from one-half to one-third of the incidence of ordinary beta hemolytic streptococci. In both glomerular nephritis and rheumatic infection more throat cultures were positive for minute hemolytic streptococci than for ordinary beta hemolytic streptococci. In many individuals suffering from these diseases, only minute hemolytic streptococci were recovered from throat cultures during the period of investigation. In view of the well known association of these two diseases with beta hemolytic streptococcus infection we feel that these findings assume an added importance. In two instances these minute organisms have been the sole etiological agent in purulent infections in human beings.

*M15. Observations on an Epidemic Infection with Hemolytic Streptococcus in an Isolated Group of Rheumatic Subjects.* A. F. COBURN, Columbia University, New York.

An outbreak of respiratory disease at The Pelham Home during the spring of 1934 made it possible to study the herd response of 25 rheumatic children to infection with a single pathogenic agent. This organism gave the fermentation reactions of *Streptococcus pyogenes*, according to Holman's classification, and was a strong toxin producer. Familiarity with the patients' previous rheumatic attacks led to the conclusion that this strain was effective in initiating a more intense activity of the rheumatic process than had been previously experienced by these individuals. No evidence was found either for the existence of a refractory state of the host or for protection from rheumatic fever by

good living conditions. There was a close correlation between the development of the rheumatic recrudescence and the stimulation of the immune mechanism as judged by serial antistreptolysin determinations of the blood serum.

*M16. Sensitivity to Nascent Phage as a Character for the Differentiation of the Beta Type of Streptococci.* ALICE C. EVANS, National Institute of Health, Washington, D. C.

Sensitivity to the 4 types of streptococcus phage described by the writer in Public Health Reports (now in press), offers a new point of view from which the relationships of hemolytic streptococci from various sources may be studied. The usefulness of phagological reactions as a supplement to other characters may be illustrated by the aid they have given in the classification of *Streptococcus equi*.

Failure to ferment lactose is the character by which the streptococcus of strangles is generally differentiated from other hemolytic streptococci, yet some investigators state that certain strains of *Streptococcus equi* ferment lactose. When this disagreement is examined in the light of phagological reactions an explanation is at hand.

*Streptococcus equi* is distinguished from other streptococci by its sensitivity to all of the 4 types of phage in the nascent state (that is, in the presence of a sensitive strain). In a large collection of strains now being studied, certain strains which fermented lactose must be regarded as belonging to a variety of *Streptococcus equi* on account of their ability to produce strangles in horses and their sensitiveness to the 4 types of phage. Five out of 22 strains from strangles fell into the lactose fermenting variety.

Not one of the collection of over 300 strains of hemolytic streptococci from a great variety of human diseases fully agreed with any of the streptococci from strangles.

*M17. A New Method of Staining Bacteria and its Application to a Study of Streptococci and the Viruses of Poliomyelitis and Encephalitis.* EDWARD C. ROSENOW, The Mayo Foundation, Rochester, Minn.

The method consists essentially of staining deeply the thoroughly fixed film with a basic dye, such as methyl violet, washing with water, adding in turn, Gram's iodine solution and a 2 per cent aqueous solution of safranine, and washing with water. Counterstaining with safranine after decolorization with alcohol, as in the Gram method, or treatment

either with the iodine solution alone or with the safranine solution alone, does not suffice. The combination of iodine and safranine is necessary to stain the delicate forms as found in filtrates, and the capsules of mature organisms.

By this method unmistakable oval cocci and diplococci, usually singly but occasionally in short chains, have been found consistently in filtrates of emulsions of glycerolated spinal cords of persons and monkeys that died of acute anterior poliomyelitis; in filtrates of the brains of rabbits and mice that succumbed to inoculations of the viruses and streptococci from poliomyelitis, epidemic encephalitis and herpes-encephalitis; in direct smears of spinal fluid of persons and monkeys in the acute stage of poliomyelitis, and in filtrates of cultures of the streptococcus in mediums such as "K" medium, Noguchi's medium and chick-mash medium. Organisms were not demonstrable in control filtrates and spinal fluids. Cultures of filtrates were usually negative.

All species of cultivable bacteria thus far stained by this method have revealed capsular envelopes of varying thickness and density.

*M18. The Serological Differentiation of Pathogenic and Non-Pathogenic Strains of Hemolytic Streptococci from Parturient Women.*

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The differentiation of hemolytic streptococci which are likely to do harm during childbirth from those which are not is based on previous studies showing that broad groups of hemolytic streptococci can be distinguished serologically by the polysaccharide common to each group.

In Queen Charlotte's Hospital, London, hemolytic streptococci were obtained from 13 of 855 women ante partum, and from 85 of another 837 post partum; only one woman from these two groups had definite puerperal infection. Forty-five additional strains from 45 women with puerperal sepsis were also studied. These strains were classified into groups by the precipitin reaction using extracts, prepared by heating the streptococci with N/20 HCl, and antisera from rabbits immunized with formalinized cultures. All strains from patients with definite puerperal infection (except one associated with *Staphylococcus aureus*) belonged to Group A; whereas only one Group A strain was found among the cultures from afebrile patients, and all other strains from afebrile patients fell into groups other than Group A. It is concluded, therefore, (1)

that hemolytic streptococci may be harbored in the vagina before or after delivery without causing disease provided they belong to serological groups other than Group A, (2) that Group A strains are the only hemolytic streptococci capable of causing definite puerperal infection in the human species, and (3) that infection almost invariably occurs if Group A hemolytic streptococci are present in the vagina.

The serological method of identifying hemolytic streptococci potentially pathogenic for man is also applicable epidemiologically wherever non-pathogenic streptococci of other groups may be encountered simultaneously with Group A strains, as in the separation of human and bovine strains in milk during epidemics of septic sore throat, or in milk borne epidemics of scarlet fever, or in routine examinations of milk to exclude pathogenic hemolytic streptococci.

*M19. The Dissemination of Human Pathogenic Streptococci Through the Cow's Udder.* D. J. DAVIS, University of Illinois College of Medicine, Chicago.

The numerous milk borne outbreaks of disease have focused attention on the mechanism of infections of the cow's udder. Tubercle bacilli, *Brucella abortus* and possibly paratyphoid bacilli reach the udder from the blood stream or tissues of the cow. Other bacteria, notably streptococci and possibly staphylococci, may reach the udder through the lactiferous ducts of the teats. Outbreaks of septic sore throat traced to milk at times have followed teat injuries. There is no evidence that hemolytic streptococci reach the udder from the blood or tissues. It is assumed, therefore, that streptococci have been implanted on the skin of the teat from some human source and have ascended the duct to the milk cisterns. Experimentally hemolytic streptococci causing outbreaks of sore throat when placed on the teat will cause a definite streptococcus mastitis in a short time.

Sections made of the cow's teat reveal the dense squamous cornified epithelium lining the lactiferous duct around which is the muscle sphincter. The epithelial surface of the duct which varies in length from 0.6 to 1 cm. is rough and irregular furnishing small pockets and crevices in which the milk may be retained. It is along such a channel that the streptococci by growth, possibly aided by manipulation, ascend. The sphincter is adequate in most teats to prevent leakage. Not infrequently, however, it becomes incompetent and as the tension of the milk in the cisterns increases the milk trickles through creating a direct route through the meatus to the exterior where bacteria may enter.

As to whether a negative udder pressure and, therefore, suction during the process of milking may occur was tested in a series of 13 milch cows by inserting the end of the teat in a dye (carbol fuchsin) as the pressure on the teat was released. The cows were then immediately slaughtered and the teats examined. The dye did not ascend in the lactiferous duct in any instance more than one half the distance to the cistern, even in a leaking teat.

In an udder in the site of experimental mastitis no changes of any importance were noted about the meatus or along the duct epithelium to indicate involvement of the lymphatics or subepithelial tissue. The main body of the udder in experimental human streptococcus mastitis reveals extensive inflammatory changes.

The streptococci used in the experiments, isolated from outbreaks of septic sore throat, were encapsulated and revealed large moist watery or mucoid growth. These streptococci, sometimes called *Streptococcus epidemicus*, ferment trehalose but not sorbitol. We assume they are human in origin. According to studies of Dawson, these streptococci may represent mucoid variants.

