STUDIES ON THE ALLERGIC AND ANTIGENIC
ACTIVITY OF SONIC FILTRATES OF BRU-
CELLA Abortus. I. Live, School of Veterinary
Medicine, University of Pennsylvania,

Sonic filtrates of Brucella abortus were pre-
bpared by exposing dense suspensions of
the organisms for two hours to vibrations of
audible frequency at 9,000 cycles per
second. The magnetostriction oscillator
of Chambers and Flodorf was used. Rab-
bits and guinea pigs were sensitized by an
injection of about 900,000,000 live organisms.
An intravenous injection of the organ-
isms into rabbits resulted in a more uniform skin
sensitivity to the intracutaneous injection
of sonic filtrate than an intraperitoneal
injection. Guinea pigs were sensitized by an
intraperitoneal injection of the organ-
isms. The animals usually showed satis-
factory allergic skin sensitivity to the sonic
filtrate when tested one month after
injection. In some instances an intracutane-
ous injection of as little as 0.0015 mg of
protein in the filtrate produced skin re-
actions in sensitized animals. The allergic
skin reactions were usually at their height
48 to 72 hours after injection of sonic filtrate,
and persisted in some cases for as long as
10 to 14 days. The infected animals de-
volved agglutinins, precipitins, and op-
sonins as determined by serological tests.
Single intracutaneous injections of as high
as 0.05 mg of protein in the sonic filtrate did
not sensitize non-infected rabbits to subse-
quent skin tests. However, they developed
agglutinins as a result of the injection of
filtrate, and the agglutinative titers as well
as the length of time for which they per-
sisted were directly proportional to the
quantity of protein injected.

The stability of sonic filtrates of B. abortus was studied by comparing the al-
lergic activity of lyophilized material with
that of non-lyophilized filtrate. Tests at
frequent intervals over a period of two
years did not show any change in the ac-
tivity of the untreated filtrate. Of three
preservatives used, 0.5% phenol, 0.25%
formalin, and 1:10,000 dilution of merthio-
late, none was found to impair the allergic
activity of sonic filtrate in the course of
comparisons over a period of one year.

THE ANTIGENIC STRUCTURE OF HEMOPHILUS
PERTUSSIS. E. W. Flodorf, A. Bondi,
Jr., Harriet M. Felton, and A. C. Mc-
Guinness, Department of Bacteriology
and Pediatrics, School of Medicine, Uni-
versity of Pennsylvania, Philadelphia,
Pa.

Hemophilus pertussis is of a single sero-
logic type. Only organisms freshly isolated
from active cases of whooping-cough, known
as strains in phase I, are of clinical sig-
nificance.

Two toxins occur in H. pertussis in all
phases; one toxin is thermolabile and the
other is thermostable. These toxins alone
are apparently not adequate for establish-
ment of immunity by their use as toxoidal
immunizing agents unsupported by reagents
to produce antibacterial immunity as well.
Vaccines of killed whole organisms are be-
lieved to be successful in production of
active prophylaxis as the result of the anti-
bacterial antibodies which are elicited.
Because of the lack of thermolabile toxin
present in such vaccines now currently
used, one would not expect much from
toxin as a reagent in a test for suscepti-
bility to whooping-cough. It might dis-
tinguish those who have had the disease,
but it would not distinguish those who
have been vaccinated from the susceptible
individuals. In clinical trials, the skin
test using agglutinin is being confirmed
as of value for determination of suscepti-
bility.

CLINICAL RESULTS OF THE USE OF AG-
GLUTINOGEN FROM PHASE I HEMOPHILUS
PERTUSSIS AS A SKIN TEST FOR SUSCEPTIBILITY TO WHOOPING-COUGH. Harriet M. Felton and E. W. Flosdorff, Departments of Pediatrics and Bacteriology, School of Medicine, University of Pennsylvania, Philadelphia, Pa.

Results were given of a clinical study with a total of 776 cases, using the purified agglutinogen as a skin test for susceptibility to whooping-cough. The test seems to classify immune and susceptible individuals according to the histories of disease and vaccination in a satisfactory manner. This material in single skin test doses produces a marked increase in the agglutinating titer of individuals with existing immunity or partial immunity to pertussis. Repeated doses produce a reversal of the test and a detectable titer in individuals who had no initial immunity. A small institutional epidemic was studied and the results indicate that susceptibility can be accurately predicted by the use of this skin test.

THE ANTIGENIC STRUCTURE OF HEMOPHILUS PARAPERTUSSIS AND ITS PROBABLE CLINICAL SIGNIFICANCE. A. Bondi, Jr., Harriet M. Felton, and E. W. Flosdorff, Departments of Bacteriology and Pediatrics, School of Medicine, University of Pennsylvania, Philadelphia, Pa.

