LIMITS OF EFFECTIVENESS OF STREPTOMYCIN IN ARTHRITIS OF RATS. H. M. Powell, Lilly Research Laboratories, Indianapolis, Indiana.

We have reported recently in a separate communication that streptomycin is an excellent chemotherapeutic agent against arthritic infections caused by pleuropneumonialike organisms in the rat. The culture we have used has been reported upon previously (J. Lab. Clin. Med., 1944, 47, 523; J. Bact., 1944, 47, 523), and Dienes has reviewed this whole group (J. Bact., 1945, 60, 441). Our former results, which were practically 100 per cent "cures," included use of 1,000 or 3,000 units of streptomycin per dose for 9 to 12 doses covering 3 or 4 days, and our test animals were white rats of about 100 g weight.

Amplified tests using less streptomycin, etc., now show that (a) 10 units streptomycin t.i.d. for 4 days is not effective; (b) 100 units t.i.d. for 4 days is a border-line therapy, curing only about three-fourths of the rats; (c) again, as previously noted, either 1,000 or 3,000 units t.i.d. for 3 or 4 days are effective; and (d) 3,000 units, but not 1,000 units, t.i.d., for 3 days with therapy starting one day after infection is effective. All chemotherapy heretofore reported, and herewith reported under (a), (b), and (c), started within an hour after intravenous infection, whereas therapy (d) began one day after infection.

A further experiment has been done in which the test culture has been injected into the foot pads, and 3,000 units streptomycin t.i.d. for 4 days has proved an effective therapy. Streptomycin appears to be a better chemotherapeutic agent than myochrysin against our pleuropneumonialike organisms.

BACTERIOLOGICAL ASPECTS IN MINIMIZING TRANSFUSION REACTIONS. Iva Dietz, Elkhart County Blood Bank, Elkhart, Indiana.

The important factors often overlooked or not stressed in blood transfusions are given careful consideration. Techniques for scrupulous cleaning of reusable apparatus are demonstrated; the importance of pyrogen-free water and solutions, and sterilization methods are explained.

TOXICITY OF STREPTOTHRICIN. Alfred R. Stanley, Research Department, Commercial Solvents Corporation, Terre Haute, Indiana.

Streptothricin produced in the pilot plant, and recovered in the laboratory as the sulfate and hydrochloride, was tested for toxicity to rabbits. Intravenous injection of either 5,000 or 20,000 units per kilogram of body weight twice a day resulted in a destruction of the mucous lining of the stomach, mottling of the liver and kidneys, and death of the animals. The same effects were produced by either salt. Feeding orally in capsules produced the same results. When two 60,000 to 85,000-unit capsules were given per day, the rabbits stopped eating on the second or third day, the nose was filled with mucus, and the chin was wet from drooling, indications of gastrointestinal disturbance.

Topical application of 30,000 units per day on scarified skin gave the same results as feeding, whereas the same application on unbroken skin had no effect. Intradermal injections of 6,000, 4,000, and 2,000 units produced hemorrhagic areas which increased in size for 6 days.

TESTING OF GERM-FREE ANIMALS FOR CONTAMINATION. James A. Reyniers, Laboratories of Bacteriology, University of Notre Dame, Indiana.

The term "germ-free" as applied to animals must mean freedom from demonstrable microbial contaminants, within the limitations of the techniques which can be applied to determining their presence. Since the techniques are usually adequate for demon-
strating the presence of recognized microorganisms, the problem really resolves itself into a theoretical question concerning the possible presence of those forms which cannot be demonstrated by microscopic, cultural, or chemical techniques. The presence of such microorganisms constitutes a special problem for future investigation.

The paper discusses this theoretical problem and offers examples from the work done in this laboratory on rats, guinea pigs, and chickens. It also includes the routine used for determining the presence of contaminations either in the living form or at necropsy. The final answer to the problem rests in the future and on a basis of being able to breed animals germ-free through a number of generations so that a pure strain can be developed.

**Production of Streptothricin in Shake Flasks and 80-Gallon Tanks.** R. E. Bennett, Research Department, Commercial Solvents Corporation, Terre Haute, Indiana.