In most outbreaks of udder borne septic sore throat, no scarlet fever appeared. Erysipelas as a complication occurs in a few cases. In udder borne scarlet fever, as in the outbreak in New Jersey, most cases were typical and relatively few cases of septic sore throat appeared.

These accidental human experiments of udder borne disease on such a vast scale and of such great virulence should be valuable criteria in determining the specificity of streptococci. Practically all biological and laboratory tests except possibly the specific toxin test of scarlet fever streptococci are more or less variable. This is true even of hemolysis, as Pilot has shown.

*M20. Laboratory Infections Due to Brucella in the United States.* K. F. MEYER, Hooper Foundation, University of California, San Francisco.

Seventeen organizations or institutes, in answer to a questionnaire, reported for the period 1922 to 1934 a total of 57 clinically recognized infections directly traceable to routine or research work with brucella organisms. The number of cases stands entirely in proportion to the extent of the investigative work which has been conducted by the laboratory. Five well known centers of brucella research had over a period of from 7 to 12 years, from 5 to 10 infections. In 45, 6 per cent of the clinical cases, the causative organism was isolated in blood cul-



tures. As might be expected from previous experiences, *Br. melitensis* was the offender in 53.8 per cent, the suis type in 42.3 per cent and the abortus bovis type in 3.9 per cent of the successful cultures. The course of the disease was mild in about one-half of the patients and the duration from 1 to 2 weeks. About 20 per cent passed through the disease in an ambulatory stage. Unfortunately, an equal number had a stormy, prolonged illness. The melitensis infections have been particularly serious, in one instance leading to a brucella cholecystitis. It may be mere coincidence but the infections were much more severe in the women than in the men. Preventive vaccination, as might be expected, afforded no protection.

*M21. Brucella Infections in White Mice.* CHARLOTTE SINGER AND E. B. SHAW, Hooper Foundation, University of California, San Francisco.

In a comparative series it was found that inoculation with 300 million *Brucella* bacteria of the suis type, human origin, was fatal to white mice within four days. Inflammatory and degenerative changes in the mice surviving 30 million, 3 million and 300,000 bacteria were entirely proportional to the number of organisms administered.

Mice immunized intraperitoneally with vaccine survived a mean inoculation of 400 million organisms of suis type. All controls died. There were no abortions; there was relatively little anatomical change, principally in spleen and lymph nodes. Infection persisted for 14 months.

In a similar series cross protection was tested with small numbers of organisms. The immunized animals re-infected with small inoculum freed their organs slightly better than controls. Judging by the persistence of infection the melitensis is slightly more invasive than suis or bovis types. There is no striking difference in the sterilization of tissues irrespective of the number of organisms inoculated. Positive cultures were obtained for 104 days (longest period tested). Immature mice 3-4 weeks old were more capable of disposing of small and large doses of living organisms of any type, regardless of whether the mothers were infected or immunized.

*M22. The Preparation of Diphtheria Toxoid Adsorbed on Calcium Phosphate.* AUGUSTUS WADSWORTH, JAMES J. QUIGLEY AND GRETCHEN R. SICKLES, Division of Laboratories and Research, N. Y. State Department of Health, Albany.

When the early work of Roux and Yersin in 1899 and the more recent work of Abt and of Smith was repeated, it was found necessary to develop the procedure in certain particulars in order to insure practical results in the recovery of diphtheria toxoid, and also of the toxin, by precipitation on calcium phosphate. The following method was adopted and has been found to give a yield of 90 per cent or over in flocculating units: To 100 cc. of diphtheria toxoid are added 10 cc. of a 10 per cent solution (1 gram) of calcium chloride, followed by 20 cc. of a 10 per cent solution (2 grams) of disodium phosphate. An excess of calcium chloride is essential to avoid the elution of the toxoid. Complete adsorption requires about 20 minutes at room temperature. The supernatant liquid (pH  $6.2 \pm$ ) is removed; the precipitate is washed twice with water, three times with physiological salt solution and suspended in salt solution. The resulting product should be free from calcium chloride with the pH approximately 7.0. The Kjeldahl nitrogen is reduced about 90 per cent. Calcium and phosphorus determinations indicate that the precipitate consists of about 70 per cent tricalcium phosphate and 30 per cent dicalcium phosphate. This preparation remains in suspension for a longer period than does alum-precipitated toxoid. Recovery is always about 90 per cent, while less than 10 per cent of the original nitrogen is present. The final reaction is about neutral; that of the alum preparations is acid. For flocculation tests, the precipitated toxoid is dissolved in diammonium citrate solution (pH 5.0).

A toxoid was prepared by the usual method of Ramon from a toxin produced in a modified infusion-free peptone medium containing maltose and sodium acetate, by a strain of the diphtheria bacillus the toxigenic activities of which had been developed, after isolation in this laboratory, to a degree equivalent to that of the standard strain of the diphtheria bacillus, Park-Williams No. 8. The toxoid was precipitated by the foregoing method with a recovery of 100 per cent of the flocculating units. The antigenic activity of this calcium-precipitated toxoid, as shown by the antitoxic content of the blood serum of guinea pigs after immunization, was equal to that obtained with alum preparations of toxoids produced by this strain and by the standard No. 8.

*M23. A Method for the Production of Staphylococcus Toxin and Toxoid.*

GEORGE F. LEONARD AND AUGUST HOLM, Biological Laboratories, E. R. Squibb and Sons, New Brunswick, N. J.

Increased interest in the use of a *Staphylococcus* exotoxin and toxoid

in the prevention and treatment of staphylococcal infections has demonstrated the need of a simplified method for its preparation on a production scale.

The culture is grown in a semi-synthetic culture medium, in an atmosphere of carbon dioxide-oxygen, and in an anaerobic drum which has been especially devised for this purpose. Methods are described in detail for the production and titration of the toxin, and for the evaluation of the antigenicity of the toxoid.

*M24. Studies on the Standardization of Gas Gangrene Antitoxin (Oedematiens).* IDA A. BENGTON, National Institute of Health, Washington, D. C.

A study of 17 strains of *Cl. oedematiens* indicates rather wide variation in the toxigenic properties. The filtrates of certain strains failed to kill mice in 0.5 cc. amounts while the filtrates of others were fatal in 0.01 cc. amounts. One of the most toxigenic strains was used in the preparation of a large volume of toxin which was to be used as a standard toxin. The medium used consisted of 3 parts of beef infusion broth and one part of chopped beef to which was added 5 per cent of sterile normal horse serum. The reaction of the broth was adjusted to pH 8.4. After the addition of the meat and sterilization the reaction fell to pH 7.0. After 3 days' incubation the growth was filtered and the toxin was precipitated by saturating the filtrate with ammonium sulphate. The yield of toxin was over 700 grams from 60 liters of filtrate.

The minimal lethal dose of the dried toxin for a 20 gram mouse was in the neighborhood of 0.02 mgm. The "test dose" of toxin against 0.02 of one international unit of oedematiens antitoxin as proposed by Dr. Madsen of the Statens Seruminstitut of Copenhagen was found to be 2 mgm. (100 M.L.D.'s of the toxin). Tests were carried out on mice (intramuscular inoculation) and on guinea pigs (intracutaneous inoculation).

*M25. Agglutination with B. proteus X19 in Specimens Submitted for Other Serological Tests.* RUTH GILBERT AND MARION B. COLEMAN, Division of Laboratories and Research, New York State Department of Health, Albany.

Since typhus fever and Rocky Mountain spotted fever have been recognized with increasing frequency in this country, and relatively few cases have been reported as occurring in New York State, an investigation was undertaken to determine the incidence of agglutination with

*B. proteus* X19 in blood submitted to be examined for evidence of typhoid or undulant fever. Reactions were obtained with *B. proteus* X19 with about 10 per cent of approximately 5,000 specimens examined. The data available indicate that most of the patients whose sera agglutinated this microorganism were not believed by the attending physician to have typhus or Rocky Mountain spotted fever at the time the specimens were collected. The results of the study suggest the need of a thorough investigation when reactions with *B. proteus* X19 occur.

*M26. Oral Heterophile Immunization.* GEORGE E. ROCKWELL AND HERMAN C. VAN KIRK, Department of Bacteriology, University of Cincinnati.