By means of agglutinative absorptive tests it has been shown that cross agglutination between H. parapertussis and H. pertussis in Phase I is due to a common minor antigen. The wide incidence of parapertussis agglutinins among the general population as shown by Miller has been confirmed. These agglutinins are specific, in large measure, presumably as a result of specific infection by H. parapertussis as shown by means of agglutinin absorption. This suggests some prevalent disease in children and adults specifically related to H. parapertussis which clinically and diagnostically is not recognized as such. Although intensive immunization increases the agglutinative titer against the heterologous species, it is not known whether such agglutinins are effective in cross protection against either disease.

THE USE OF THE SYRIAN HAMSTER AS A LABORATORY ANIMAL. Harry E. Morton, Department of Bacteriology, School of Medicine, University of Pennsylvania, Philadelphia, Pa.

The Syrian hamsters have been found to be a satisfactory laboratory animal for the study of numerous viruses, as follows: encephalitis (Linnette, 1941; Broun, LeGier, Mesera, and Muether, 1941), equine encephalomyelitis (Shahan and Creech, 1942), influenza (Taylor, 1940; Taylor and Parodi, 1942, and Wheeler and Nungester, 1942), lymphocytic choriomeningitis (Smedal and Wall, 1942), mare abortion (Anderson and Goodpasture, 1942), and poliomyelitis (Plotz, Reagan, and Hamilton, 1942). They have also been found useful in the study of dental caries (Arnold, 1942), leptospirosis (Morton, 1942), various vitamin deficiencies (Routh and Houchin, 1942), and are susceptible to the human, bovine, avian, and vole strains of tubercle bacilli (Griffity, 1941) and to leishmania infections (Adler and Tcherno-

merets, 1941). We have found the animals hardy, free from the usual laboratory infectious diseases, and capable of bearing young when only 67 days old. The gestation period is 21 days with 6 or 7 young comprising an average litter.

THE EFFECT OF PROMIN ON EXPERIMENTAL TUBERCULOSIS IN THE RABBIT. Max B. Lurie, The Henry Phipps Institute, University of Pennsylvania, and Joseph Stokes, Jr., The Children's Hospital, Philadelphia, Pa.

Twelve rabbits were fed twice daily with particulate chow which had been thoroughly mixed with a solution of promin in water containing Karo syrup. The amount of promin consumed by each rabbit varied between 350 to 750 mg per kilo per day. These rabbits, together with an equal number of control animals, were infected intracutaneously with 0.2 mg of a virulent strain of bovine tubercle bacilli (Ravenel). The control animals were given the same
diet, in exactly the same manner, in the same quantities including the syrup, except that no promin was added. The progress of the disease at the site of inoculation and in the draining lymph nodes was carefully measured at weekly intervals. During the first four weeks following the infection no significant difference was observed in the progress of the tuberculosis at these sites between the promin-treated and control animals. On the thirty-second day after inoculation the local lesion in both groups had ulcerated. The pus from each lesion was evacuated and finely ground crystals of promin were sprinkled, at weekly intervals, into the wounds of the experimental animals while glucose, the constituent of promin outside the diphenol-sulfone group of the drug, was likewise sprinkled into the lesion of the control animals after the evacuation of its pus.

It was found that all of the local lesions of all of the experimental animals without exception had healed completely by the 15th week after infection while not a single local lesion in the control animals was healed at the end of 140 days, when all surviving animals of both groups were sacrificed. The extent of the disease in the lymph nodes draining the site of inoculation was about five times greater in the control animals than in the rabbits treated locally with promin; apparently because of the drainage of large amounts of the drug from the primary lesion to the local nodes. A similar though less pronounced retardation of the tuberculous process in the lungs, kidneys, pleura, bones, and joints and other internal organs was clearly discernible in the promin-treated group. The concentration of promin in the blood of these animals varied between 2 to 6 mg per cent. There is evidence that the glucose administered to the control animals was not responsible for the failure of the local tuberculosis to heal. The efficacy of promin in the local treatment of tuberculosis in rabbits is evident from these experiments.


LABORATORY STUDIES IN SPOTTED FEVER IMMUNE SERUM. Florence Fitzpatrick, Sharp and Dohme Laboratories, Glenolden, Pa.

NEW JERSEY BRANCH (THEOBALD SMITH SOCIETY)

NEW BRUNSWICK, N. J., NOVEMBER 5, 1942

CULTIVATION OF HEMOPHILUS INFLUENZAE IN YOLK-SAC OF DEVELOPING CHICK EMBRYO. E. Alture-Werber, Division of Microbiology, The Squibb Institute for Medical Research, New Brunswick, N. J.

