the photographic exposures can be made short enough to avoid blurring from brownian movement. Measurements of Bacillus megatherium will illustrate the method.


The Robinow technique for staining bacterial nuclei was modified to yield reproducible results with Escherichia coli (strain B). The length of each step in the procedure (fixation in OsO₄ vapor, hydrolysis in HCl, neutralization in buffer, staining in Giemsa solution) must be carefully chosen, for best results, with organisms from both liquid and solid media.

Changes in nuclear morphology during the growth of a culture were studied. Cells from fully grown cultures appear lightly but uniformly stained; nuclear differentiation begins to appear soon after transfer to a fresh medium while cell elongation without division takes place. In actively growing cells, the nuclei seem to divide before the bacterial cell itself does, so that the common longer cell forms show at least two nuclei, while the small, recently divided individuals contain one nucleus only.

The nuclear structures of abnormal cells produced by various methods have also been examined.


The Massachusetts Institute of Technology Graduate House dining service has been under technical supervision since 1943. Eating and drinking utensils are soaked and thoroughly desoiled in clean soapy water at 110-120°F, and passed through a mechanical dishwashing machine for washing and rinsing. The temperature of the water varies between 160-180°F and usually is at 170°F. The total time in the dishwasher is 35 seconds. All utensils are allowed to dry in the air. Bacteriological results have been consistently satisfactory and approximated sterility. In the course of the work it became obvious that the excellent bacteriological results were due to the effective preliminary desoiling of all utensils. Since the temperature of the wash and rinse water could be regulated, a comparative study, using rinse water at 145-150°F and rinse water at 160-180°F, was undertaken. All other factors were kept constant. Five test runs over weekly intervals clearly demonstrated that “sterile” utensils can be obtained with rinse water at 145-150°F, as with rinse water at 160-180°F, if the preliminary desoiling is complete and effective. The emphasis in restaurant sanitation should therefore be on adequate preliminary desoiling of all utensils rather than on the use of high temperature rinse waters.

Relatively little attention has usually been directed to the temperature of the reconstituting fluid when plating readily soluble dried milk products. However, the use of warm water (35–55 C) has been employed by a number of workers to improve the dispersion of the dried products. The procedure we adopted is to add water at 50 C to the powder which has been weighed into a wide-mouthed jar or bottle, shake the mixture vigorously fifty times, let stand five minutes (in a bath) at 50 C, again shake vigorously fifty times, then dilute in water blanks at room temperature. We have found that the number of viable cells in spray-dried skim milk cultures of Lactobacillus bulgaricus are greatly increased if the temperature of the reconstituting fluid is 50 C rather than 21–25 C. It is essential that the reconstituting liquid be at 50 C when added to the powder rather than that the mixture be warmed to 50 C from room temperature after the liquid is added.

Reconstitution at 50 C instead of at room temperature shows a proportionately greater increase in viable cells with older cultures than with freshly dried ones. This heat treatment has not resulted in an increase in the count of viable cells in a dried culture of lactic streptococci, but rather a decrease. The counts on commercial spray and roller-dried milk powders has at times given increased counts and at others decreased counts by reconstitution in water at 50 C.


Although the Lancefield method for the production of streptococcal grouping serum usually yields excellent antiserum for groups A, B, and C, considerable difficulty has been encountered by many investigators in attempts to produce antiserum for group D by this method. Following numerous attempts, a method has been devised by the authors which has produced good serum in most instances. Cells of 18-hour cultures of Lancefield strain C-1 grown in 100 ml of glucose veal infusion broth are removed by centrifugation and extracted at room temperature for 30 min in 50 ml of acetone. Extraction is twice repeated, following which the centrifuged cells are dried in vacuo over H2SO4 at 10 C for 24 hours. The dried cells are then suspended in 0.85 per cent saline containing 0.2 per cent formalin and held at 10 C for 48 hours. This stock antigen is then diluted 1 to 20 with saline solution and used in the rapid immunization of rabbits. Following an initial dose of 0.1 ml, dosage is rapidly increased to a maximum of 1.0 ml daily and injections are continued until a total of 40 ml have been administered. Injections are continuous unless the physical condition of the animal warrants an occasional brief period of rest. Antiserum produced in this manner is used with freshly prepared testing antigen.

Using a modification of the White and Sherman sodium azide penicillin medium for isolation of the enterococci, studies have been made to determine the possibility of using these microorganisms as an index of fecal pollution. The medium was modified by doubling the penicillin content and adding 0.001 per cent methylene blue. Isolates from the medium were verified by the following tests: catalase production, salt tolerance, growth at 10 C and 45 C, and reaction on litmus milk. Only those gram-positive streptococci which met these requirements, as suggested by Sherman's studies, were considered to be typical enterococci.

Enterococci were isolated from raw sewage, fresh and salt water. When compared with the numbers of coliform bacteria present in the same samples, the enterococci are usually present in much smaller quantities. In polluted waters, coliform bacteria persisted for a greater distance from the source of pollution than did the enterococci. Studies are now in progress to determine the survival period in sewage and water and the seasonal quantitative variations which might occur.