The object of these experiments is to show that oral administration of certain Forssman's antigens will stimulate the development of a heterophile immunity to such an extent that it offers a formidable protection against certain virulent organisms. The experiments also indicate the duration of this protection.

Rabbits were given orally one billion heat killed, rough variant type I pneumococci (D.R.-I) in 5 cc. of water one hour before the rabbits were fed in the morning. Their heterophile immune response and the duration of this response was shown first by frequent titration of their serum for sheep cell hemolysin; secondly by intradermal inoculation with several lethal doses of rabbit virulent type II pneumococcus.

Five days after the last of 8 oral treatments the rabbits showed a heterophile titration from 240 to 480 units per cubic centimeter of serum. But after a rest period of 9 to 12 days this titration would drop to 30 or 60 units. If after a 12 day rest period another oral treatment was given within 12 hours, the heterophile titration would increase from 30 to 120 units or better.

When the above rabbits were inoculated intradermally with several lethal doses of rabbit virulent type II pneumococci the following results were obtained:

- (1) Those that received this inoculation within 5 days of the last oral treatment *lived*.
- (2) Those that received this inoculation 9 days or more after the last oral treatment *died*.
- (3) Those that had a rest period of 12 days after the last oral treatment before inoculation, if given another oral treatment with the inoculation, had about 25 per cent mortality.

M27. *The Wassermann Reaction and the Kahn Test in Leprosy.* M. H. SOULE, Hygienic Laboratory, University of Michigan.

The specificity of the various laboratory tests for syphilis with the sera of individuals infected with Hansen's bacillus has frequently been questioned. There is considerable evidence that leprosy interferes with these methods. By way of explanation it has been stated that a positive reaction in leprosy lies in the simultaneous occurrence of syphilis or yaws and in mistakes in clinical diagnosis. As long as the amelioration of leprosy appeared to be hopeless there was little need for more than passing scientific interest into the part that the disease might play in non-specific serological reactions. With the accumulation of evidence that chaulmoogra oil and its derivatives have unquestioned therapeutic value it becomes increasingly important for the serologist to be able to furnish the clinician with data which will aid in the wise interpretation of the tests for syphilis in the presence of leprosy. With this end in view the sera from 669 cases of leprosy were tested by means of the Kolmer Wassermann complement fixation reaction and the Kahn precipitation test. These two procedures were selected because of a familiarity with them and the fact that experience has shown the advisability of using a Wassermann test along with a precipitation test of proven sensitiveness and specificity. In every instance a special effort was made by the clinicians to establish the absence of syphilis or yaws. The data are presented in the following table:

*The Kolmer-Wassermann and Kahn reactions with the sera of lepers (Filipinos), presenting no evidence of syphilis or yaws*

TEST	NUMBER OF SERA TESTED	STRONGLY POSITIVE	POSITIVE	DOUBTFUL	NEGATIVE	PER CENT NEGATIVE	PER CENT POSITIVE
Kolmer .....	615*	109	5	14	487	81.5	18.5
Kahn .....	615	121	70		424	69.0	31.0
Sera in which the two reactions were identical .....		100	5		407		

\* 526 males and 89 females.

It is concluded that leprosy *per se* is responsible for the positive reactions.

M28. *Native Versus Denatured Bacterial Antigens.* H. M. POWELL AND W. A. JAMIESON, The Lilly Research Laboratories, Indianapolis.

Following experimental devitalization of bacteria with the non-coagulating antiseptic, Merthiolate, as a method for preparation of more nearly natural antigens, we have continued by the methods of Krueger, accomplishing both devitalization and fragmentation of bacteria by mechanical means comprising grinding and ultrafiltration. Such ultrafiltrates preserved with Merthiolate consist almost entirely of undenatured bacterial antigen ("U B A").

Utilizing carefully selected cultures of *H. pertussis* and other bacteria corresponding to those from which ordinary vaccines are made, we have prepared U B A in increasingly large quantities for clinical evaluation in pertussis, paranasal sinusitis, gonorrhea, etc. The earliest clinical reports on such U B A have appeared or are in press.

Laboratory standardization of different antigens has been on the basis of nitrogen value, and pertussis U B A of 10 mgm. nitrogen per 100 cc., for example, have appeared to be of an optimum concentration for human use. Precautions against denaturation as suggested by Dr. Krueger comprise the use of alkaline buffered bacterial suspending medium, grinding at slow speed and ultrafiltering at a rapid rate, both at cool temperatures, and finally control of all preparations for unsuspected denatured protein by isoelectric precipitation.

As compared to ordinary vaccine, U B A is quite free of bacillary metabolic products and culture media ingredients. It is quite atoxic and optimum human doses represent much greater numbers of original bacteria than do regular doses of bacillary vaccine. The immunological response to U B A should be specific to native bacillary substance, and seemingly cannot be evaluated on the basis of conventional serum antibody production.

*M29. Simultaneous Immunization with a Mixture of Ten Kinds of Laked Blood.* EDNA DELVES, John McCormick Institute for Infectious Diseases, Chicago.

Precipitin, agglutinin and hemolysin production following the simultaneous immunization of 8 rabbits with an antigenic mixture of 10 kinds of laked blood has shown that rabbits may respond to such multiple immunization by producing precipitins for a large number of different blood proteins and hemoglobins at the same time that they produce agglutinins and hemolysins for red blood cells. By absorption experiments the agglutinin and lysin for a particular kind of red blood corpuscle could be removed with little or no reduction in the agglutinins for other corpuscles or in the precipitins for homologous and heterol-

ogous antigens; and a particular hemoglobin precipitin could be removed by specific absorption with little or no reduction in the precipitins for other hemoglobins or in the agglutinins and lysins for homologous and heterologous corpuscles.

*M30. The Fate of Specific Antigen in the Immunized Animal: Extent of Its (1) Temporary Retention in Area of Injection, (2) Diffusion from this Area, (3) Destruction in this Area, and (4) Destruction following Diffusion.* R. L. KAHN, E. B. McDERMOTT AND S. D. SATTLER, University of Michigan, Ann Arbor.

When specific antigen is injected into a tissue of an immunized animal, some fixation takes place locally between the tissue and the antigen, probably by colloid chemical union of serum and antigen. The capacity of the tissue to combine with antigen determines the extent of local retention of the antigen and is dependent upon at least three conditions: (1) the state of immunity of the animal, (2) the tissue injected, and (3) the dose of antigen employed.

The antitoxin-toxin method of measuring the reacting capacities of different tissues of an immunized animal to specific antigen was employed in the present investigation. Animals are first immunized with horse serum and the capacities of the tissues to combine with or retain injected antigen are determined by the use of standardized horse serum diphtheria antitoxin in place of horse serum, the antitoxin being regarded as specific antigen.

(1) If into the skin of a rabbit immunized by two doses of horse serum, 1000 units of horse serum antitoxin and 50 M.L.D. of diphtheria toxin, are injected simultaneously about 5 cm. apart, the animal will succumb to the toxin. If instead, the antitoxin is injected into the skin 24 hours before the toxin, 750 units are sufficient to save the animal from toxin death. The fact that 750 units of antitoxin, when injected 24 hours before the toxin, are sufficient to save the rabbit from toxin death, would indicate that when the 1000 units were injected simultaneously with the toxin, approximately 250 units were temporarily held back in the injected area. This temporarily retained antitoxin apparently did not reach the rapidly diffusing toxin fast enough. It appears that temporary retention of part of the antigen takes place, when the total quantity injected somewhat exceeds the antigen-combining capacity of the tissue.

(2) When the excess of antitoxin, such as 1500 units, is injected, simultaneously with 50 M.L.D. of toxin, into the skin of a horse serum-

immunized rabbit, antitoxin diffuses from the area of injection with sufficient rapidity to neutralize the toxin *in vivo*. In another experiment, 5 rabbits similarly immunized with horse serum are injected subcutaneously with 0.01, 0.1, 1.0, 5.0 and 10.0 cc., respectively, of this serum. The rabbits receiving from 0.01 to 1.0 cc. doses show inflammatory responses of increasing intensity and no horse serum in the blood stream. The rabbit receiving 5.0 cc. will show only a mild local response and the one receiving 10.0 cc., no local response. Horse serum is present in the blood stream in both of the latter. The diffusion of antigen from a tissue of an immunized animal can not be said to result solely from the injection of an excessive dose. When a small dose is injected, some slight diffusion must also take place, even though not detected thus far. This view is based on the assumption that the increase in immunity of an animal receiving repeated injections of antigen into some tissue, is due to traces of antigen that reach the circulation.