Six- and seven-day-old chick embryos proved to be very susceptible hosts for H. influenzae. Given these organisms by the yolk sac method in a highly diluted culture 6 to 60 organisms were sufficient to kill all embryos. Virulence tests suggested that even a single organism would infect and kill the embryo. The bacilli multiply and spread rapidly in the egg and kill the embryo in 2 to 3 days. Yolk and amniotic fluid are teeming with bacilli. The susceptibility to H. influenzae appears to diminish with the age of the embryo. Microscopic preparations of the head of the embryos showed that H. influenzae invades the blood vessels and causes severe hemor-

phages. No meningitis was observed in these sections.

CHEMOTHERAPEUTIC STUDIES WITH HEMOPHILUS INFLUENZAE IN MICE AND CHICK EMBRYOS. E. Alture-Werber, Division of Microbiology, The Squibb Institute for Medical Research, New Brunswick, N. J.

Mice were infected intraperitoneally with 10,000 to 100,000 organisms suspended in mucin. Of mice fed on sulfonamides mixed in Sherman diet 48 hours before infection, 74% were protected by 1% sulfathiazole and 91 by 0.1% sulfadiazine. Ten mice fed on 1% promin survived. The untreated infected controls had a survival rate of 13%. Of mice treated intraperitoneally immediately after infection with the sodium salt of the compounds, 42% of those with sulfathiazole (600 mg./kg. daily) and 80% of those with sulfadiazine (500 mg./kg. daily)
survived. The untreated controls belonging to this group showed a survival rate of 10%.

Six- and seven-day-old chick embryos infected with 6 to 60 H. influenzae bacilli by the yolk sac method and treated 30 to 45 hours later with various amounts of the sodium salts of the drugs by the same route gave the following results: most of the embryos survived for the 8-day period of observation when treated with 1 mg. to 3 mg. sulfathiozole. One mg. to 3 mg. of sulfadiazine protected most of the 6- and 7-day embryos. Smaller doses of the drug delayed the death of the embryos. Promin had no influence on the course of infection. Untreated infected chick embryos have a mortality rate of 100%; their yolk and amniotic fluid are teeming with bacilli, while in treated eggs, amniotic fluid and embryo, depending on the protective power and dosage of drug, are little or not at all affected by the infection.

**Fungi Tolerant to Extreme Acidity and High Concentrations of Copper Sulfate.** E. L. Starkey and S. A. Waksman, Agricultural Experiment Station, Rutgers University, New Brunswick, N. J.

Two fungi were isolated from acid solutions (pH 0.2 to 0.7) containing 4 per cent copper sulfate. One form was found to be closely related to Cephalosporium and was identified as Aconitum velatum Morgan; the other was a dark green fungus belonging to the Dematiaceae; accurate identification was impossible since it did not produce any true spores.

Both fungi were tolerant to extremely high concentrations of hydrogen ions and copper sulfate. They grew quite well in a synthetic medium with pH 0.3, 0.4, 0.5 and 1.0 and made limited development even at pH 0.1. The green fungus likewise grew at pH 0. The most acid medium which supported growth had an acidity of approximately 2.5 normal H₂SO₄. Both of the fungi were able to grow in media saturated with copper sulfate, although they grew better in media to which no copper salt was added. They developed quite well in saturated solutions at pH 2.0 to 0.3 and produced some growth at a reaction level of approximately pH 0.

These fungi appeared to be as tolerant to acidity as any organism known so far; it is believed that these two organisms show the greatest tolerance to the combined effects of high concentrations of copper sulfate and of extreme acidity yet recorded.

**Tumor Formation by Attenuated Crown-Gall Bacteria in the Presence of Growth-Promoting Substances.** Armin C. Braun and Thomas Laaskar.

An attenuated culture of Phytoponas tumefaciens formed large tumors on tomato plants when supplemented with growth-promoting substances. The growth substance reaction was not specific. The three synthetic growth substances used as well as the plant growth hormones served as stimulating agents. Fragments of these artificially induced tumors when implanted into healthy tomato plants developed into typical large tumors in 4 to 5 weeks. The tumor tissues have now successfully been carried through 5 successive passages in tomato plants involving a period of six months. One clone of tumor tissue was isolated that was entirely free of crown gall bacteria. Fragments of this bacteria-free tissue grew rapidly into large neoplastic growths similar to the bacteria-containing tumors. The host cells when acted upon by the growth substances alone were found incapable of inducing the formation of tumors. It would appear, therefore, that the attenuated culture is capable of altering the host cells to tumor cells. The attenuated culture alone, however, is unable to further stimulate these altered cells to any appreciable extent. When supplemented with growth substances these altered cells are capable, like those stimulated by the virulent culture, of uncontrolled growth in the host.