Feces of man and certain domestic and wild animals were examined quantitatively for the presence of enterococci. Fecal samples of the latter were taken from animals which had been trapped in their natural environment. Although the numbers of coliform bacteria per gram of fresh feces were roughly constant, there was marked variation in the enterococci count. The numbers of enterococci per gram varied from zero in the muskrat to a maximum of 37,000,000 in the raccoon.


Use of a sodium lauryl sulfate tryptose nitrate broth reduces the time required for the presumptive test for coliform bacteria in water to twelve hours or less. Employing the principle of nitrate reduction instead of lactose fermentation, a new, more rapid, and possibly more reliable basis for the presumptive test was introduced.

Since the publication of the results of the test on raw and treated waters, the investigations have been extended to include sea water, crabmeat, and oysters. The results of the test applied to twelve samples of oysters, forty-eight samples of crabmeat, and fifty samples of sea water were comparable to those obtained by the standard method for determining coliform bacteria.

The data obtained thus far indicate that it is not necessary to confirm tests which are nitrite-positive within eight hours or less. Since the majority of samples tested gave positive tests for nitrites between six and eight hours, it is clear that the method may be four to eight times more rapid than the standard procedure.
Sixty additional samples of water have been tested using the medium as a secondary medium for confirmation after preliminary growth in lactose broth. Eighty-eight per cent of the samples that were gas-positive in lactose broth produced nitrates in four hours or less. Ten hours was the longest incubation required of the remaining samples to produce nitrates. All samples that were nitrite-positive were also positive in the standard confirmatory media.

A6. The Cellulose-decomposing Bacteria in the Rumen of Cattle. R. E. Hungate,
State College of Washington, Department of Bacteriology and Public Health, Pullman, Wash.

A method for growing the cellulose-digesting bacteria in the rumen of cattle has been developed. Quantitative estimates of the number of these bacteria have been made and their numbers found to range from 20,000 to 1,000,000,000 per ml. Cellulose decomposition is very active, clear spots developing in the agar within three days. Two chief types of bacteria have been pure-cultured. One is a gram-negative streptococcus and the other a rod form. Slight amounts of gas may be produced but in general the metabolism results in the production of acid without gas. It is believed that the isolated organisms are responsible for most of the cellulose decomposition which takes place in the rumen.

A7. A Survey of the Coliform Status and Suggested Standards for Coliform Control of Pasteurized Milk in a Large City. Leon Buchbinder and John W. Fertig, Department of Health, Bureaus of Laboratories and Food and Drugs; Columbia University, School of Public Health of the Faculty of Medicine, New York, N. Y.

In the absence of standards for coliform control of pasteurized milk other than those based almost solely on empirical grounds a survey of the coliform status of pasteurized milk in New York City was conducted for a one-year period so that reasonable standards might be established. Quart samples from each of forty-six pasteurization plants were studied once weekly. Three volumes from each sample were examined simultaneously: 1 ml, 20 ml, and the remainder of the quart. A total of about 2,150 samples were studied on desoxycholate agar. It was found that the percentage of samples positive for coliforms varied markedly with season. The July peaks for the three volumes in descending order are 86 per cent, 58 per cent, and 24 per cent, whereas the December lows are 59 per cent, 25 per cent, and 3 per cent, respectively. The 20-ml volume data were used to suggest practical standards. The numbers of coliform organisms per ml which were exceeded by 25 per cent, 20 per cent, and 10 per cent, respectively, of the samples for each four-week period were examined. It was found that the critical numbers for the several percentages in order were 0.1, 0.1, and 0.5 in the six cool months and 0.5, 1, and 2 in the six warm months. Comparison of the findings for summer and winter in individual plants suggests the existence of a definite pattern. Trial standards of 0.1 with a zone of grace to 0.3 for the cool months and 1 with a zone of grace to 2 for the warm months were established. Tables have been prepared which indicate the volume of samples required to differentiate
between the upper and lower limits of the zones with a 90 per cent probability of accuracy.


Cheddar cheese was made from good, fair, poor, and very poor milks. One half of each lot of milk was pasteurized. The bacterial flora of each cheese was determined at 1 day, 2 weeks, and 1, 2, 3, 4, and 6 months. The bacterial counts of raw milk cheese were from 2- to 1,000-fold greater than those of the corresponding pasteurized milk cheese, depending on age of cheese and quality of milk.

During the first month of curing, the counts of raw milk cheese decreased approximately 10-fold. Subsequently, depending on quality of milk, the counts increased greatly or remained relatively constant. During the first month, the counts of pasteurized milk cheese decreased from 50- to 100-fold and subsequently remained relatively constant or decreased slightly. Cheese made from poor and from very poor milk, raw or pasteurized, contained many more bacteria than did cheese from good and from fair milk.

Cocci from the lactic starter predominated in young cheese, regardless of quality or pasteurization of milk. After one month, the flora of pasteurized milk cheese consisted almost entirely of enterococci, and that of raw milk cheese of lactobacilli, enterococci, and a few diversified types.