**Review of Literature on the Cardiolipin Antigen in the Serodiagnosis of Syphilis.** Margaret Higgenbotham, South Bend Medical Laboratories, South Bend, Indiana.

**Texas Branch**

**Austin, Texas, May 10 and 11, 1946**

**Effect of Para-Aminobenzoic Acid on the Intestinal Flora of Guinea Pigs.** Dorothy M. Whitney and Ludwik Anigstein, Department of Preventive Medicine and Public Health, University of Texas School of Medicine.

Recent investigations on p-aminobenzoic acid (PABA) gave evidence of its wide range of action as an essential metabolite for bacterial growth, as a detoxicant, as a possible catalyst, and as a rickettsiosstatic agent.

Since parenteral administration of PABA on experimental spotted fever was found ineffective, it was felt that a study of its effect on the intestinal flora by oral administration might throw light on the mechanism of its action.

The intestinal flora of normal guinea pigs was studied; this was followed by examination of the same animals after PABA was given. The fecal material was examined in direct smears, and aerobic cultures were made on various solid and liquid media.

Untreated guinea pigs showed a predominance of gram-positive bacilli and cocci in both direct smears and cultures. A total of 70 cultures, 54 of which were gram-positive, were isolated from a dozen apparently healthy guinea pigs. Of the 16 gram-negative strains, 11 were of the coliform type. There was a marked decrease of all bacteria after treatment with massive doses of PABA. After an interval of 4 PABA-free days the growth was still markedly restricted. No gram-negative, lactose-fermenting bacilli were found.

**A Practical Key for Rapid Identification of the Common Species of Aerobic Sporeforming Bacilli.** Kenneth L. Burdon, Department of Bacteriology and Immunology, Baylor University College of Medicine, Houston, Texas.

Previously suggested keys to the genus *Bacillus* based on a single morphological characteristic (spore size) or upon variable cultural features are inaccurate and impractical. The simple scheme presented here utilizes for differentiation a variety of properties found to be constant and readily demonstrable. It lists first the three species that ferment glucose, maltose, and mannitol (in bromoresol purple tryptose agar butt-slants). Among these, *Bacillus subtilis*, Ford, is identified by the fact that it grows well within 24 hours when an inoculated slant is incubated in a 56 C water bath. The two remaining species (*Bacillus megatherium* and *Bacillus circulans*) are distinguished by their general morphology and appearance when the fat is stained, as well as by their characteristic growth on potato slants. *Bacillus cereus* and *Bacillus mycoides*, which ferment glucose and maltose but not mannitol, are separated by the distinctive character of the myceloid giant colony formed by the
latter species on gelatin agar plates. The two species that ferment glucose but not maltose (*Bacillus mesentericus* and *Bacillus subtilis*, Marburg) are differentiated by the inability of the former to hydrolyze starch. Finally, *Bacillus brevis* is recognized by its failure to attack glucose.

**Some Unusual Salmonella Types Found in Texas.** MacDonald Fulton, University of Texas School of Medicine, Galveston.

A report was made of 7 isolations of *S. panama* from man, 2 of *S. rubislaw* from two dysenteric monkeys, 3 of *S. habana* from diarrhea in adults and children, and 1 of *S. bareilly* from a child with diarrhea. Previous isolations of these species elsewhere were reviewed. These are the first strains of *S. rubislaw* to be reported from the monkey, and the second occurrence of *S. habana* to be reported anywhere.

**A Presumptive Medium Differentiating Paracolon from Salmonella Cultures.** Martha Chilton, University of Texas School of Medicine, Galveston.

Carbohydrates fermented by various paracolon strains but not by Salmonella types were combined to give a new multisp- sugar broth. Fermentation in this broth within 24 hours indicated that a culture was probably a paracolon bacillus, if the use of polyvalent Shigella antiserum and a test for urea hydrolysis has excluded Shigella and *Proteus*, respectively. The medium contained 0.5 per cent each of adonitol, esculin, salicin, and sucrose. Kovacs' test for indole, performed on all cultures negative at 24 hours, further increased the detection of paracolon strains by this medium. In conjunction with the fermentation of lactose in agar slants containing 10 per cent carbohydrate, over 80 per cent of 252 paracolon strains tested were identified in 1 day.