**CONNECTICUT VALLEY BRANCH**

Yale School of Medicine, New Haven, November 28, 1942

Cultivation of a Bacteria-free Strain of *Trichomonas Fetus*. W. N. Plastridge, Department of Animal Diseases, Storrs Agricultural Experiment Station, Storrs, Connecticut.

A bacteria-free strain of *Trichomonas fetus*
was isolated postmortem from the uterine exudate of a cow affected with pyometra. The strain has been maintained for a period of 8 months in a medium composed of beef infusion, 1.0 per cent peptone, 1.0 per cent glucose, 0.07 per cent agar and 2 per cent sterile inactivated ox-serum, and adjusted to an initial pH of 7.2. All of the ingredients mentioned were necessary for growth. The size of the inoculum required for growth in the freshly prepared medium may be as small as 1 motile trichomonad per 10 ml of medium. Counts as high as 12,000,000 motile trichomonads per ml were obtained. Cells grown in the clear medium provide satisfactory suspensions for serological tests.

The presence of a utilizable carbohydrate was found to be essential for growth. Good development, with acid production was obtained in a basal medium to which were added Andrade's indicator and 1.0 per cent of one of the following monosaccharides: galactose, glucose, levulose and mannose. Moderate growth took place in a medium prepared with arabinose, and slight growth in the one containing xylose. No development occurred in media prepared with rhamnose, maltose, lactose, sucrose, raffinose, dextrin, glycogen, inulin, starch, glycerol, dulcitol, mannitol and sorbitol.

The optimum initial pH of the medium was found to be between 7.0 and 7.2; the optimum concentration of serum was between 2.0 and 20.0 per cent, and of glucose 1.0 per cent.

Attempts to grow the culture in the presence of staphylococci and coliform organisms failed. The inclusion of different concentrations of crystal violet and sodium azide in the medium did not permit the successful cultivation of T. fetus in the presence of the organisms mentioned.

A Study of the Spread of H. influenzae, Type B. Roswell D. Johnson and Mildred D. Fousek, Department of Pediatrics, Yale School of Medicine.

In 1931 Pittman described a "smooth" variant of Hemophilus influenzae which showed capsular formation and was the form to be found in clinical invasions. This organism she designated type "b." By 1939 Alexander developed a rabbit antiserum against this organism. From 1922-1939, 22 cases of influenzal meningitis were treated here with 100% mortality, and, from 1939-1942, 20 cases were treated with this serum and sulfonamides with but 3 deaths, two of these in infants under 6 months of age. Because of an apparent increase in the morbidity rate due to H. influenzae type "b," cultures of the throat and nasopharynx in families of children harboring the organism were made to find the carriers.

Ten families were studied, comprising a total of 46 individuals, of whom 14 were 8 years of age or less. Of this younger group, 7 (50%) were positive, while in the group over 8 years of age (including parents), there were 32 individuals with but one mother positive.

Six families studied as controls, with a total of 24 members (6 of whom were under 6 years of age) were all negative.

A convalescent hospital was studied, where 1 child had a febrile illness. Cultures of his nose and throat revealed the type "b" influenza bacillus. The rest of the ward was cultured, and positive cultures obtained in 0 of 5 infants, 3 of 4 toddlers, 4 of 4 older children (to ten years), and 1 of 6 nurses, a total of 8 out of 19 individuals. Repeated one week later, one additional child previously negative was positive, and another nurse, a newcomer, was positive.

Seventeen older children in an adjoining ward were all negative, although they were cared for by the same nurses.

The organism apparently attacks children more frequently than it does adults, but close contact with the latter, as in the case of mothers or nurses, may induce a carrier state in them. Local treatment of the carriers may be considered, but is not being done at present.

Non-Hemolytic Variants of Hemolytic Streptococci. Paul L. Boisvert and Mildred D. Fousek, Department of Pediatrics, Yale School of Medicine.

Recently non-hemolytic variants of hemolytic streptococci were recovered from three children with scarlet fever. In two instances only non-hemolytic colonies were present in cultures from the nose and throat. Cultures from one child showed both hemolytic and non-hemolytic colonies.

These strains were members of Lancefield's human pathogenic Group A, and their
fermentation and biochemical reactions were those of human pathogens. They belonged in Griffith's Type 14, a type which is prevalent in New Haven at this time.

The organisms were non-hemolytic on original cultivation on rabbit's blood agar plates incubated aerobically. Incubation of blood agar plates in an atmosphere of CO₂ produced slightly hemolytic colonies. The strains were hemolytic in blood broth after several transfers, and subcultures on blood agar plates produced either hemolytic and non-hemolytic colonies or only hemolytic colonies.