The bacteriological quality of the milk, both raw and pasteurized, was an important factor affecting the quality of the cheese. With high-grade milk there was very little difference in quality between the raw milk and the pasteurized milk cheese, but with low-grade milk the raw milk cheese was inferior.

A9. A Survey of Antibiotic Production by Representative Aspergilli, Penicillia, and other Fungi from a Culture Collection. ALBERT KELNER, University of Pennsylvania, School of Medicine, Philadelphia 4, Pa.

Over 100 species and strains of Aspergillus and Penicillium, as well as some members of the genera Gliocladium, Trichoderma, Scopulariopsis, Metarrhizium, and Paecilomyces were studied for antibiotic production. The cultures were selected by Dr. Charles Thom as representative of the groups and subgroups of the genera. Most of them came from the collection of the Northern Regional Research Laboratory, Peoria, Illinois. Each mold was grown in surface culture in at least four media, glucose peptone yeast extract broth, corn steep lactose broth, Czapek-Dox and Raulin-Thom broths. The crude filtrates were tested for activity against Staphylococcus aureus, Escherichia coli, Eberthella typhosa, and Pseudomonas aeruginosa.

The problems encountered in making the survey will be presented. The results obtained will be discussed with special reference to the question of
whether antibiotic production is a characteristic of a particular strain or the species as a whole.

A10. Eumycin—a New Antibiotic Active Against Pathogenic Fungi and Higher Bacteria, Including Bacilli of Tuberculosis and Diphtheria. Edwin A. Johnson and Kenneth L. Burdon, Baylor University College of Medicine, Department of Bacteriology and Immunology, Houston, Texas.

By exposing agar plates to dust and isolating bacterial colonies inhibiting growth of neighboring mold colonies, strains of Bacillus subtilis were obtained from which we have extracted a new antibiotic ("eumycin") active against filamentous pathogenic fungi and higher bacteria. To date maximum yield has been obtained from cultures five days old at 30 C in buffered yeast extract proteose peptone broth. The substance is soluble in butyl alcohol, ethyl alcohol, and acetone, but not in ether or amyl acetate. By precipitation from the original broth with acid, extraction of the precipitate with alcohol, evaporation of the alcohol, and re-solution with dilute NaOH to pH 7.0, the active material is greatly concentrated. The final (Berkefeld-filtered) solution is nearly colorless, heat-stable in acid, unstable in alkaline solutions beyond pH 8.0. It has low toxicity for mice. Eumycin has no action on typhoid or colon bacilli, and only slightly inhibits staphylococci. In concentrations of 0.1-0.3 mg (dry weight) per ml of medium it prevents entirely, or definitely inhibits, the growth of Trichophyton mentagrophytes, Microsporum gypseum, Epidermophyton floccosum, and related species of fungi, Actinomyces, and Mycobacterium tuberculosis (avian and human types). Its effect on species of Sporotrichum and Hormodendrum is slightly less, and it fails to inhibit Monilia or Cryptococcus. Greatest bacteriostatic activity is exhibited against Corynebacterium diphtheriae, for as little as 0.005 mg per ml stops growth. Studies of possible therapeutic value in experimental infections are in progress.

A11. Two Antibiotics Produced by Actinomyces Isolated from Soil. Albert Kelner, Walter Kocholaty, Renate Junowicz-Kocholaty, and Harry E. Morton, University of Pennsylvania, School of Medicine, Departments of Physiological Chemistry and Bacteriology, Philadelphia 4, Pa.

Two antibiotics active not only against gram-negative but also many gram-positive organisms have been obtained from Actinomyces isolated from soil. Chemical studies thus far have made it possible to differentiate the two antibiotics from one another and from other known antibiotics. One antibiotic is produced by an actinomyces, A-10, which has been identified tentatively as belonging to the A. lavendulae group; the other antibiotic is produced by a strain, A-105, tentatively identified as a variant of A. erythreus or a new species. Cultural conditions for maximum yields of the antibiotics will be discussed. Chemical purification has resulted in a 40-50-fold increase in activity calculated on a

A. J. SALLE and GREGORY J. JANN, University of California, Department of Bacteriology, Los Angeles, Calif.

Subtilin, an antibiotic extracted from Bacillus subtilis, has been shown to be antagonistic chiefly against gram-positive bacteria. Acid-fast organisms, including Mycobacterium tuberculosis and a number of pathogenic higher fungi, are also susceptible. The agent is bacteriostatic in high dilutions and germicidal in greater concentrations.

Subtilin is a polypeptid, and is digested by proteolytic enzymes, such as pepsin, trypsin, and pancreatin. It shows its greatest activity at pH 2.2 and becomes slowly inactivated with decreasing acidity. At pH 7.0 it retains 94 per cent of its original activity; at pH 8.0 about 80 per cent; and at pH 9.0 about 65 per cent. Subtilin is relatively nontoxic when tested by the tissue culture technique. It is approximately 20 times more toxic to Staphylococcus aureus than to chick heart tissue, a remarkably low toxicity.