(3) The destruction of antigen within the injected area of a tissue of an immunized animal was demonstrated by two methods: (a) A method which showed the gradual disappearance of antigen from the area of injection; and (b) the antitoxin-toxin method above. By the latter method it was shown that unless the amount of antitoxin injected exceeds the specific combining capacity of the tissue, the antitoxin does not diffuse appreciably from the injected area in horse serum-immunized rabbits. This would indicate local destruction of the antigen, presumably by proteolysis.

(4) Protein antigen, injected intravenously with consequent ready diffusion rapidly disappears, most likely by proteolysis. Fifty units of antitoxin injected intravenously are insufficient to protect a horse serum-immunized rabbit from 50 M.L.D. of toxin, injected simultaneously into the skin, while 5 units of antitoxin are sufficient to protect a normal rabbit under the same conditions. Seventy-five units would have saved the animal, suggesting that the 50 units must have undergone such a change as to render them incapable of neutralizing the toxin. If the antitoxin is given 7 days before the toxin, as many as 7500 units are insufficient to protect a horse serum-immunized rabbit from 50 M.L.D. of toxin. These findings indicate that, as in the case of localized antigen, diffused antigen must also be destroyed with considerable rapidity in specifically immunized animals.

*M31. Lessons in Laboratory Diagnosis from the Recent Outbreak of Amebiasis.* FRED O. TONNEY, Board of Health, Chicago, Ill.



The common amoebae of the human intestinal tract, viz.: *E. histolytica*, *E. coli*, *E. nana*, *Iodamoebae butchlii* and *Diendamoeba fragilis*, are treated from the standpoint of the principal diagnostic points for quick identification, in the order of their importance. The trophozoites of each form are considered under the heading of motility, the nuclei, cell inclusions, the character of protoplasm and size. The cysts of each amoeba are taken up in the same manner, under the headings, chromatoid bodies, the nuclei, size and general considerations.

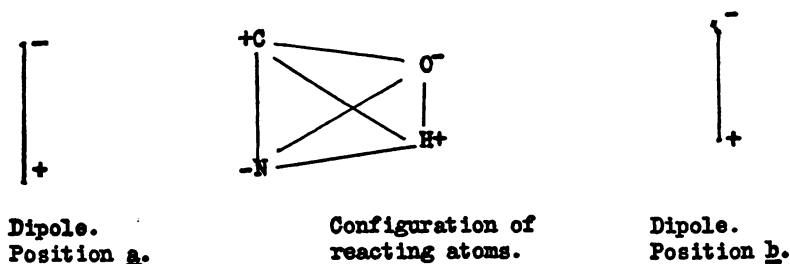
Other parasitic forms and cellular bodies found in feces are: *Trichomonas intestinalis*, *Guardia hominis*, *Chylomastix mesnili*, *Blastocystis hominis*, rounded epithelial cells, large macrophages, mucous cells, fat globules and vegetable cells.

There follows a laboratory guide, in outline form, of the essential differential points needed for the practical diagnosis, based on the author's recent experiences.

*M32. Calculated Energy Effects as Suggestive of a Mechanism of the Influence of Antigen on Antibody Formation.* ALLEN E. STEARN, University of Missouri, Columbia.

The work of Landsteiner and others has shown the importance of specific chemical groups on an antigenic molecule in determining the nature of the antibody formed, and thus, ostensibly, in the mechanism of antibody formation. Unless the antibody is itself a hydrolytic product, or modified hydrolytic product, of the antigen (in which case it is practically impossible to visualize any basis for antigen-antibody reaction from considerations of energetics), then the latter must function in some enzymatic capacity in the formation of antibodies. Energy calculations show that this is a real possibility. If we grant the claim of Northrop that at least certain enzymes are protein in nature, then some of the chemical groups present in proteins must be able to exhibit prosthetic activity. The function of any enzyme is to lower the activation energy of a reaction. If the enzyme be a hydrolytic one, then the lowering of the activation energy for hydrolytic decomposition and that for re-synthetic "dehydration" must be precisely the same, which of course means a catalyzing effect on the formation of polypeptids, etc. In this paper an investigation is made, from energy considerations, to see whether any of the chemical groups commonly associated with proteins (or groups of similar nature) might lower the activation energy of the hydrolytic breaking of a carbon-nitrogen bond, the one involved in

the peptid linkage, and if so what the mechanism of the effect might be, bearing in mind that these groups themselves should not be altered. The chemical groups specifically studied were  $\equiv\text{C}-\text{O}-$ ,  $\equiv\text{N}=\text{O}$ , and  $=\text{N}-\text{H}$ . These are all electric dipoles, and it is here shown that by a proper placing of these (or similar) dipoles near a reacting configuration of atoms, the reaction velocity, depending on dipole strength, orientation, etc., may be either decreased or increased by amounts as much as several thousand times the "uncatalyzed" velocity in the cases investigated. The effect described is, of course, not limited to the groups studied but is a general one. Diagrammatically it can be pictured as follows:



So long as the dipole does not approach close enough to become part of the reacting system its effect is a purely electrostatic one, and this may be either to decrease or increase the potential energy of the four atom reacting system. As diagrammed above, the dipole in position *a* would increase the potential energy of the reacting system by tending to pull the C—N away from the O—H, and thus it would act as a *negative* catalyst. Were this dipole in position *a* turned end for end the effect would be reversed. Thus the C—N would be repelled and the O—H attracted by it, causing them to come more easily together, i.e., facilitating their approach and catalyzing their reaction positively. As drawn in position *b* the dipole will be seen to catalyze positively the reaction. Quantitatively the quantum mechanical approach as outlined by the author last year was employed. The present paper does not treat specifically the mechanism of the placing of these dipoles. Ostensibly this will come from a chemotactic or haptophoric bonding between other groups of the enzyme molecule and other groups of at least one of the reacting molecules. It is planned to treat later this problem specifically.

**M33.** *The Spectrographic Identity of Horse Euglobulin and Pneumococcus Antibody Solution Purified According to Felton.* CALVIN B. COULTER, ELVIN A. KABAT AND FLORENCE M. STONE, College of Physicians and Surgeons, Columbia University, New York.

Using a hydrogen discharge tube as a source of continuous illumination in the U. V., and a quartz spectrograph, it has been found possible to resolve the ultraviolet absorption spectra of various proteins into 10 distinct bands, whose maxima have the same wave lengths in all the proteins examined. These were crystalline egg and serum albumen, thyroglobulin, horse euglobulin and pseudoglobulin. The intensities of the individual bands, however, vary in the different proteins so that it is possible to characterize a given protein by its spectral absorption curve. The absorption curves given by (a) normal horse euglobulin and (b) pneumococcus antibody solutions prepared according to the method of Felton (Felton, L. D., Jour. Immun., **21**: 341, 1931) and containing 65-70 per cent of specifically precipitable nitrogen are identical.

**M34.** *Some Oxidation and Reduction Reactions of Pneumococcal Hemolysin.* BARNETT COHEN AND HARRY SHWACHMAN, The Johns Hopkins Medical School, Baltimore.

1. With a wider variety of reagents, we have confirmed the observation of Neill (J. Exptl. Med., **39**: 745, 1924) that pneumococcal hemolysin activity may be inhibited by oxidation and restored by reduction. Oxidizing agents such as air,  $\text{H}_2\text{O}_2$ , iodine, ferricyanide, benzoquinone,  $\text{SeO}_2$ , azochloramid all cause more or less reversible inactivation. Reducing agents such as  $\text{Na}_2\text{S}_2\text{O}_4$ ,  $\text{H}_2\text{S}$ , cysteine, reduced glutathione, thioglycolate, cyanide and ascorbic acid reactivate the oxidized hemolysin.