These non-hemolytic variants are of some importance to the clinical bacteriologist since such colonies might be overlooked. Although the colonies are not hemolytic they appear otherwise to be typical matt colonies of hemolytic streptococci. We can attribute no special qualities to these variants.


The value of Salmonella typing in a public health laboratory is discussed and the methods used in Connecticut are presented. A public health laboratory should render at least a minimum typing service by which human types may be differentiated from animal types and the main somatic groups (B, C, D and E) may be recognized. The methods involved are no more complicated than those for pneumococcus typing. Additions to or departure from this basic plan may be desirable in a given locality but should be made only after a sufficiently long experience to obtain a general idea of the types commonly encountered. The assistance of a National Salmonella Center should be utilized in any case.

In Connecticut, Salmonella typing, originally the minimum stated above, was started in 1939. The results of this service extending over 45 months for a total of 358 cultures have shown that: 43.3 per cent of all new Salmonella isolations were strains of animal origin, belonging to 23 serotypes; 56.7 per cent were of human origin, represented by 2 serotypes, S. typhi and S. schottmuelleri. The most common serotypes were as follows: typhi, 40.8 per cent; schottmuelleri, 15.9 per cent; typhimurium, 14.8 per cent. Only 1.1 per cent of the serotypes fell into somatic groups other than B, C, D and E. Organisms isolated for the first time from man were S. california, S. pullorum, S. worthington, S. kentucky and two new types, S. hartford and S. simsbury. Concerning the animal strains, the carrier state in man was rarely prolonged either in convalescents or in healthy individuals and the chronic carrier state is by no means so frequently found as with types of human origin.

Typing of a Salmonella culture by means of antigenic analysis is the only procedure by which the laboratory can furnish an authentic report and save time and effort in the ensuing epidemiological investigation by stressing the relative importance in each case of (1) looking for the human carrier or (2) establishing a chain of events leading from the infected or carrier animal to man. The Biochemical and Serological Relationships of the Organisms of the Genus Proteus. Robert Rustigian and C. A. Stuart, Biological Laboratory, Brown University, Providence, R. I.

Biochemical and serological studies were made with several hundred Proteus, proteus-like and other cultures to determine the cardinal characteristics of the genus Proteus.

Culturally P. vulgaris members of the genus could not be distinguished from P. mirabilis since all motile strains readily swarmed on nutrient agar and eosin methylene-blue agar. Two strains each of P. vulgaris and P. mirabilis were isolated which were non-motile and formed compact colonies. Swarming of P. vulgaris or P. mirabilis was never observed on SS agar, but well isolated colonies generally developed black centers in from 24 to 48 hours. Except for one fecal strain all P. morganii isolates were motile and 18 of 76 strains exhibited swarming under suitable conditions.

The outstanding physiological criteria for the genus Proteus appeared to be urea decomposition and slight gas formation
(bubble to ten per cent) in fermentable carbohydrates even after several days incubation. P. vulgaris, 55 strains, P. mirabilis, 143 strains, P. morganii, 76 strains, and one presumably new type tentatively called "P. entericus", 37 strains, (Cope and Kilander, 1942) decomposed urea and produced small gas volumes. Strains of P. ammoniacus and P. americanus which also decomposed urea could not be distinguished biochemically or serologically from P. mirabilis. P. icthysmius and P. hydrophilus failed to decompose urea and after several days incubation produced large volumes of gas (50 per cent or more) in fermentable carbohydrates and it is recommended that these types be classed as aberrant coliforms.

Both P. vulgaris and P. mirabilis produced acid and gas in glucose, sucrose and some strains in salicin. Lactose and mannitol were not attacked. Biochemically P. vulgaris differed from P. mirabilis in that it fermented sucrose and mannitol in 24 to 48 hours and formed indole. A majority of P. vulgaris strains were Voges-Proskauer negative and freshly isolated strains generally failed to grow on citrate agar. On the other hand, a majority of P. mirabilis cultures gave a positive Voges-Proskauer reaction and utilized citrate. Atypical biochemical reactions were encountered, particularly with old laboratory cultures of P. vulgaris and P. mirabilis. P. morganii fermented glucose, and occasionally sucrose but failed to ferment lactose, maltose, mannitol and salicin. Indole was formed, but negative reactions were obtained for H₂S production, gelatin liquefaction, Voges-Proskauer test and citrate utilization. "P. entericus" formed acid, occasionally acid and a bubble of gas in glucose and mannitol and some strains acid in salicin, most strains produced acid in sucrose slowly while lactose and maltose were not fermented.

