THE SERODIAGNOSIS OF INFECTIOUS DISEASE. Augustus Wadsworth, Division of Laboratories and Research, New York State Department of Health, Albany.

Serologic tests in the study of infectious disease involve qualitative analyses and quantitative titrations. Agglutination has definite qualitative significance in typhoid fever and other infections, depending upon the titration of the activity; further differentiation with "O" and "H" antigens forecasts lines of future investigation.

Complement fixation possesses all the sensitivity of agglutination and is not so limited in the character of the antigens that may be used with it. Precipitation is the simplest procedure. The series of comparative tests here and abroad might suggest that precipitation in general is the more satisfactory in the serodiagnosis of syphilis. The difficulty in arriving at a true evaluation, however, lies in the fact that in the qualitative determination there is lacking that accurate quantitative analysis which is necessary for diagnosis.

Quantitative procedures in complement fixation which have recently been developed are therefore most timely. The data obtained with them, which will be published in detail, justify the conclusion that in the report of the 1936-1937 series of the United States Public Health Service the results with the precipitation tests, despite the fact that the first general impression may favor them, do not quite equal those obtained with complement fixation as reported by Doctor Gilbert from this laboratory, No. 27. (Jour. Amer. Med. Assoc., 1937, 109, 425.)

BASIC PRINCIPLES GOVERNING THE FIXATION OF COMPLEMENT AND THEIR APPLICATION TO PRACTICAL TESTS. Frank Maltaner, Division of Laboratories and Research, New York State Department of Health, Albany.

Studies with bacterial or protein antigens and their homologous antisera and with tissue extract antigens and syphilitic sera demonstrated constant relationships between the specific activities of serum, antigen, and complement which provide a rational basis for the performance of complement-fixation tests in general and allow an accurate titration of the immune reaction. Thus, the change in activity of complement is directly proportional, first, to the quantity of antigen present—provided a satisfactory excess of immune serum is present—and, secondly, to the amount of immune serum, provided a satisfactory excess of antigen is present. The relationship be-
tween the amounts of antigen and immune serum which cause the same change in complement activity is constant.

The point of 50-per-cent hemolysis is the most accurate for evaluating the changes which occur in complement-activity. By interpolation of values between 10- and 90-per-cent hemolysis to that of 50-per-cent, equally accurate results are obtained. Three to five tubes suffice for titrating sera of low or moderate activity whereas highly active sera require a more extensive titration. The titer of the serum may be expressed in terms of the change in complement-activity or in direct relation to antigen.

THE QUANTITATIVE DETERMINATION OF THE FIXATION OF COMPLEMENT BY IMMUNE SERUM AND ANTIGEN: FURTHER STUDIES WITH TUBERCLE ANTIGEN AND IMMUNE SERUM. Elizabeth Maltaner, Division of Laboratories and Research, New York State Department of Health, Albany.

Previous studies of the fixation of complement by tubercle antigen and immune serum have been extended to include observations in the region of serum, antigen, and complement excess.

In experiments covering a wide range of complement concentration there was a direct proportion between complement and immune serum when antigen was present in excess, and between antigen and complement when immune serum was present in excess. By determining the ratios of the slopes of the straight-line graphs representing these relationships, constant values for the specific reactive capacity of antigen with immune serum were obtained. The activity of serum and antigen in relation to each other and to complement may be accurately evaluated on the basis of the constant relations which exist between them. The complement-fixation test for tuberculosis described in 1925 is being revised on the basis of these studies to provide a quantitative determination of the reaction.

QUANTITATIVE COMPLEMENT-FIXATION TESTS WITH SPECIMENS SUBMITTED BY THE UNITED STATES PUBLIC HEALTH SERVICE IN EVALUATION OF SERODIAGNOSTIC TESTS FOR SYPHILIS. Elizabeth Maltaner, Division of Laboratories and Research, New York State Department of Health, Albany.