2. The following evidence leads to the inference that a thiol grouping may be essential to the lytic activity.  $\text{Cu}_2\text{O}$ , which reacts rapidly with SH-compounds to form mercaptides, rapidly and reversibly inactivates the hemolysin.  $\text{C}_6\text{H}_5\text{HgOH}$  and  $\text{ClCH}_2\text{HgCl}$ , which also can form mercaptides, also inactivate the hemolysin reversibly.  $(\text{CH}_2\text{I})_2\text{Hg}$ , which cannot form a mercaptide, does not inactivate. The rates of inactivation by certain of the oxidants are suggestive of their respective actions on thiol compounds.

3. These observations which were recently reported (J. Biol. Chem., **107**: 257, 1934) are in remarkable agreement with the findings by Hellerman and Perkins (J. Biol. Chem., **107**: 241, 1934) on the behavior of the hydrolytic enzymes, urease and papain.

4. The foregoing experiments were directed toward the elucidation of the specific chemistry of the lysin. In addition, the results of some preliminary tests on the suspected active thiol grouping made with the oxidants of reversible oxidation-reduction systems are of interest. It was found that inactivation occurs with 2,6-dichlorophenol indophenol ( $E'_0 = +0.19$ , pH 7.4) at a rate comparable with that produced by ferricyanide ( $E'_0 = +0.43$ ). With 1,4-naphthoquinone-2-sulfonate ( $E'_0 = -0.13$ ) the inactivation is very much slower, while with phthiocol ( $E'_0 = -0.21$ ) it was not detectable. These observations are offered with all reserve, but they are highly suggestive.

*M35. The Fraction of the Pneumococcus Antigenic for Human Beings.*

LLOYD D. FELTON, Harvard Medical School, Boston, Mass.

The work is an extension of that reported last year in an attempt to isolate the immunizing substance or substances of the pneumococcus antigenic to man. Various water-soluble fractions of the pneumococcus were tested in human beings by a single subcutaneous injection of 2 mgm. dissolved in physiological salt solution. All preparations were sterilized by passing through a Mandler filter. Reactions following injection of this group of samples may be considered mild. However, with one sample 0.7 per cent of a group of 3200 individuals developed what might be called a severe reaction. None in the group however were so incapacitated as to interfere with their regular activity. Immunity was tested for by the estimation of the amount of protective antibody in the serum 14 days to 3 months after injection, and in a few cases after 10 months. In a small group of over 200 persons, protective antibody was found in all individuals 14 days after injection, in most individuals after 3 months, and in the few tested after 10 months. The amount of antibody found as compared to that already present varied on the average from a 17-fold to a 98-fold increase. All fractions studied were high in polysaccharide content as indicated by the amount of hydrolyzable sugar present and the precipitin reaction. It has not been determined whether the antigenicity is due to this polysaccharide alone, or to a polysaccharide combined with some other chemical, or due to some other cell constituent or product.

*M36. Studies on the Specific Characteristics of Syphilitic Blood Proteins.*

SETH T. WALTON, City Health Service, Charlotte, N. C.

A series of experiments has been carried out with the purpose of showing the relationship of the condition of the protein equilibrium in

blood serum to the progress of diseased conditions, taking syphilis as a type of disease which has long been classified into a number of more or less well-defined stages clinically.

Studies on some of the physical properties of syphilitic blood proteins has shown that there is a change in the character of the protein molecules in immunity as shown by a different behavior. The surface tension, solubility, refractivity, ionization effects, and cataphoretic behavior of antibody proteins all point to a marked change in the physical state of immune molecules. Since each microorganism presents a different type of surface, with a different chemical and electrical pattern, a correspondingly different antibody is called forth for each.

It may well be assumed that the process of building up an immunity in a body against certain invading microorganisms is equivalent to a rearrangement of the atomic groups in the serum protein molecules in order to produce new molecules which have the power of arresting the enemy. The end products and the extent of this change are dependent upon the character and quantity of the invading agent.

*M37. Purification and Concentration of Diphtheria Toxin.* MONROE D. EATON AND S. BAYNE-JONES, Yale University Medical School, New Haven, Conn.

In the course of a study of bacterial toxins and a search for chemical antidotes, diphtheria toxin has been purified and concentrated by the following methods.

I. Fractionation with ammonium sulphate at one-third and three-fourths saturation. The fraction obtained with three-fourths saturation contained most of the toxin. Material from this fraction was absorbed on  $\text{Al}(\text{OH})_3$ , eluted with 2.4 per cent disodium phosphate at pH 8.6–9.0, and again fractionated with ammonium sulphate. In successive states this fraction was precipitated with phosphotungstic acid and cadmium chloride. The final solution was dialyzed, and concentrated in vacuo at 20–30°C. Analyses and tests of the solutions were as follows: mgm. N per  $L_F$  unit of crude toxin, 0.150; mgm. N per  $L_F$  unit of purified toxin, 0.0005; mgm. N per M.L.D. of fresh crude toxin, 0.003; mgm. N per M.L.D. of crude toxin as old as the purified product was 0.006; mgm. N per M.L.D. purified toxin, 0.00005 to 0.0001. The flocculating time of the original toxin was 40 minutes at 45°C. The purified toxin, diluted to contain 72  $L_F$  units per cubic centimeter flocculated in 2 minutes at 25°C. The purified toxin contained approximately 13 per cent N. It coagulated when heated in an acid solution,

gave a positive biuret test and dried down to a yellow glassy residue. The lead acetate test for sulphur was negative and the Molisch reaction was negative or weakly positive. It contained traces of heavy metals. The usual qualitative tests for copper were negative.

II. Precipitation with acetic acid at pH 3.8 according to Watson and Wallace and Watson and Langstaff. Fractionation with ammonium sulphate separated this material into one part which contained some of the toxin but flocculated slowly with diphtheria antitoxin and another part with more of the toxin which flocculated rapidly.

Final yields by these methods have been about 10 per cent of the original amount of flocculating material (toxin and toxoid in the crude broth filtrate). About half of this flocculating material was changed to toxoid during purification. The other half appeared to be unchanged toxin. Solutions remained stable in toxicity during 8 months. The flocculation reaction has been a very convenient, rapid and accurate method for indicating the toxic material in solutions obtained at all stages of these processes of purification.

*M38. Two Antigens of High Molecular Weight: Hemocyanins of Limulus polyphemus and Busycon (Fulgur) caniculatum.* SANFORD B. HOOKER AND WILLIAM C. BOYD, Evans Memorial, Boston.

The authors have proposed a theory connecting the molecular weight of the antigen with the proportion by weight of antibody to antigen in precipitates made at the equivalence point. In attempting to obtain evidence for or against this hypothesis, it seemed desirable to investigate antigens at the extremes of the molecular weight range. The respiratory pigments of two species, *Limulus polyphemus*, and *Busycon caniculatum*, provide examples of proteins of extremely high molecular weights, 2 million and 5 million, respectively. The latter is the highest molecular weight ever reported. That these values, obtained by Svedberg's ultracentrifugal method, are of the right order of magnitude is shown by the recent measurements of the Adairs and Roches, using the osmotic pressure technic.

The hemocyanins were prepared by repeated precipitation at the isoelectric point. Sera from rabbits injected with them exhibited high titers, gave very rapid flocculation and contained, as determined by the Dean and Webb method of optimal proportions and by chemical analysis, several milligrams of antibody per milliliter; one serum contained as high as 8 mgm. per milliliter. Precipitates made at the equivalence point (also a few at other points) were prepared, washed

and analysed for copper and nitrogen. From the copper values the amount of antigen in the precipitate could be calculated and this checked in a general way with that estimated by immunological test of the supernatant and washings; from this and the total N the ratio of antibody to antigen was computed. The values at the optimum were found to be for *Limulus*, 1.55 ( $\pm 0.045$ ), and for *Busycon*, 0.68 ( $\pm 0.049$ ). These agree fairly well with the theoretical values calculated by the authors from their formula, e.g., for *Limulus*, 1.47, for *Busycon*, 0.97. The value for *Busycon* apparently differs from the theoretical by an amount which is statistically significant.

*M39. The Biological Activity of a Few Chemical Constituents of Brucella Cells.* I. FOREST HUDDLESON AND ALFRED D. HERSHEY, Department of Bacteriology and Hygiene, Michigan State College.