P. vulgaris, P. mirabilis, P. morganii and "P. entericus" exhibited serological heterogeneity in that several antigenic types were established within each species. The incidence of agglutination was relatively high with strains of each species, indicating a frequent distribution of common antigens. No simple serological division could be made between vulgaris and mirabilis since common H and O antigens were not infrequently encountered. In addition there were H antigens common to mirabilis, "entericus" and morganii, and H and O antigens common to vulgaris and morganii.

Other than the serological distinction of the X strains sero-typing in the genus Proteus does not appear to be of any taxonomic or practical value.

It is suggested that urea decomposition and slight gas formation be considered as two of the cardinal characteristics of Proteus. It is recommended that the term "morganii" be employed solely for the organisms described as P. morganii (Morgan's Bacillus No. 1) and that its usage to designate various "morganii types" be discontinued.

THE TEXAS BRANCH
THE UNIVERSITY OF TEXAS, AUSTIN, OCTOBER 31, 1942

THE EFFECT OF HEAT STERILIZATION ON THE GROWTH-FACTOR ACTIVITY OF PYRIDOXINE FOR STREPTOCOCCUS LACTIS R. E. Snell, Department of Chemistry, University of Texas, Austin, Texas.

Pyridoxine has been recognized for some time as an essential growth-factor for certain species of lactic acid bacteria. In investigating the growth of Streptococcus lactis

* We feel it imperative to include this reference since these authors were the first to describe this organism completely. Am. J. Pub. Health, 1942, 32, 352-354.

R on a pyridoxine-free medium, it was found that crystalline pyridoxine (vitamin B₆) was almost inactive in permitting growth when heat sterilization was avoided. Autoclaving media which contained pyridoxine increased its growth-promoting activity for this organism from 10 to 100 times depending on the time and temperature of heating. The same effect was achieved in varying degrees by autoclaving pyridoxine at neutrality with individual amino acids. Cystine was the most effective in this respect, while glycine was somewhat less effective. The effect is not a specific one given
only by amino acids, since activation is also effected by heating pyridoxine with various other compounds.

The Relation of Structure to Septic Action of Certain Chlorinated 4-Phenylphenols. C. M. S. Sawyer, J. L. Abernethy, and O. B. Williams, Departments of Chemistry and Bacteriology, The University of Texas, Austin, Texas.

The phenol coefficients of five chlorinated 4-phenylphenols (4-hydroxybiphenyls) have been determined using the standard F. D. A. method of determination. The organisms used were Staphylococcus aureus and Eberthella typhosa. From these results it becomes evident that two chlorines ortho to the hydroxyl group are more effective than one. Preliminary tests show that against E. typhosa a remarkable increase in activity exists when the chlorine atom is substituted in the most remote position to the hydroxyl group, namely the 4'-position. However, introduction of a second chlorine in the 3'-position decreases the activity, and a third chlorine in the 5-position further decreases the activity.

Observations on Salmonella typhimurium. Oleta Beck and W. B. Coffee, State Health Laboratory, Austin, Texas.

An epizootic has existed among the guinea pig stock of the State Health Laboratory caused by Salmonella typhimurium, and this infection still persists enzootically among the stock. No pathogenic organism other than this Salmonella species was encountered, and the possibility of a virus involvement was investigated with negative findings. The causative organism is capable of being disseminated by roaches of the genus Periplaneta americana but not of the genus Blatella germanica. The arthropods appear to harbor the organism in the intestine as well as mechanically on the appendages.

Triatoma sanguisuga (Le Conte) and Triatoma ambiguca (Neiva) as Natural Carriers of Trypanosoma cruzi in Texas. D. J. Davis, National Institute of Health, Washington, D. C., T. McGregor and T. DeShazo, State Dep't. of Health Laboratories, Austin, Texas.

Virus-Neutralizing Antibody Content of Human Sera Following Equine Encephalomyelitis Vaccination. J. V. Irons, S. W. Bohls, and Dorothy Albert, Bureau of Laboratories, Texas State Department of Health, Austin, Texas.

Fifteen laboratory workers received "equine encephalomyelitis" vaccine prepared for humans, in two one ml. doses each a week apart. Sera from eleven laboratory workers were examined in neutralization tests both before and after vaccination. Negative findings were obtained before vaccination. Neutralizing antibodies protective against 100 to 1000 mouse-brain infective units were obtained following Western type vaccination with persons working intimately with this virus. Feeble or even negative responses were obtained with controls who were not working with this virus.

Some Physiological Factors in Spore Production. A. E. Hayward, Department of Botany and Bacteriology, The University of Texas, Austin, Texas.