A unit of subtilin has been determined and is defined as that amount present in 1 ml of the highest dilution (expressed in mg) capable of killing S. aureus in 10 min at 37 C (FDA phenol coefficient method).

Subtilin exhibited great activity in vivo. White mice were infected with a virulent culture of pneumococcus type III and treated after varying periods. Control animals died within 24 hr; all treated animals survived after receiving very minute amounts of the antibiotic. Guinea pigs infected with B. anthracis recovered after treatment with subtilin; control animals died within 36 hours. The antibiotic has shown a definite suppressive effect on the course of experimental tuberculosis in guinea pigs.


Certain antibacterial agents are capable of being inactivated by cysteine. This report presents evidence that the bacteriostatic action of certain antibacterial agents may be reversed by cysteine.

By use of the Warburg technique it is possible to show with certain antibacterial agents that the cessation of oxygen uptake by the test organisms may be resumed upon the addition of cysteine to the bacteriostatic system. Another group of antibacterial agents, including penicillin and the active principle of Asarum reflexum, do not permit this reversal of bacteriostasis following treatment with cysteine.

A14. The Action of Clavacin, a Clavacin Isomere, and Related Compounds on Tetanus Toxin. BRUNO PUTZER and THOMAS C. GRUBB, Research Laboratories, Vick Chemical Company, Flushing, N. Y.
The synthesis of a clavacin isomere and related compounds was recently reported from these laboratories. It was believed of interest to determine the action of these compounds on tetanus toxin since Neter showed that clavacin neutralized this toxin in vitro. The clavacin, prepared according to Raistrick’s method, isoclavacin, dimethylisoclavacin, and \( \alpha\)-keto-\(\beta\)(\(\beta\),\(\beta\)-dimethylacryl)-butyrolactone were mixed with 1:50,000 dilution of tetanus toxin in nutrient broth (pH 7.0), incubated one hour at 37 C, and injected subcutaneously into the hind legs of mice. Clavacin prevented tetanus and death of all mice, thus confirming Neter’s report. Neither isoclavacin nor the two related compounds prevented tetanus or prolonged the lives of the animals beyond that of the toxin controls which died within 96-110 hours. Clavacin differs structurally from isoclavacin only in the position of one double bond. However, this simple shift in the position of the double bond produces a profound change in several of the chemical characteristics of clavacin. While the tetanus toxin-neutralizing activity of clavacin is apparently a function of the position of the double bond, which chemical group or groups in the molecule affected by the position of the double bond are responsible for the toxin-neutralizing properties cannot be postulated from the present evidence.

A15. The Inhibitory Action of Saliva on the Diphtheria Bacillus. The Antibiotic Effect of Salivary Streptococci. Richard Thompson and Madoka Shibuya, University of Colorado School of Medicine, Department of Bacteriology, Denver, Col.

This report is concerned with the inhibitory action of saliva on Corynebacterium diphtheriae; the role of salivary streptococci in this inhibition; and several factors which influence it. The method used involved placing standard drops of the material to be tested on pour plates containing suitable dilutions of bacilli and observing the zones of inhibited growth around the drops.

Pure cultures of “mitis” type of the viridans streptococci isolated from saliva inhibited the growth of the bacilli in the same manner as did fresh saliva. Removal of the streptococci from saliva by centrifugation, heat, or the bactericidal effect of copper abolished the inhibitory power. The actions of saliva and of pure cultures of streptococci were affected in identical fashion by several factors. Both actions were best demonstrated when the tryptose content of the medium was between 0.2 per cent and 0.5 per cent, and were completely eliminated on medium containing 2 per cent tryptose. Both actions were antagonized by certain organisms present in saliva, especially staphylococci. The actions of both saliva and cultures of streptococci were increased by their suspension in nutrient media rather than in saline. The increased activity was associated with increased growth of the streptococci. The inhibitory action of saliva was destroyed by 56 C in approximately the same time as was the action of pure cultures of streptococci. The destruction paralleled the diminution of the numbers of streptococci.

It is concluded that the inhibitory action of saliva against \( C. \) diphtheriae demonstrated by the method used is due to inhibitory streptococci present in the saliva.
A16. Changes in the Bacterial Flora of the Throat and Intestinal Tract During Prolonged Oral Administration of Penicillin. Miriam Olmstead Lipman, James A. Coss, and Ralph H. Boots, Edward Daniels Faulkner Arthritis Clinic of the Presbyterian Hospital and the Columbia University, College of Physicians and Surgeons, Department of Medicine, New York, N. Y.

A bacteriological study on the throat and intestinal flora of ten rheumatoid arthritis cases, prior to and during the oral administration of half a million to a million units of penicillin daily over a period of months, has demonstrated a rapid and striking change. The prepenicillin throat cultures of all cases except one, which harbored Friedländer's bacillus, have shown the predominance of gram-positive diplococci, sensitive to penicillin. In the majority of cultures taken during the course of penicillin therapy, gram-negative organisms, resistant to penicillin, have predominated. Coliform bacteria, in no instance found prior to penicillin therapy, have appeared in most of the cultures during therapy. Changes in the intestinal flora, though less striking, have been definite. Non-hemolytic streptococci, recovered from most of the stool specimens before penicillin, have been found only infrequently during treatment. The relation of the concentration of penicillin in the serum to the sensitivity of the organisms isolated has been determined.