Following the official examination in the series of tests sponsored by the United States Public Health Service, the specimens were studied by a quantitative complement-fixation test. The numerical value obtained with the new method is a direct index of the titer of the serum. Titers greater than 10 are not differentiated; those less than 2 are classed as negative findings. The highest titer obtained with the control sera was 1.5. Sixty-seven of 191 syphilitic sera had titers greater than 10, 31 had titers of 7 to 10, 55 of 4 to 6, 19 of 2 to 3, and 19 of less than 2; seven of the latter were greater than 1.5.

Methods of reporting reactions as ±, +, 2+, 3+, or 4+ did not provide a reliable quantitative evaluation of the results. In general, specimens which reacted with both non-cholesterolized and cholesterolized extracts had high titers in quantitative tests with cholesterolized antigen. Reports made with different methods in the Federal series varied considerably with sera of low titer; the few disagreements observed with sera of high titer were important in indicating the danger of false or misleading reports due to prozone reactions.
REPORT ON A PRELIMINARY PRECIPITATION TEST AS AN AID IN THE SERO-DIAGNOSIS OF SYphilIS. Rachel Brown, Division of Laboratories and Research, New York State Department of Health, Albany.

Specimens submitted by the United States Public Health Service, 1936-37, were examined by the precipitation test of Wadsworth and Brown (Jour. Immunol., 1936, 31, 155) which is used in the New York State laboratory routinely in conjunction with an oversensitive complement-fixture test to select specimens requiring further examination. Whenever sufficient material was available after the completion of other tests, the sera which gave partial or no precipitation but definite complement fixation were retested in dilution to detect prozone reactions.

With 198 sera from cases of syphilis, precipitation was marked (2+ - 4+) with 93.9 per cent and partial (+ - +) with 2 per cent. No reaction of any degree occurred with 100 specimens from nonsyphilitic cases. This was a greater number of reactions than was recorded with the same sera from cases of syphilis with any of the precipitation methods used in the control laboratories in instances where no reactions were obtained with sera from nonsyphilitic cases.

The amount of antigen originally used in the test has been doubled in order to reduce the number of prozone reactions.


Specimens of human sera (447) have been titrated quantitatively for complement-fixture with two gonococcus antigens: I—a filtered extract of frozen and thawed cells; II—a broth culture filtrate. Both antigens were purified and concentrated by ultrafiltration.

Titers of 198 sera from persons with past or present history of gonorrhea (four days to forty years): Over 2.0 with both antigens, 113; over 2.0 with antigen I only, 3; over 2.0 with antigen II only, 15; below 2.0 with both antigens, 67. Thirty-eight had titers of between 5.0 to 10.0, 16 had titers of over 10.0.

Titers of 249 sera from persons with no recorded history of gonorrhea: Over 2.0 with both antigens, 32; over 2.0 with antigen I only, 1; over 2.0 with antigen II only, 19; less than 2.0 with both antigens, 197. Of these 52 reacting sera, the majority of which (35) had titers of between 2.0 and 3.0 with one or both antigens, 34 were from cases of syphilis, 3 from rheumatic fever, 2 from rheumatoid arthritis, 2 from cancer, 1 from epithelioma, 1 from erysipelas, 9 from pneumonia. Sera from 41 healthy persons did not react appreciably with either antigen.

QUANTITATIVE COMPLEMENT-FIXATION TESTS WITH SERUM AND SPINAL FLUIDS FROM MENINGOCoccus MENINGITIS CASES, CONVALESCENTS, AND CONTACTS. Grace M. Sickles and Christine E. Rice, Division of Laboratories and Research, New York State Department of Health, Albany.

Serum from patients convalescent from three to six weeks from group-I-III meningococcus meningitis was titrated by a simplified quantitative complement-fixture technique with extracts and culture filtrates of meningococci concentrated and purified by ultrafiltration. All cases had been serum treated. Of five sera from these convalescent carriers, four reacted to
titers of 3.0 to 7.4 with extracts and filtrates of group-I-III meningococci and to a lesser degree, titers 1.2 to 2.8, with group-II filtrates; the fifth was of low titer, 1.9, with group-I-III extracts. The reactions of sera from two convalescents who were no longer carriers and from seven contact carriers from whom group-II or atypical group-II meningococci had been isolated, were minimal.