This report presents results of an investigation of the effect of nutrilites and pentose and hexose sugars on Bacillus subtilis (Kahn) grown in a vitamin-free casein hydrolysate medium. Thiamin, nicotinic acid, pantothenic acid, riboflavin, pyridoxine, and biotin did not serve to increase the percentage sporulation. Inositol was found to have a slight stimulatory effect. A correlation between the percentage sporulation and configuration of the carbohydrate molecule is indicated. Further investigation of this phase of the problem is now in progress.

Ornithodoros talaje (Guerin-Meneville), a Possible Vector of Relapsing Fever in Texas. T. McGregor, R. B. Eads, and D. C. Thurman, Texas State Department of Health, Bureau of Laboratories, Austin, Texas.

Ornithodoros talaje has been found naturally infected with spirochetes in an endemic relapsing fever area in Southwest Texas. Ornithodorus turicata is apparently the principal vector of relapsing fever in Texas. After small groups of O. talaje were fed on
white rats, a characteristic relapsing spirocheta
tosis occurred. O. turicata was not found in the im-
mediate locality where infected O. talaje were col-
clected. In another locality specimens of O. talaje were not infected, although these were collected from the same rat nest in which infected O. turicata were found.


Strains of gonococcus from 100 patients have been tested for sulfonamide sensitivity by plating on Proteose-starch-agar containing sulfonamides. The range of sulfathiazole tolerance is wide, varying from 0.0005 to 5 mgm. per cent, and corresponds in general to the clinical response to sulfa-
thiazole therapy.

Sulfonamide-resistant variants of sensitive strains may be selected by plating heavy suspensions on inhibitory concentrations of sulfonamide-agar. The colonies which develop are 5-10 or 50-100 times as resistant as the parent strain, and on plating these, occasional variants obtained are 1000 times as resistant as the original, thereby indicating near-logarithmic, step-wise acquisition of tolerance. Although great differences in strain capacity to produce resistant variants were observed, the num-
ber of variants produced by a single strain in the presence of different sulfonamides does not vary significantly, a fact supporting the theory that acquisition of sulfa-

amide-resistance is a sudden, spontaneous "mutation", occurring continuously, but becoming evident only when conditions are suitable for selective propagation of the resistant variant cells.

Further observations: 1), a sulfonamide-fast variant selected in the presence of one sulfonamide is proportionately resistant to others; 2), the bacteriostatic efficiency ratio — sulfathiazole : sulfapyridine : sulfanil-
amide—for parent strains and variants tested is usually 100:10:1 or 50:5:1; 3), simultaneous production of variants of increased and decreased tolerance may occur.

The Rh Antigen in Spontaneous Abortions and Erythroblastosis Foetalis. Sol Haberman, William Buchanan Blood, Plasma, and Serum Center, Baylor University Hospital, Dallas, Texas.

One clinical case of erythroblastosis and eight cases of spontaneous abortion were tested for the presence of the Rh factor in the parents and the foeti. In the eight cases of spontaneous abortion, syphilis, sepsis, eclampsia, and injury were ruled out as etiological factors. The erythroblastosis case showed the Rh antigen in both the father and the child, while the mother was negative for the antigen and had a weak Rh agglutinin titer. In one case of abortion, the foetus was aborted at six months of gestation. The father and the foetus had the Rh antigen in their erythrocytes, while the mother was negative for the antigen and had a weak Rh agglutinin in her serum. In two of the cases, the Rh antigen was present in the father and the foetus, while the mother was negative in each instance. No agglutinins were found in the mother's serum or the foetal serum. Two other cases showed positive Rh antigens in the father and none in the mother. The foeti of these cases were not available for tests. Three cases of abortion failed to demonstrate the proper incidence of the Rh antigen in the parents or foeti to incriminate this antigen in the cases. The proper distribution of the Rh antigen in the parents and the foeti in three cases of abortion, and the proper dis-

tribution in the two cases in which the foeti were not available, lend some evidence to the theory that the Rh factor may be con-
cerned in repeated spontaneous abortions.

Identification of Proteus ammoniae and Related Species. Preston E. Harrison, Dept. Bacteriology, Hygiene and Preventive Medicine, Baylor University College of Medicine, Dallas, Texas.

Of 17 cultures of Proteus ammoniae and 41 cultures of Proteus morganii studied, most P. ammoniae strains were isolated from urine while most of the P. morganii cultures were obtained from feaces.

On S-S agar P. ammoniae produces a small colony with a black center whereas P. morganii produces a colorless colony.
P. ammoniae produces a spreading growth on moist agar while P. morgani does not.