*Activity of cells before chemical treatment:* Brucella cells in the wet or dry state, when injected into normal guinea pigs in amounts as small as 0.02 gram, will produce a rapidly fatal toxemia.

*Activity of autolysate from cells:* When the cells are permitted to undergo autolysis from their own enzymes in the presence of toluol at 37°C., a colloidal constituent is recovered in solution which, after removal of the cell residue by centrifuging, has been found highly toxic for guinea pigs. It produces a rapidly fatal toxemia similar to that produced by fresh or dried cells. The toxic substance is non-dialysable. The chemical separation of the toxic substance has been undertaken. It has the properties of an albuminoid.

*Nucleoprotein fraction:* This fraction gives rapid specific precipitation with precipitating sera. There is no specificity in the precipitability of fractions prepared from each of the three species with sera produced by each of the three species.

The nucleoprotein fraction when injected intradermally in a suitable dilution will elicit a local, or local and systemic reaction in brucella sensitive human beings. It has not been found satisfactory for detecting brucella allergy in the guinea pig, rabbit, monkey or cow.

The nucleoprotein fraction prepared from a reversible agglutinating strain of *Br. abortus* (agglutinating with normal sera but not with specific sera) is not precipitable in any dilution with precipitating sera. It is capable of eliciting a specific allergic reaction in brucella sensitive human beings. It is optically inactive.

*S substance:* The S substance, a non-protein, non-polysaccharide

substance prepared from *Br. melitensis*, is precipitable in a dilution of over 1:400,000. It is non-toxic for experimental animals. Repeated injections of 1.0 cc. of one per cent solution into guinea pigs, stimulates the formation of precipitins which precipitate the solution up to 1:1600 dilution, but no agglutinins for the cells can be detected. Immune opsonins are produced in the guinea pig.

Studies conducted on the Favilli precipitating polysaccharide obtained for *Br. abortus* show that if the chemical separation is carried far enough, the precipitating substance may be obtained free from polysaccharide. The precipitating substance is of the same nature as the S substance from *Br. melitensis*.

*Polysaccharide fractions:* The polysaccharide fraction recovered from the three species of *Brucella* is inactive biologically.

*M40. Application of the H—O Technique of Agglutination to Certain Clostridia.* ELIZABETH MCCOY AND L. S. MCCLUNG, University of Wisconsin, Madison.

The H—O technique for serological agglutination of bacteria allows some separation of the factors concerned and therefore reveals new meaning of the agglutination reaction. This is excellently illustrated by an analysis of the difference, yet interrelations, of three species of motile anaerobes, *Cl. acetobutylicum*, *Cl. felsineum*, and *Cl. roseum*. Cross agglutination with differential absorptions shows the following: (1) H factors, separate and species specific, (2) an O factor common to the three species, and accounting for the entire group reaction in two cases, and (3) a complex group reaction involving both H and O fractions between *Cl. acetobutylicum* and *Cl. roseum*. The latter is an interesting case in that the H factor is not a common group H but represents possession by one organism of some of the specific H of the other species.

It was also shown that within one species strains may vary quantitatively with respect to the species specific H factor. That the variant strains were deficient but not devoid of their specific H was shown by agglutinogenic action, by absorptive capacity for H-antibody, and by positive agglutination in a pure H serum of a strain not similarly deficient. The presence of strains of this type in three of the anaerobic species studied so far, *Cl. acetobutylicum*, *Cl. thermosaccharolyticum*, and *Cl. bifermentans*, indicates that this may be a common phenomenon.

*M41. Heat Labile and Heat Stable Antigens in the Production of Agglutinins for Various Spore-bearing Anaerobes.* L. S. MCCLUNG AND ELIZABETH MCCOY, University of Wisconsin, Madison.



A technique for the production of antisera against the stabilotropic and labilotropic antigens of spore-bearing anaerobes has been developed. Heat-treated cells were chosen for the somatic antigen and the flagellar antigen was the supernatant obtained by long centrifugation, after mechanical agitation, of a suspension of young cells grown in glucose-tryptone broth. Sera prepared against untreated cells were included for comparison. Rabbits were the experimental animals and usually 5 intravenous injections were given on alternate days. The animals were bled on the fourth to sixth day after the last injection and the serum was stored at low temperature. The appropriate antigens for the various tests were prepared in large volumes in normal saline and also refrigerated. No chemical preservatives were used in either antigen or serum. Test readings were made after overnight refrigeration following a four hour incubation period at 52–54°C. The results obtained were entirely comparable in type of agglutination to the descriptions in similar studies of the *Salmonella* and other groups by the English and German investigators.

Various motile anaerobes have been studied with the technique developed. Perhaps the most interesting are the non-pathogenic species, *Cl. acetobutylicum* and *Cl. thermosaccharolyticum*. The thermophilic species, cultivated at 60°C., possessed a heat labile antigen apparently analogous to that of the mesophilic species. *Cl. welchii* was chosen as the non-motile species to be investigated. Somatic sera were prepared from massive doses of heat-treated cells or cells washed free of toxin.

*M42. Purification of Suspensions of the Virus of Vaccinia.* C. A. BEHRENS AND F. A. NIELSEN, Purdue University, Lafayette, Ind.

An efficacious procedure of purifying dermo- and neuro-virus emulsions has been developed.

This method is based upon the separation of the virus from the extraneous material by precipitating the tissue at its iso-electric point.

Acetic, carbonic, citric, lactic, succinic and tartaric acids were found to have very little harmful effect on the virus when used in the small amounts necessary to reach the iso-electric point of the tissue.

Water clear suspensions of the virus have been prepared which have high titers and from which 75 to 85 per cent of the protein has been removed.

*M43. An Immunological Study in Laboratory Animals of Thirteen Strains of Equine Encephalomyelitic Virus.* BEATRICE F. HOWITT, Hooper Foundation, San Francisco, Calif.

A comparative immunological study was made of 13 different strains of equine encephalomyelitic virus (Meyer, Haring and Howitt). The 11 strains isolated from different parts of the United States could be divided serologically into two groups, an eastern and a western. Of the two foreign viruses, the one from Argentina was classified with the western series while that from Russia was immunologically distinct from the other two groups and varied in several other characteristics.

There was no *in vitro* cross neutralization nor *in vivo* protection between serums of any group when tested against the heterologous viruses.

Animals immunized to each member of the 3 divisions showed a constant tissue immunity within their own groups when tested intracerebrally but cross injection experiments were not conclusive, except for the Russian strain. A certain percentage of guinea pigs immune to the American western strains showed immunity when tested with the eastern and vice versa. There was no cross immunity between the Russian strain and either of the two American groups.

The American eastern strains of virus were more invasive and potent than the western, both by intracerebral and by intradermal inoculation of guinea pigs. The Russian virus was invasive for rabbits intracerebrally but not intravenously.

Comparison of potency was best demonstrated by titration methods.

A discussion is given of the possibility that the strains of virus isolated from horses in the different localities have sprung from a common root stock and should therefore be considered as varieties or subvarieties of the identical virus causing the same clinical disease.

*M44. Poliomyelitis. In Vitro Neutralization Tests Using Normal Adult and Convalescent Human Serums.* BEATRICE F. HOWITT, Hooper Foundation, San Francisco, Calif.

During the 1934 outbreak of poliomyelitis in northern California, serum was collected both from recovered cases of the disease and from normal adult individuals. The convalescent serums were given therapeutically and the normal pools prophylactically. *In vitro* neutralization tests were made on the pooled lots and on the individual serums with the following results.

Sixteen (66.6 per cent) of 24 undiluted normal serums from profes-

sional donors neutralized a standard amount of poliomyelitic virus while 8 (33.3 per cent) lacked potency.

Pooled undiluted serum from members of the medical staff as well as from normal adult volunteers, all neutralized the same amount of virus. Seven of them neutralized in a 1:10 dilution, 4 out of 6 in a 1:40 while none was positive after diluting 1:80. Sixteen pools of convalescent serum were tested and all but one neutralized when undiluted. Titrations of the latter showed a slightly lower degree of potency than did the normal serums.

Selected lots of serums from house staff members over and under 35 years of age, respectively, as well as from normal adult volunteers of the same age groups all neutralized the virus in a 1:40 dilution of serum.