Following Cowan's report that synthetic rubber inactivated penicillin solutions, we have studied the effects of a number of synthetic and natural rubbers upon the stability of penicillin and streptomycin. The antibiotic solutions were placed in suitable lengths of sterile rubber tubing and allowed to stand at room temperature. At appropriate intervals, samples were withdrawn for assay. Twelve samples of synthetic rubber and four samples of natural rubber were tested. Of these, four samples of synthetic rubber and one sample of natural rubber inactivated penicillin completely in 24 hours. Two other samples of synthetic rubber caused marked reductions in the activity of penicillin during the same period. In six hours buna s rubber caused a 50 per cent reduction, and two other samples of synthetic rubber caused 20 and 30 per cent reductions in the activity of penicillin.

None of the samples of rubber tested caused any reduction in the activity of streptomycin.


Barley malt possesses two disadvantages as a conversion agent for grain mashes. These are the high bacteria count and the excessive time required
for conversion of limit dextrins. Previous work on the use of mold amylase preparations revealed that they convert limit dextrins more rapidly than barley malt and result in higher yields of alcohol. Production of mold amylases by the submerged culture process would eliminate the problem of bacterial contamination.

Twenty-three strains of the genera *Aspergillus*, *Mucor*, *Penicillium*, and *Rhizopus* known to produce amylases were subjected to screening tests to determine their amylase production in submerged culture. The effect of pH, aeration, medium, and incubation time was studied to ascertain the conditions necessary for maximum amylase elaboration. The best preparations as determined by amylase content were further evaluated by the yeast fermentation of grain mash using these preparations as conversion agents.

It was found that the most critical factors were pH, medium composition, and time of incubation. The best of the cultures tested is an isolate believed to be a variety of *Aspergillus flavus*. Forty-eight hours incubation of this culture in a medium consisting of 3 per cent distillers' dried solubles at an initial pH of 6.0 results in a preparation with very high diastatic activity. This preparation has been successfully used as a replacement for barley malt in the conversion of grain mashes.


Studies were made to develop submerged culture methods for preparing fungal amylases. The object was to devise procedures adaptable to large-scale production, especially in connection with grain alcohol distillery operations.

A large number of molds, therefore, were cultivated under aeration in a medium composed of thin stillage and corn meal. Enzyme production was determined both by dextrinization of starch and by the ability of the culture liquors to replace barley malt as the saccharifying agent in the alcoholic fermentation of grains.

Of over 350 cultures tested, only seven produced practical concentrations of alpha amylase under the conditions employed. Of these, *Aspergillus niger* NRRL 337 was superior, both in producing alpha amylase and in replacing barley malt. The medium finally adopted consisted of thin stillage supplemented with 1 per cent corn meal and 0.5 per cent calcium carbonate. On this medium *A. niger* NRRL 337 gave amylase potencies equivalent to 600–800 alpha amylase saccharification units per ml of mycelium-free culture liquor in 72 hours. Since these culture liquors contained about 3 per cent solids, on the dry substance basis they were approximately 12 times as potent in dextrinizing power as barley malt. In the alcoholic fermentation of corn, use of culture liquors at the rate of 10 to 15 per cent of the mash volume resulted in alcohol yields of 5.2 to 5.4 proof gallons per bushel as compared to 5.0 to 5.2 obtained with commercial barley malt.

A filtration technique has been developed for recovering and maintaining a bacteriophage culture isolated from Aerobacter aerogenes fermentations of acid-hydrolyzed corn mash in a 2,3-butylene glycol pilot plant. This method was developed after noting that there was a great loss in bacteriophage titer upon using a Seitz filter for recovery. As a filter pad in the Seitz filter carried a negative charge, it was believed that the phage particles were being adsorbed from the solution. This was based on the assumption that the phage particles were charged positively. Upon the addition of CaCO₃ to the solution the titer of the phage was not lost during filtration and this procedure enabled a stock phage culture to be maintained in the laboratory. It is believed that the addition of CaCO₃ to the solution changed the charge on the Seitz filter pad from negative to positive, allowing the phage particles to pass through.

The bacteriophage cultures have been classified in respect to the sensitivity and immunity of strains of bacteria to the phage. Stock phage cultures are being carried in the laboratory and bacterial cultures have been immunized against them.

A21. Studies on the Nutritional Requirements of Actinomyces griseus for the Formation of Streptomycin. Geoffrey Rake and Richard Donovick, Squibb Institute for Medical Research, Division of Microbiology, New Brunswick, N. J.