Lactose, sucrose, maltose and mannitol are not fermented by either species. All P. ammoniae strains ferment glucose, galactose, trehalose, xylose, and are able to utilize citrate, liquefy gelatin, produce H2S, peptonize milk and rapidly decompose urea, but do not attack levulose and mannose, and fail to produce indole. P. morgani cultures ferment glucose, galactose, levulose and mannose, and produce indole, but do not attack trehalose and xylose, and fail to utilize citrate, liquefy gelatin, produce H2S or peptonize milk. Urea is decomposed slowly.

**Salmonella and Salmonella-like Types from Texas.** MacDonald Fulton, Baylor College of Medicine Salmonella Center, Dallas.

A collection of 93 strains including 31 from Texas was typed using Kauffmann's sera. The most frequent type was *Salmonella typhimurium*. The Texas types also included *typhi, panama, newport, london, reading, kunzendorf, enteritidis, san diego, cholerae suis, onderstepoort, shanghai*, and 2 strains related to *tel aviv*. Many strains submitted for study appear to be "morgan" bacilli, paracolon bacilli, proteus, and similar types, some of which have antigens corresponding to those of *Salmonella*. These other groups must be more specifically defined in order to decide the limits of the genus *Salmonella*.

**Jaundice Following Yellow Fever Vaccination.** A. Packchanian, Department of Public Health and Preventive Medicine, The University of Texas Medical School, Galveston, Texas.

**Joint Meeting, New York City Branch and the Theobald Smith Society (New Jersey Branch)**

College of the City of New York, December 29, 1942

(Most of the papers listed below were prepared for the Annual Meeting of the Society at Columbus, and abstracts of such papers were published in the January issue of the Journal on the pages listed after each title.)


**The Evaluation of Antiseptics and Other Anti-Infectious Agents.** Ward J. MacNeal and Nancy C. Farnsworth, New York Post-Graduate Medical School and Hospital, New York, N. Y. (J. Bact., 45, 41.)

**The Mouse-Protective Test as a Uniform Method of Assay for Antibacterial Sera.** Paul A. Little, Lederle Laboratories, Pearl River, N. Y. (J. Bact., 45, 70.)

**Formation of Tyrothricin in Submerged Cultures of Bacillus brevis.** J. L. Stokes and C. R. Woodward, Jr., Merck and Co., Rahway, N. J. (J. Bact., 45, 29.)

**Cultivation of Actinomycetes under Submerged Conditions with Special Reference to the Formation of Streptothricin.** H. B. Woodruff and J. W. Foster, Merck and Co., Rahway, N. J. (J. Bact., 45, 30.)

**Anti-Biotin Activity of Methionine.** C. Virginia Fisher and Gustav J. Martin, Warner Institute for Therapeutic Research, New York, N. Y. (J. Bact., 45, 33.)

**Correlations between Oxidation-Reduction Potentials and the Development of Sulphate-Reducing Bacteria.** R. L. Starkey and Kent M. Wight, New Jersey Agricultural Experiment Station, New Brunswick, N. J. (J. Bact., 45, 39.)

**The Nature of the Changes in Optimal Temperature of Luminescence Caused by Enzyme-Sulfanilamide and Enzyme-
URETHANE EQUILIBRIA. Frank H. Johnson and Henry Eyring, Princeton, N. J. (J. Bact., 45, 24.)


DEMONSTRATION OF SEROLOGICAL TYPES WITHIN THE NON-HEMOLYTIC PASTEURELLA. Paul A. Little and B. M. Lyon, Lederle Laboratories, Pearl River, N. Y. (J. Bact., 45, 58.)

CULTIVATION OF THE VIRUS OF MOUSE ENCEPHALOMYELITIS (THEILER'S VIRUS) IN THE CHICK EMBRYO. Wolcott B. Dunham and Sue Parker, New York Post-Graduate Medical School and Hospital, New York, N. Y. (J. Bact., 45, 80.)


THE NATURE AND ACTION OF GRAMICIDIN AND TYROCIDINE AND OF THEIR ACTION ON BACTERIA. Rollin D. Hotchkiss, Hospital of the Rockefeller Institute for Medical Research, New York, N. Y. (J. Bact., 45, 64.)

THE NATURE AND ACTION OF PENICILLIN. Gladys L. Hobby, Karl Meyer, Eleanor Chaffee and Martin H. Dawson, College of Physicians and Surgeons, Columbia University, and Presbyterian Hospital, New York, N. Y. (J. Bact., 45, 65.)

THE MECHANISM OF SULFONAMIDE POTENTIATION THROUGH IONIZATION AND OXIDATION. Franz C. Schmelkes, Wallace and Tiernan Products, Inc., Belleville, N. J. (Paper to be read by Dr. Orville Wyss.) (J. Bact., 45, 67.)