**The Selective Action of Penicillin in the Isolation of Brucella abortus from Milk.** Helen A. Lacy, L. J. Rode, and V. T. Schuhardt, Brucellosis Research Project of the Clayton Foundation at The University of Texas, Austin.

Pooled milk samples from the four quadrant of the udder were collected in sterile test tubes by hand milking from 564 cows. The cream from these samples was plated in duplicate on the usual gentian violet tryptose agar (GVTa) and on this medium containing 1 Oxford unit of penicillin per cubic centimeter (PGVTa). Brucella abortus colonies were isolated a total of 87 times from the two media. In 56 instances *B. abortus* was isolated on the PGVTa and not on the duplicate GVTa. In only 1 instance was the reverse of this situation true. In 20 instances the PGVTa plates showed more than 50 *B. abortus* colonies, whereas not one of the GVTa plates showed more than 50 colonies. In 12 instances the PGVTa plate showed more than 50 colonies of *B. abortus*, whereas not a single colony was found on the duplicate GVTa plate. *B. abortus* was isolated from 86 (15.24 per cent) of the 564 samples on PGVTa and from 29 (5.14 per cent) of the samples on GVTa.

**Single Spirochete Infections in Experimental Relapsing Fever.** Martha Wilkerson and V. T. Schuhardt, The University of Texas, Austin.

Microcapillaries approximately 1-inch long and 10 micras in diameter were filled with diluted blood serum containing spirochetes. These were mounted in saline and examined with the 4-mm objective using dark-field illumination. Those capillaries containing one spirochete were placed in the lumen of a hypodermic needle which had been partially plugged with agar and which was attached to a syringe containing saline. The contents of the syringe and needle were then injected into the peritoneal cavity of a rat.

Four of eleven rats so inoculated developed relapsing type infections. The infection sequences were followed by the examination of uniform (0.01 ml of a 1:20 dil.), daily, dark-field preparations of tail blood. The incubation period ranged from 5 to 7 days, and the number of relapses varied from 1 to 3. In general the infection sequences were similar to those resulting from tick bite infections.

**Sero logical Aspects of the Relapse Phenomenon in Rats Infected with**
Single Spirochetes (Borelia recurrentis var. turicatae). V. T. Schu- hardt and Martha Wilkerson, The University of Texas, Austin.

Antisera, collected from each of four rats 30 days after infection with single spirochetes, showed spirocheticidal activity against all antigenic varieties tested (attack passage, various relapse passage, and brain passage spirochetes of single-cell-infected and tick-infected rats). Thus, it would appear that each spirochete accomplishes all the possible antigenic variations characteristic of that particular strain during the course of the infection in the white rat.

Antisera collected from infected rats on the second day after the termination of each attack and relapse and on the thirtieth day after infection showed a constantly rising spirocheticidal titer against various strains of the spirochetes. This tends to confirm the concept that once spirochetes have taken part in an attack or relapse that particular antigenic variety cannot recur in subsequent relapses.

Antitickettshal Activity of Para-Amino-Benzoic Acid (PABA): Evaluation and Significance of PABA Blood Levels in Guinea Pigs with Reference to Spotted Fever Treatment. Ludwik Anigstein and Dorothy M. Whitney, Department of Preventive Medicine and Public Health, University of Texas School of Medicine.

An attempt was made in this study to investigate the correlation between the mode of administration of p-aminobenzoic acid (PABA) and its plasma concentration in the infected host with the course of spotted fever.

Significant differences have been found in the mode of administration since the compulsory oral or parenteral supply of the drug, resulting in free PABA levels as high as 43 to 69 mg per cent, had little effect on the progress of the disease. Natural feeding, providing a frequent intake or an almost constant supply of PABA in small doses, corroborated previous results. Under these conditions, 0.3 g of PABA per 100 g body weight per day was found effective, particularly when protein (soya) and calcium were added.