Spinal fluids from five cases of serum-treated meningitis were titrated for the presence of meningococcus antigen. Of three specimens from which group-I-III meningococci had been cultured, two gave no fixation, one a very strong reaction, titer 18.0, with group-I-III rabbit serum and a weak reaction with group-II rabbit serum. This patient subsequently died. The fourth specimen from which group-II meningococci had been isolated fixed complement with group-II serum, titer 7.5, but not appreciably with group-I-III serum. From a fifth specimen without significant reactivity with either group-I-III or -II sera, an atypical group-II meningococcus was obtained on culture.

A Note on the Biological Properties of Strains Isolated from Cases and Carriers in an Outbreak of Meningococcus Meningitis. Sophia M. Cohen, Division of Laboratories and Research, New York State Department of Health, Albany.

A comparative study of strains from patients, convalescents, and contact carriers in an outbreak of meningococcus meningitis in New York State revealed marked differences which, in this limited series, appeared to be related to the serological group and to the source of the strains.

The cultures from the eight patients were classified as group I-III by agglutination and precipitation (immune-serum-agar plate) tests. Strains from two of three convalescent carriers corresponded to those isolated during the acute stage of the disease; that from the third was related to group I-III according to the agglutination reactions but lacked precipitative activity with group I-III sera. Five of six contact carrier strains, isolated late in the outbreak, fell into group II or '"X" related to II; the sixth was intermediate and showed some relationship to group I-III in agglutinative activity and to group II in precipitation reactions.

In general, among the limited number of strains tested, those from patients and convalescents were of higher virulence for mice and remained viable longer in sodium-chloride solutions than the contact carrier strains. Marked differences in toxigenic activity were not demonstrated. The atypical intermediate strains from two of the carriers, one a convalescent and one a contact, possibly represented modified group I-III strains.


The Action of Bacterial Toxins on the Tissues of Cold-Blooded Animals. Myrtle Shaw, Division of Laboratories and Research, New York State Department of Health, Albany.

Further studies of the effect of bacterial toxins on cold-blooded animals are reported. To determine the effect...
of toxins on regeneration of planaria, bacteria-free worms were necessary. This was accomplished by repeated washings in sterile water, continued until sterility tests showed no bacterial contamination. These planaria were cut transversely, both halves placed either in a hanging drop, or in tubes of suitable medium containing diluted toxin. Purified diphtheria toxins did not affect the regeneration of anterior and posterior regions but crude toxins were injurious.

Tadpole heart and liver tissues were cultivated in vitro in frog plasma and Tyrode's solution. A like medium containing crude diphtheria toxin diluted 1:10 (50 M.L.D.) or similarly diluted crude botulinus toxin type B had no inhibitory effect on the growth.

Similar studies were made on Fundulus embryos, removed aseptically from the egg, at the hatching stage. Fragments of tissue were explanted in drops of medium with diluted sea water as a base. Considerable migration of cells occurred within twenty-four to forty-eight hours. Crude diphtheria or botulinus toxins did not inhibit migration if used in dilutions higher than that in which broth itself inhibited. Botulinus toxin purified and concentrated by ultrafiltration did not inhibit migration in a 1:10 dilution.

Oxidation-reduction Potentials and a Method of Determining Them in Skin. Calvin C. Torrance, Division of Laboratories and Research, New York State Department of Health, Albany.

In order to determine the O/R potentials of skin, it is necessary to inject both oxidized and reduced solutions of sterile dyes. The dyes must be dissolved and sterilized by boiling immediately before use. In the method developed here, they are reduced in tubes designed to maintain anaerobic conditions and protected from light. A rubber stopper carries a glass filter which extends below the surface of the liquid and an outlet tube with cotton filter. Before sterilization, a pinch of platinumized asbestos is placed in the tube and sufficient distilled water is added to raise a petrolatum seal above the filter. The sterile dye solution is added with a syringe through rubber tubing attached to the filter tube. In preparing the solution of the dye, allowance must be made for the water in the tube. Hydrogen gas is passed down the filter stem until the color of the dye is bleached. The reduced dye can then be aspirated directly through the filter into the syringe used for injection without contamination or reoxidation. Thionin, cresyl blue, galloxanin, methylene blue, and indigo-tetrasulfonate, -trisulfonate, and -disulfonate were found useful in studies of skin.