Titration of convalescent serums pooled in lots from cases having had poliomyelitis in 1934 and 2, 4, 10, 20 and over 20 years previously showed a positive neutralization in a 1:20 dilution for all the pools except the 20 year group which failed to neutralize in a 1:10 dilution. The pool from the oldest age group, however, was potent when diluted 1:10. Several individual serums from recently recovered cases showed less potency than those from convalescents of longer duration, even over 40 years.

*M45. Immunization of Monkeys by Means of Poliomyelitis Virus Adsorbed to Alumina-gel.* F. B. GORDON, JAMES A. HARRISON AND N. PAUL HUDSON, Department of Hygiene and Bacteriology, University of Chicago.

Rhoads has reported the successful immunization of monkeys by means of virus adsorbed to alumina-gel. Observations in other fields indicate that the antigenicity of a substance may be enhanced by combining it with a colloidal carrier.

We subjected 6 normal monkeys to a series of subcutaneous injections of poliomyelitis virus adsorbed to alumina-gel (Willstätter, Type C). One animal died of poliomyelitis during the course of immunization. The undiluted serum of each of the remaining 5 neutralized the virus *in vitro*. One of the animals was killed accidentally, leaving 4 which were given intranasal inoculations of virus; 3 developed poliomyelitis and the fourth later succumbed to an intracerebral virus inoculation.

A comparable series of 6 normal monkeys was immunized by subcutaneous injections of the eluate of virus-alumina-gel complex. All survived the immunizing injections and the serum of each neutralized the virus. All were given an intranasal inoculation of virus and 5 developed

poliomyelitis. The sixth was also refractory to a subsequent intracerebral virus inoculation.

For control purposes 2 normal animals were injected subcutaneously with alumina-gel which had been shaken with normal monkey nervous tissue, the technic being the same as that used for preparing the virus-alumina-gel complex. Both serums failed to neutralize the virus. After intranasal inoculation only one animal was attacked but the second succumbed to a later intracerebral virus inoculation.

*M46. Purification of the Virus Neutralizing Fraction of the Serum of Poliomyelitis Convalescents.* JAMES A. HARRISON, Department of Hygiene and Bacteriology, University of Chicago.

A number of workers have shown that bacterial antibodies may be adsorbed to such inert materials as kaolin, chalk, ferric hydroxide, etc. We have attempted to adsorb to an alumina-gel the virus neutralizing substance of convalescent human poliomyelitis serum.

The supernatant of slightly acid (pH 6.5) mixtures of aluminum hydroxide (Willstätter, Type C) and convalescent human serum contained an insufficient amount of antibody to neutralize a test dose of virus in 10 of 13 trials. The alkaline (pH 7.4) washings of the gel adsorbates neutralized test doses of virus in 9 of 11 trials. In the only quantitative experiment tried, the eluate was neutralizing in approximately as high a dilution as was the original serum sample. Similar eluates of normal monkey serum did not neutralize the virus. Biuret tests run on serial dilutions of gel-absorbed serum and eluted antibody indicated a considerable diminution of protein in both fractions.

*M47. Failure to Infect Monkeys with Poliomyelitis Virus through Isolated Intestinal Loops.* EDWIN H. LENNETTE AND N. PAUL HUDSON, Department of Hygiene and Bacteriology, University of Chicago.

Four *rhesus* monkeys, with Thiry or Thiry-Vella fistulae at various levels of the intestinal tract, were given virus instillations into the isolated bowel loops. Two of these animals received a total of 40 cc. of 20 per cent crude virus-cord emulsion over a period of one week; all received, over a 4-month period, from 8 to 10 grams of *whole* glycerolated or fresh virus-cord. After a brief rest period, 3 consecutive daily instillations of 10 per cent virus-cord emulsions were given, each instillation being preceded by a preliminary flushing out of the isolated bowel with phosphate buffer solution, pH 5.0. No poliomyelitis occurred at any stage of the prolonged treatment with virus.

Neutralization tests on undiluted sera taken 44 days after the last administration of virus were negative. Saline extracts of the isolated bowel segments and of the regional lymph nodes failed to inactivate the virus.

*M48. An Investigation into the Mechanism of Adult Immunity against Poliomyelitis.* MAURICE BRODIE AND WILLIAM H. PARK, Department of Bacteriology, New York University and Department of Health, City of New York.

Two hypotheses have been offered for explaining the relative insusceptibility of adults to the virus of poliomyelitis. The first of these maintains that a specific immunity develops, the result of exposure to the virus, while the second presupposes that the resistance develops irrespective of the etiological agent and is merely a physiological change occurring with age.

Accordingly the serums of a series of 14 monkeys, whose ages ranged between 15 months and 15 years, equivalent in the human scale to between 8 years and middle age, were tested against a minimal completely paralyzing dose of virus. None of the serums neutralized the virus. Moreover, two of the older and larger animals were unable to withstand, per kilo of weight, any more virus than younger and smaller monkeys. Thus the experimental animal, which has little or no opportunity for exposure to the virus, fails to develop immunity with growth.

On the other hand 27 out of 34 serums, obtained from adults, where exposure to the virus was likely, showed considerable neutralizing substance. Moreover those with a history of frequent exposure to the virus showed a slightly higher antibody average content than those who had no knowledge of contact. On the other hand a series of 32 children, whose ages ranged between 1 and 7 years and who had probably had insufficient exposure to the virus to develop immunity, failed to show except in a few instances, an appreciable amount of antiviral substance. Thus immunity as evidenced by the neutralizing power of the serum for the virus, seems to develop only where there is an opportunity for exposure to the virus. Additional evidence was obtained by the production in monkeys of antiviral substance, upon repeated intranasal instillation of virus into the nares of animals with cut olfactory nerves.

In keeping with the development of immunity from specific exposure to the virus, was the fact that the immunity was not related to that of diphtheria and so immunity to each was probably the result of its own nonspecific incitant. In a series of 32 children and 19 adults, the Schick

test and presence of poliomyelitis neutralizing substance did not correlate. Likewise, in a small series of 26 adults, 11 of whom were in the A group and 15 in the O group, the incidence of antiviral substance was approximately the same for either group.

In keeping with the epidemiological evidence of others our experiments suggest that this immunity developed only after prolonged exposure to the virus.

*M49. Active Immunization of Experimental Animals and Children Against Poliomyelitis with Formalin Inactivated Virus Suspension.* MAURICE BRODIE, Department of Bacteriology, New York University and Department of Health, City of New York.

In previous communications it was pointed out that definite immunity could be developed against the virus of poliomyelitis using virus rendered non-infective by formalin. However, the amount of formalin used produced considerable skin irritation. Since then it has been shown that virus suspension inactivated with either 0.2, 0.1 or 0.05 per cent formalin at incubator temperature, was also antigenic for monkeys, and at the same time, the two latter strengths gave practically no skin irritation. The majority of the animals showed both humoral immunity as tested by the neutralizing power of the serum for the virus of poliomyelitis, and tissue immunity, that is resistance to intracerebral inoculation of active virus.

The inactivated antigen produced no reaction whatsoever; neither symptoms, temperature rise nor cerebrospinal fluid changes developed upon repeated inoculations of large doses given both intracerebrally and intraperitoneally, each representing the equivalent of thousands of infective doses of living virus. Moreover during vaccination the animal suffered no untoward local or systemic reactions and so it was felt that the vaccine could be given to humans with perfect safety.

However, before doing so upon children, it was deemed advisable to try it upon ourselves to determine whether the vaccine produced any disagreeable local or general reactions. Accordingly, 6 volunteers from the Bureau of Laboratories, Department of Health, New York, were given 3-cc. doses of 10 per cent virus suspension, inactivated with 0.1 per cent formalin for 16 to 48 hours. These were given: three, 1 dose; two, 2 doses and the third, 3 doses. The second inoculation was given 11 days after the first, and the third, 8 days after the second. After inoculation there was some soreness at the site of injection, lasting but a few minutes and probably due to the formalin. Three of these injected

had some induration, which lasted but a few days and was not painful or uncomfortable. In no instance was there any systemic reaction.