In the plasma of normal and spotted fever guinea pigs not treated with any drugs, a substance was revealed which gave a color reaction matching PABA. This substance (DS) appeared only in traces of fasting guinea pigs; its level rose after normal feeding. No conjugated values were found.

Proteinase Production by Bacillus subtilis. J. R. Stockton and Orville Wyss, The University of Texas, Austin.

Some Effects of Sterilizing Glucose in Culture Media for the Gonococcus, with Special Reference to Cysteine. C. E. Lankford, The University of Texas Medical Branch, Galveston.

Observations on Drug Sensitivity of Coliform Organisms during Administration of Phthalylsulfathiazole or Succinylsulfathiazole with Penicillin. R. I. Wiss, E. J. Poth, and Mary P. Slattery, The University of Texas Medical Branch, Galveston.

Field and Laboratory Investigations of Epidemic Influenza during the Winter of 1945-46. J. V. Irons and Oleta Beck, State Department of Health, Austin.

The Use of Anti-Human-Globulin Serum as a Developing Test for Inapparent Antibodies. Sol Haberman, J. M. Hill, and Katharyn Willis, Baylor University Hospital and Southwestern Medical College, Dallas.

An Aerobacter sp. Producing a Yellow Pigment. Robert I. Wise, Mary P. Slattery, and E. J. Poth, The University of Texas Medical Branch, Galveston.

Bactericidal Action of Bromine. Orville Wyss and J. R. Stockton, The University of Texas, Austin.

Influence of Ether Anesthesia on the Course of Five Experimental Neurotropic Virus Diseases. Christine Zarafonitis, S. Edward Sulkin, and Cleo Housman Terry, Southwestern Medical School, Dallas.
PROPHYLACTIC, THERAPEUTIC, AND CURATIVE TESTS IN ATYAN MALARIA WITH N1-(5-CHLORO-2-PRIMIDYL) METANILAMIDE. Wendell Gingrich and Eugenia Worley Schoch, University of Texas Medical Branch, Galveston.

SURVEY OF TYPHUS RICKETTSIAE IN RAT ECTOPARASITES. Lill R. Skinner, Ruth

CENTRAL NEW YORK BRANCH
Ithaca, New York, June 20, 1946

'TRIPE-SUGAR IRON AGAR (HAJNA) AND LACTOSE-SUCROSE-SALICIN BROTH AS AIDS IN THE IDENTIFICATION OF SALMONELLA. Erwin Neter, Children's Hospital and University of Buffalo.

Since certain Proteus and paracolon bacilli in Hajna's triple-sugar iron agar produce reactions similar to those of Salmonella, this culture medium was supplemented by a single broth containing 1 per cent lactose, 5 per cent sucrose, 1 per cent salicin, and phenol red as indicator (L.S.S. broth). In L.S.S. broth 37 Salmonella strains, representing 18 types, and 13 strains of Proteus morganii failed to produce acid.

All 24 strains of Proteus mirabilis, recently isolated from feaces of children with diarrhoea, produced acid, 14 within 24 hours, 20 within 48, 22 within 72, and 2 after 72 hours. Eight strains, producing the Salmonella type of reaction in T.S.I. agar, formed acid in L.S.S. broth. Of 21 strains of paracolon bacilli, 17 caused acid formation in L.S.S. broth, namely, within 24 hours (10 strains), 48 hours (13 strains), and 72 hours (17 strains), respectively; 4 strains did not acidify the medium within 3 weeks. L.S.S. broth as an aid in the rapid and economic identification of Salmonella is discussed.

STUDIES OF A GLYCOLYTIC STIMULANT WITH STREPTOCOCCUS PAFCALIS. C. E. Foust and I. C. Gunsalus, Laboratory of Bacteriology, Cornell University.

During the course of differential fermentation studies, the rate of fermentation of cell suspensions and dried cells of lactic acid bacteria was found to decrease rapidly when the cells were stored. Yeast extract and a number of other natural materials were found to stimulate glycolysis of the cells.

Yeast extract and acid-hydrolyzed yeast extract have been fractionated and the nature of the active material has been partially determined. A number of the as-yet-unidentified growth factors described in the literature possess properties in common with the glycolytic stimulant observed in these studies.