Beef extract, or corn steep liquor, is shown to be unnecessary for the formation of streptomycin by Actinomyces griseus grown in a medium consisting of soybean meal, glucose, sodium chloride, and water. The absence from a soybean medium of an inorganic salt, such as sodium chloride, leads to very little streptomycin formation and it is suggested that beef extract may supply some of the necessary salts. Preliminary studies indicate that sulfate ion may be substituted for chloride ion but that magnesium does not satisfactorily replace the sodium ion. In shake-flask cultures the volume of medium per flask significantly affects the yields of streptomycin obtained.


Because of variations in streptomycin titers under controlled aeration rates, it seemed desirable to determine the influence of this factor on oxidation-reduction (OR) values and streptomycin production.

Sterile beef extract broth, 350 ml, and sterile 50 per cent glucose, 7.5 ml, were placed in each special glass fermentation flask. The flasks and contents were then incubated at 28 C, and sterile air was introduced at rates varying between 0.5 and 6 volumes per volume of media per minute (vpm). Three groups of
experiments were carried out: sterile media control, media inoculated with Actinomyces griseus, inoculated media containing 0.1 per cent ferro-ferricyanide. The ferro-ferricyanide was added to obtain closer agreement of duplicate electrode readings in the same culture.

The OR of sterile media varied between 310 and 375 mv regardless of the rate of aeration. In inoculated media during the first 24 hr the OR dropped from 344 to 228 mv. With continued incubation and aeration rates of 0.5 and 1 vpm the OR dropped to 80 and 160 mv, respectively, in 96 hours. The streptomycin titers were less than 50 units per ml. With 2 to 6 vpm the OR values were between 200 and 255 mv and the titers were approximately 75 units in the same interval of time.

The ferro-ferricyanide favored more uniform electrode readings without otherwise influencing the reactions.


Corn steeping liquor is a valuable nutritive material recently given much publicity by its use in the penicillin fermentation. Earlier it was used as a nitrogen and mineral nutrient for yeast production and was known to the trade as "yeast compound." It has been found valuable as a minor adjunct in many fermentations. Essentially it is an extract of corn solubles under acid conditions, pH 4-4.5, in the presence of dilute sulfuric acid and lactic acid. Laboratory and plant study shows that during processing an active microbial population, chiefly lactic acid bacteria and yeasts, assists in the extraction. It contains approximately 8 per cent nitrogen, amino N/total N 0.5; and is high in essential amino acids and minerals, and most of the B complex vitamins. Its value in antibiotic production is in part at least due to the extensive fermentation it has undergone during the wet corn milling process. In addition to its frequently demonstrated powers of enhancing yields in mold fermentations, it is an effective medium for many bacteria. It may replace peptone as a source of available nitrogen, or beef, yeast malt, or other extracts as essential adjuncts. Specially refined products are available for biological work. Further preliminary processing is desirable for specific uses such as use in a clear agar medium.


Cottonseed meal is at least as good as corn steep liquor for penicillin production in submerged culture by P. chrysogenum strains Demerec X1612 and Wisconsin Q176. Without added chemical precursors, cottonseed meal is considerably superior to corn steep liquor. A number of characteristics of the cottonseed meal medium are discussed. P. chrysogenum requires an adaptation to
lactose for most rapid and efficient utilization of lactose and production of penicillin.


The production of penicillin in the presence of beta radiation was investigated with both surface and submerged (shaker) cultures. The radiation source was radioactive phosphorus, $P^{32}$, in the form of phosphoric acid added to the production mediums before inoculation, to give activities ranging between 0 and 100 microcuries per ml of medium.

The culture mediums and methods employed were essentially those developed at the Northern Regional Research Laboratory. *Penicillium notatum*, NRRL 1249.B21, and *Penicillium chrysoogenum*, NRRL 1951C, were used for surface and submerged penicillin production respectively. Assays were carried out by the standard cup method using *Staphylococcus aureus*, FDA 209P, as the test organism.

The presence of beta radiation in the medium appeared to inhibit penicillin production; at radiation levels below 100 microcuries test samples usually assayed lower than, although within the experimental error ($\pm$ 25 per cent) of, the control assays. In submerged cultures, amounts of penicillin significantly lower than the controls were not found until an activity of about 100 microcuries per ml of production medium was reached. In surface cultures, activities only up to 4.0 microcuries were tried, and no significant effects were noted.


Several groups of compounds have been tested in shake flasks with *Penicillium chrysoogenum*, strain X-1612, to determine the effect on penicillin production. The basal medium was of known chemical composition containing lactose, glucose, acetate, and mineral salts. The amount of antibiotic produced was checked by routine assays with *Staphylococcus aureus*, *Bacillus subtilis*, and *Salmonella enteritidis*.

The common fatty, hydroxy, and dibasic acids have shown no significant stimulation of penicillin yields. Likewise very little effect has been evident with most of the amino acids used. l-Leucine and certain of the sulfur-bearing amino acids have sometimes given increased yields but the effect has not been consistent. A series of aromatic compounds related to phenylacetic acid and phenylethylamine have markedly stimulated penicillin yields. Phenoxyacetic acid and the meta- and para-halogen derivatives of phenylacetic acid have been quite effective. P-nitrophenoxyacetic and p-aminophenoxyacetic acids increase the activity of the antibiotic for *B. subtilis* and *S. enteritidis*. In most cases the amide derivatives were just as effective or better than the corresponding acids.
A27. Studies on a Spirochate Found in the Blood of Sick Turkeys. W. R. HIN- 
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re, Department of Veterinary Science, Davis, Calif.