PROCEEDINGS OF LOCAL BRANCHES

CONNECCTICUT VALLEY BRANCH

YALE MEDICAL SCHOOL, BRADY AUDITORIUM,
310 CEDAR STREET, NEW HAVEN, CONNECTICUT,
DECEMBER 4, 1937

The Fibrinolytic Test in Clinical Use. Paul L. Boisvert, Department of Pediatrics, School of Medicine, Yale University, New Haven, Connecticut.

The streptococcal fibrinolytic test of Tillet and Garner had promise of being a practical serological test for recent hemolytic streptococcal infection. Recently, however, Waaler has re-
ported that the test is also positive (i.e. the plasma clot is resistant) in pneumonia caused by organisms other than the hemolytic streptococcus.

The fibrinolytic test has been used clinically in our pediatric laboratory for over a year. Although in young infants variable results have been obtained, the test is generally positive in children recovering from a hemolytic streptococcal infection. We have also observed positive tests in the majority of patients in the pediatric age group with pneumococcal pneumonia. However, repeated tests on these patients have revealed that the test is positive only during the acute febrile stage of the disease, and rapidly becomes negative. This reaction is quite different from that seen in children with hemolytic streptococcal infections. In the latter the test is negative during the active infection and generally becomes positive at the time of the patient’s recovery.

The results indicate that a series of tests may be of clinical diagnostic value. The simplicity and inexpensiveness of the test add to its practical value.

THE ACTION OF INTESTINAL BACTERIA ON ASCORBIC ACID (VITAMIN C).
William B. Esselen Jr., Department of Bacteriology and Physiology, Massachusetts State College, Amherst, Massachusetts.

The purpose of this work was to determine whether certain intestinal bacteria destroy vitamin C, and if they do, what explanation may be offered for this action. The results obtained are in contradiction to those previously reported by other workers. Intestinal bacteria (Four strains of *Escherichia coli-communis*, seven of *Escherichia coli-communior*, and one strain each of *Aerobacter aerogenes*, *Salmonella pullorum*, *Salmonella aer-trycke*, *Salmonella enteritidis*, *Eberthella typhi*, *Bacillus subtilis*, and *Proteus vulgaris*) exert a protective action on the readily oxidized ascorbic acid. This protective action appears to vary directly with the suitability of the medium for bacterial growth and the numbers of organisms present. The effect may be due to: (1) increased acidity produced by the organisms, (2) their reducing power, (3) the removal of oxygen from the media by them, or (4) metabolic products produced. It was shown that the lowering of pH by the bacteria is not a factor in preventing the destruction of ascorbic acid. Furthermore, no correlation was established between the ability of the organisms to reduce methylene blue and the stability of ascorbic acid in their presence. Carbon dioxide, but not hydrogen, exerts a protective action on ascorbic acid, similar to that produced by bacteria growing in media containing readily fermentable carbohydrates. It is suggested that the ability of intestinal bacteria to retard the destructive oxidation of ascorbic acid may be due to their ability to produce carbon dioxide.

THE EFFECT OF VACCINIA IMMUNE SERUM IN REDUCING THE NUMBER OF COUNTABLE LESIONS ON THE CHORIO-ALLANTOIC MEMBRANE OF THE DEVELOPING CHICK EMBRYO.
Elisabeth Osterman and Rachel E. Hoffstadt, Department of Bacteriology, University of Washington, Seattle, Washington. (Miss Osterman now a graduate student in the Department of Bacteriology, Yale University, New Haven, Connecticut.)