The blood serums of all 6, obtained before immunization, were tested for antibody and the amount was determined by careful titration. A preliminary test was carried out upon the serums of the 3 who had more than one dose of vaccine and some increase in antibody was demonstrable.

The vaccine was then given to two series of children the first consisting of 12 and the second of 20 children, whose ages range between 1 and 7 years. The virus suspension used for preparation of the vaccine was cultured aerobically and anaerobically before it was treated with 0.1 per cent formalin for 12 to 16 hours, 12 hours being the time required to inactivate the virus. In the first series, 5 received a single dose of 5 cc., the others were given a second dose, either 11 or 13 days later. In the second series one half were given 5 cc. and the other half 2.5 cc. of antigen and half of each group received one and two doses, respectively. One to 2.5 cc., was given intracutaneously, the remainder subcutaneously. The material was injected into the skin of the abdominal wall.

The children were carefully observed for local and general reactions and temperatures were recorded 4 times daily. There was no apparent general reaction or discomfort and at no time any febrile manifestations that could be attributed to the vaccine. The local reaction was negligible, consisting only of some induration in those receiving the larger amounts intracutaneously. The first dose did not render the children sensitive to the second dose.

To determine the degree of immunity produced by the vaccine, the neutralizing power of each serum was tested before and 3 or 4 weeks after the administration of the vaccine. In each instance the antibody or antiviral content of the serum was determined quantitatively by estimating the number of minimal completely paralyzing (M.C.P.) doses the virus neutralized. The M.C.P. dose represents the smallest amount of virus-containing tissue that will produce a complete and rapid paralysis in a monkey of 2.5 to 4 kgm. within 13 days.

Almost all of the children showed, prior to immunization, a small amount of neutralizing substance. Up to the present only the first series have been completed and all of the children have shown an appreciable response to the antigen. The blood serums of 6 children neutralized between 100 and 200 and the other 6 between 200 and 600 additional infective doses of virus when examined 3 weeks after immunization. The immunity developed within a week.

*M50. Avian Psittacosis.* K. F. MEYER AND B. EDDIE, Hooper Foundation, University of California, San Francisco.

The course of psittacosis as observed for a period of 295 days among ricebirds and parrakeets in small cages and also in a large aviary was briefly as follows:

(1) Spontaneous psittacosis among ricebirds is a rapidly fatal disease. In exposure experiments the longest incubation time recorded was 98 days.

(2) The virus present in the organs is discharged in the urine, faecal droppings and nasal mucus.

(3) Parrakeets are less susceptible to psittacosis than ricebirds. Latent infections are quite common and of greatest importance in the epidemiology of human and avian psittacosis.

(4) Immature and unmasked parrakeets succumb more readily to the virus than the mature breeding birds.

(5) The latent infections in a flock of parrakeets may, within 6 to 8 months, be reduced to 1 to 2 per cent. These carriers are non-infectious. With the resumption of breeding operations after an elapse of from 6 to 8 months psittacosis will flare up again and parrot fever in young and even old birds will be noted.

*M51. The Specific Behavior of Bacteriophage.* PHILIP LEVINE AND A. W. FRISCH, Department of Pathology and Bacteriology, University of Wisconsin, Madison.

Recent observations made in this laboratory, namely, the specific reaction between phage and carbohydrate containing extracts of bacteria and the demonstration of qualitatively specific fractions in polyvalent phages, are in harmony with the theory that the specificity of bacteriophage and antibodies depend upon similar, if not identical, substances.

With the aid of the phage absorption technic employed, i.e., tests for residual phage in the presence of heat-killed absorbing organisms, several of the somatic factors in the *Salmonella* group could be identified. At the same time qualitatively specific differences within the suipestifer group were demonstrated since several strains, absorbing poorly from a certain anti-suipestifer phage, were found to absorb small quantities of a phage for paratyphosus B. Experiments were then made to determine whether or not these strains could also be characterized by specific antibodies. Sera were produced by injecting rabbits with boiled organisms of the several varieties selected in the tests with phage and cross



absorption experiments were performed. The results of these tests point to the existence of qualitative specific antigenic differences within the suipestifer group attributable to somatic (heat-stable) factors. It remains to be determined whether or not there is a complete correlation between the observations made with phage and those with antibodies.

*M52. A Comparison of Streptococci from the Colon with Barga's Organism.* JOHN F. KESSEL, Los Angeles County Hospital and School of Medicine, University of Southern California.

Streptococci isolated from stools and from proctoscopic scrapings of patients exhibiting symptoms of colitis and of individuals with no colitis symptoms have been compared with 5 strains of streptococci commonly designated as Barga's "diplo-streptococcus."

The streptococci observed have fallen into 10 different fermentative types when grown in media containing inulin, mannitol, salicin, lactose, saccharose and raffinose, respectively.

All the types compared in this study have produced methemoglobin on blood agar plates, have resisted a temperature of 60°C. for 30 minutes, and have exhibited similar morphologic and cultural characteristics. None has produced intestinal lesions when injected intravenously into rabbits, though kidney lesions have been a common result of such injections.

By the use of mannitol and salicin only, it is possible to refer each of these 10 types to one of the following species of Andrewes and Horder: *S. faecalis*, *S. mitis* or *S. salivarius*.

Agglutination reactions do not correlate to any appreciable degree with the 10 fermentative types listed. A more marked serologic relationship exists, however, between strains giving fermentation reactions of *S. faecalis*, *S. mitis* and *S. salivarius*, respectively.

No one type has been associated more frequently with colitis patients than with non-colitis patients.

Of the 5 strains of Barga's organism compared, one was identical with our Type I which is also identical with *S. faecalis* of Andrewes and Horder; one with Type II which is a variant of *S. faecalis*; one is identical with our Type V, *S. mitis* of Andrewes and Horder, one with Type II, a variant of *S. mitis* and two with Type VIII, also a variant of *S. mitis*. Barga's strains did not exhibit any apparent differences from our type in (1) the action of blood plates, (2) heat resistance qualities or (3) pathogenicity in rabbits.

It is, therefore, concluded that there is no bacteriologic justification

for regarding the streptococci described by Bargaen as being essentially different from other types of alpha streptococci commonly recovered from the human alimentary tract.

## AGRICULTURAL AND INDUSTRIAL BACTERIOLOGY

### A1. *A Study of Methods for Determining the Numbers of Yeasts and Molds in Butter.* E. H. PARFITT, Purdue University Agricultural Experiment Station, Lafayette, Ind.

Various media which have been used in determining the yeast and mold count of butter were compared, and it was found that potato dextrose agar was the most satisfactory in that it yielded the highest counts. The composition of potatoes, as would be influenced by potatoes grown under widely different conditions and stage of maturity, did not affect the count of yeasts and molds. No advantage was secured by adding ammonium chloride, dipotassium phosphate and calcium chloride to the basic medium.

The kind of acid used to acidulate the medium to  $\text{pH } 3.5 \pm 0.1$  was found to affect the growth of yeasts, lactic acid causing the greatest inhibition and tartaric acid the least. Lactic acid was neutralized by the growth of yeasts to a greater degree than was tartaric acid.

The maximum count was secured after 5 days incubation at  $21\text{--}25^\circ\text{C}.$ , though the count secured after 3 days incubation at  $21\text{--}25^\circ\text{C}.$  closely approached the 5-day count.

The oxidation-reduction potential of the media within the limits studied, did not appear to be the limiting factor in the growth of yeasts and molds found in butter upon the media used.

### A2. *Study of the Lipolytic Enzymes of Three Specific Organisms.* GEORGE SPITZER AND E. H. PARFITT, Purdue University Agricultural Experiment Station, Lafayette, Ind.

The lipolytic activity of the enzymes of *A. lipolyticum*, *Ps. mucidolens* and *Ps. fluorescens* were determined on an emulsion of milk fat and tributyrin. The effect of sodium chloride and acidity was also studied.

The lipolytic enzymes of the three organisms were less active on the alkaline side than on the acid side ( $\text{pH } 7.5$  to  $5.5$ ). In the substrate at  $\text{pH } 7.0$  there was definite inactivation of enzymatic lipolysis in the presence of 8.0 per cent sodium chloride and a much greater inactivation with 16.0 per cent.

The enzymes of the organism were obtained by growing the organisms