A spirochate obtained from the blood of sick turkeys has been proved patho-
genic for turkeys and chickens. It stains readily with Tunnicliff's stain; is 
soluble in 10 per cent bile, and in 10 per cent saponin. It is loosely spiraled, 
has an average of 6 spirals, and measures an average of 14 microns in length in 
stained specimens. Dark-field studies show that it is motile.

The spirochate remains viable in the blood of surviving infected chicks for 
3 to 17 days, with an average of approximately 10 days. In adult chickens and 
turkeys the maximum survival time in the blood of survivors has been 4 days. 
In infected chicks which have been killed and stored at 0 C, the organisms have 
remained viable and capable of producing infection for at least 16 days. At the 
peak of infection the organisms tend to form large clumps in the blood, and 
become granular immediately before death of the host.

Transmission with infected blood has been possible by the following routes: 
intravenous, intraperitoneal, intramuscular, intranasal, intraorbital, subcu-
taneous, and oral. A distinct rise in temperature accompanies infection. The 
mortality rate has been lower than reported in avian spirochatois in other parts 
of the world. This is believed to be the first outbreak of spirochatois in turkeys 
reported in North America.

A28. Sulfur Drugs in the Control of Shigella gallinarum Infections. D. FRANK 
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of Bacteriology, Knoxville, Tenn.

An investigation was made of the value of sulfur drugs in the control of fowl-
typhoid infection. Sodium sulfathiazoie, soluble sulfonamide no. II, sodium 
sulfamerazine, and insoluble sulfamerazine were employed. These agents were 
administered to young chicks through feed or water in amounts of 0.1 and 0.5 
per cent. The drugs checked both naturally occurring outbreaks and experi-
mentally induced infections within two or three days, but the disease frequently 
reappeared after discontinuance of treatment. In one instance, the disease 
reappeared five days after the chicks had been removed from a treatment of 14 
consecutive days.

Prolonged administration of sulfur drugs in 0.5 per cent concentration gave 
evidence of retarding the growth and weight of chicks without impairment of 
appetites. Post-mortem examination of experimentally infected chicks, sacri-
ficed after 10 days on sulfur drug treatment, revealed no gross pathology. That 
the chicks still harbored Shigella gallinarum was proved by the isolation of the 
organism from the gall bladder. The bacilli were not isolated from other organs 
of the treated chicks, as was readily accomplished when the disease was permitted 
to run its course in the absence of sulfur drug treatment.

A29. Commercial Inoculation of Legume Seed. FRED S. ORCUTT AND ALMA L. 
WHITMAN, Virginia Polytechnic Institute, Department of Biology, 
Blacksburg, Va.
Inoculation of legume seed prior to sale has not been considered to be practical since the available literature indicates that the rhizobia will not remain viable for a sufficient period to be effective. Chemicals used in disinfection of such seeds are difficult to remove and residual amounts may inhibit growth of the desired bacteria. The results of our investigations reveal that a procedure, not involving disinfectants which tend to remain on the seed, may be used, and that inoculation with desirable strains of *Rhizobium* may be satisfactory in that survival times greater than one year may be demonstrated. Therefore, it appears that large-scale commercial inoculation of legume seed may be practical.


The action of cultures from marine muds on certain organic acids has been studied to determine whether bacteria under simulated marine conditions can produce hydrocarbons or compounds that could be regarded as precursors of hydrocarbons. Mixed cultures were developed by adding marine muds to a sea water medium enriched with a small amount of yeast extract and one of the following compounds: lactic, propionic, palmitic, and glutamic acids, leucine, and phenylalanine. Calculation of carbon balances indicates that for the smaller molecular weight compounds, acetic acid was the primary end product. It was generally accompanied by small amounts of carbon dioxide and higher fatty acids such as butyric. Ether extraction of liquors from the amino acid fermentations has given a constant but very small per cent of neutral material, some of which contains sulfur or sulphydryl groups. Ether extracts from palmitic acid and phenylalanine dissimilations likewise have contained acetic acid and significant amounts of higher acids. One mud culture was able to convert phenylalanine almost quantitatively to phenylacetic acid.

In all of these studies, the marine cultures have produced acid rather than neutral compounds. The necessary oxygen for this process apparently was obtained by sulfate-reducing bacteria from the sulfate ion present in sea water. The common occurrence of H₂S in active fermentations of this nature is added evidence of the source of oxygen.

**A31. Lipid Transformations by Anaerobic Bacteria.** William D. Rosenfeld and Claude E. ZoBell, University of California, Scripps Institution of Oceanography, La Jolla, Calif.

Lipolytic anaerobes have been isolated from muds, water samples, and materials associated with petroleum deposits. Hydrolyzable substrates included glycerides and other esters of fatty acids, as well as more complex oils, and lipolysis was marked at Eh levels often more reducing than −400 millivolts.