There is little direct evidence supporting the belief that hyperimmunization of animals with vaccinia increases
the virus "neutralizing" property over that present in the serum taken after simple vaccination. An attempt was made to obtain more definite information by utilizing the method of Burnet (1936) and Koegh (1936), titrating the serum-virus mixtures on the chorionic-allantoic membrane of the developing chick embryo.

Serum was collected from two rabbits fourteen days after dermal vaccination. They were then hyperimmunized by intravenous inoculation of saline extracts of dried vaccinal membranes, serum being taken after a total of 50 mg. and 150 mg. had been injected. Two control rabbits received normal membrane extracts in the same amounts. The serum-virus mixtures were inoculated into each of ten eggs, and the lesions appearing on the countable membranes averaged. The percentage reduction in the number of lesions that appeared in the presence of serum taken after vaccination and after hyperimmunization is shown in Table I.

<table>
<thead>
<tr>
<th>SERUM</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal memb. 150 mg.</td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dermal vaccination</td>
<td>73</td>
<td>57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperimm. 50 mg.</td>
<td>83</td>
<td>82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperimm. 150 mg.</td>
<td>92</td>
<td>83</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Parallel titrations using both saline and distilled water as diluents, and observations of the mixtures both macroscopically and microscopically by dark field illumination, gave no evidence that flocculation had occurred under the conditions of the test. Therefore, flocculation could not have played a rôle in reducing the count. The results support the belief that the "neutralizing" properties of vaccinal antiserum are increased by hyperimmunization following vaccination.

**Field Studies on the Recovery of Poliomyelitis Virus. J. R. Paul, J. D. Trask, and A. J. Vignec, Department of Pediatrics, School of Medicine, Yale University, New Haven, Connecticut.**

Recovery of poliomyelitis virus from contaminated material has always been difficult. Whether or not this depends on inadequate methods is uncertain. Kramer has described the use of ether as a satisfactory bactericidal agent. Accordingly, nasal washings and stool emulsions were treated with ether and in most instances frozen and concentrated in a Florsdorf-Mudd apparatus. Intracerebral and intraperitoneal inoculations were made into monkeys.

Three criteria were adopted as evidence of positive results: (1) Production of the experimental disease, (2) Histopathology, (3) Passage into a second animal.

Success was attained in three out of four children studied in the first day of the disease, twice from nasal washings and once from the stools. Ten attempts to recover the virus were made late in the disease with one successful result.

The high percentage of positive findings reported here would seem to indicate that the method employed has considerable promise.

**The Influence of Estrogenic Hormone on the H-Ion Concentration and Bacterial Flora of the Human Vagina, with Special Reference to Döderlein's Bacillus. Louis Weinstein and Joseph H.**
Howard, Department of Bacteriology, Yale University, New Haven, Connecticut, and The Gynecology Service, City Dispensary, Bridgeport, Connecticut.

The injection of estrogenic hormone into women in the post-climacterium led to the development of a high degree of H-ion concentration in the vaginal secretions in eight out of nine cases. The only individual who did not respond was one who had undergone ovariectomy several years previous to the time of this experiment. Studies of the histological structure of the vaginal mucosa, before and after treatment with the hormone, revealed that a low pH could be correlated, in every instance, with a growth of the mucosal tissue.

Attempts to establish a correlation between the acidity of the vaginal secretions and the presence or absence of the Döderlein bacillus failed; in seven of the nine individuals examined no organism of this type was demonstrable culturally in a pH range of 3.8 to 7.0. In one instance large numbers of the Döderlein organism were present at a pH varying from 6.6 to 7.2. This was in the ovariectomized individual who did not react to hormone treatment. In the other case the aciduric vaginal bacillus was recovered, at times in fairly large amounts, at pH 6.0 to 6.8, but it was not demonstrable at other times at a pH level which was the same or considerably lower.

It is concluded that there is very little, if any, correlation between large amounts of acid in the vaginal secretions and the presence of Döderlein's bacillus. On the basis of this fact, it is felt that this organism has little value as an indicator of vaginal health or as a therapeutic agent.