Cells harvested from a phosphate-buffered glucose tryptone yeast-extract medium after 18 hours, final pH 4.5, showed a Qa of about 100; these were stimulated by the addition of yeast extract to a Qa of 200 to 400. Fructose is also fermented by these cells, though more slowly, and shows approximately the same degree of stimulation. Fractionation of acid-hydrolyzed yeast extract by butyl alcohol extraction left most of the activity in the aqueous residue. When further separation suggested that the histidine fraction possessed activity, this amino acid was tested and found to account for a portion of the yeast extract stimulation. In the presence of histidine the addition of glutamic acid or glutamine would further increase the stimulation.

Studies have been undertaken to determine whether this factor is acting directly on the glycolytic system and to locate the site of action.

THE PRODUCTION OF GASEOUS NITROGEN FROM NITRATE BY THE LEGUME BACTERIA. J. K. Wilson, Department of Agronomy, Cornell University, Ithaca.

It is known that the legume bacteria under certain conditions can reduce nitrate to nitrite. If this occurs and certain organic and inorganic compounds are present...
in an acidic environment, the nitrous acid produced will react with such compounds and gaseous nitrogen will be liberated. Slopes containing nitrate that were inoculated with the bacteria and cultured for a few days and then plugged with some of the same medium developed sufficient gas to split the agar in about 36 hours. The gas was neither oxygen nor carbon dioxide, and was not inflammable. Since the legume bacteria reduce nitrate to nitrite, and since amines were supplied in the medium, it seems reasonable to conclude that the gas was nitrogen.

THE DARKENING OF MAPLE SYRUP DUE TO BACTERIAL ACTION. C. S. Pederson and F. W. Hayward, N. Y. State Agricultural Experiment Station, Geneva.

The chief factor in grading the quality of maple syrup is color; light-colored syrup usually has a more delicate maple flavor than dark-colored syrup.

The color of maple syrup depends upon the alkalinity of the sap and the invert sugar content. The growth of bacteria causes a temporarily increased alkalinity and the inversion of sucrose, which, in turn, result in darker-colored syrup. The bacteria grow at temperatures slightly above the freezing point and cause significant deterioration in color.

Cleanliness of equipment and rapid handling of the sap, therefore, are very important factors in the production of high quality, light-colored maple syrup.

THE DISAPPEARANCE OF HEMOPHILUS PERTUSSIS FROM INFANTS TREATED WITH STREPTOMYCIN. Elizabeth Day and William L. Bradford, University of Rochester, Rochester.

THE ANTIBACTERIAL ACTION OF STREPTOMYCIN IN EXPERIMENTAL BRUCELLOSIS OF GUINEA PIGS. H. L. Gilman and W. R. LeGroux, Veterinary College, Cornell University, Ithaca.

THE RESISTANCE OF THE GONOCOCCUS TO PENICILLIN IN VITRO. Charles M. Carpenter, Leif G. Suhrland, and Martha Morrison, University of Rochester, Rochester.

SELECTION OF SALMONELLA AND SHIGELLA CULTURES FOR TYPING. W. H. Ewing, Veterinary College, Cornell University, Ithaca.

THE RELATION BETWEEN ENZYME ACTIVITY AND VIABILITY IN DISINFECTION. Martha Roberts and Otto Rahn, Laboratory of Bacteriology, Cornell University, Ithaca.


THE ANTIBACTERIAL ACTION OF STREPTOMYCIN IN EXPERIMENTAL BRUCELLOSIS OF GUINEA PIGS. H. L. Gilman and W. R. LeGroux, Veterinary College, Cornell University, Ithaca.

THE RELATION BETWEEN ENZYME ACTIVITY AND VIABILITY IN DISINFECTION. Martha Roberts and Otto Rahn, Laboratory of Bacteriology, Cornell University, Ithaca.

SELECTION OF SALMONELLA AND SHIGELLA CULTURES FOR TYPING. W. H. Ewing, Veterinary College, Cornell University, Ithaca.