The detection of lipoclasts was facilitated by the use of neutral red base, an indicator proposed by Knaysi. The progress of lipolysis was followed by using a Warburg respirometer to measure carbon dioxide evolution from bicarbonate buffer in the presence of fatty acids released by hydrolysis.

Considerable numbers of lipid-utilizing cultures are facultative in their oxygen
reducing requirement. A notable exception is the group of strictly anaerobic sulfate-reducing bacteria. Impure cultures containing sulfate reducers have actively consumed both glycerides and oils as sole carbon sources, although it has been difficult to isolate lipolytic sulfate-reducing colonies. Associative activities may thus be responsible for the transformations observed in these instances. There is evidence to suggest that sulfate reducers utilize end products of lipolysis. The production of hydrocarbonlike substances from lipids has been observed in this laboratory on several occasions. Such syntheses may have resulted from the utilization of fatty acids in an extremely reducing environment.

**A52. Effect of Growth of Microorganisms upon Formation of Peroxides, Free Fatty Acids, Aldehydes, and Ketones from Oils with Different Iodine Numbers.**

**Joseph Henja and Leslie R. Hedrick,** Illinois Institute of Technology, Department of Biology, Chicago 16, Ill.

The organisms *Pseudomonas fluorescens*, *Serratia marcescens*, *Aspergillus niger*, and *Penicillium italicum* were grown upon a 50 per cent emulsion (with and without the antiozidant NDGA) of the following oils: coconut (iodine number 8 to 10), soya bean (iodine number 130 to 140), and olive (iodine number 170 to 180). All the oils were refined, bleached, and neutral in reaction. At the beginning of the experiments and after an incubation period at 20 C for three intervals of seven days, the substrates were tested for peroxides, free fatty acids (FFA), aldehydes, ketones, and rancid odors.

In general, the growth of the organisms caused an increase in the amount of free fatty acids (FFA); but they produced a decrease in the quantity of peroxides in comparison with the amount formed in the sterile oil control. Ketones were formed only by molds, and only in oils with low molecular weight.

In coconut oil emulsion, most FFA was formed by *Aspergillus*, least FFA by *Pseudomonas*; peroxides, greatest reduction by *Aspergillus* and least reduction with *Pseudomonas*. With soya bean emulsion, most FFA was formed by *Penicillium*, least by *Serratia* (with NDGA); peroxides, greatest reduction by *Pseudomonas* (with NDGA) and least reduction with *Aspergillus*. In olive oil emulsion, most FFA was formed by *Serratia*, least FFA formed by *Pseudomonas*; peroxides, greatest reduction by *Pseudomonas* (with NDGA) and least reduction with *Aspergillus*. There was no correlation between the rancidity detected organoleptically and the results of the chemical tests performed.

**A53. The Bacteriostatic Action of Short Chain Fat Acids.**

**Orville Wyss,** University of Texas, Department of Bacteriology, Austin, Texas.

Certain of the fat acids exert a bacteriostatic action in a mineral salts glucose medium in addition to the effect observed in the presence of amino acids or peptone. This inhibition is reversed by casein hydrolyzate, by aspartate or glutamate, and by pantothenate. It is exhibited by acetate, propionate, and butyrate but not by formate or valerate or by compounds of chain lengths exceeding 5 carbon atoms. This appears to be another example of the effect of inhibitory analogues on the utilization of metabolites.
A34. The Effect of Agar upon the Germicidal Potency of the Quaternary Ammonium Salts. R. Quisno, I. W. Gibby, and M. J. Fotet, The Wm. S. Merrell Co., Department of Bacteriology, Cincinnati 15, Ohio.

During investigations of death rates of bacteria treated with quaternary ammonium salts, a discrepancy was noted between results obtained with liquid media and results obtained with media containing agar. It appeared probable that agar had partially neutralized the germicide.

To determine the effect of agar upon the germicidal potency of quaternary ammonium salts 0.2 per cent agar was included in the germicide-bacteria mixtures during the test period. Standard germicide tests without agar were included as controls. When the four quaternary ammonium salts were tested in the presence of 0.2 per cent agar, lethal concentrations were found to be three to six times greater than lethal concentrations in the absence of agar. Several investigators have reported a lack of correlation between results obtained with liquid media and results obtained with agar cup plate tests for these germicides. The neutralization effect of agar probably accounts for this discrepancy.

Standard tests for antiseptic potency call for evaluation of creams, ointments, powders, etc., by agar cup plate methods. It is evident that such procedures are inappropriate for preparations which contain quaternary ammonium germicides.

A35. The Relation of pH and Quinine to Growth and Disinfection Rates of Escherichia coli. Isaac Lewin and Frank H. Johnson, Princeton University, Department of Biology, Princeton, N. J.

During the early logarithmic growth phase of Escherichia coli in a synthetic medium, a transfer of the cells from neutral to increasingly acid media causes an initial retardation or cessation of growth, or a disinfection, followed by a resumption of growth. This subsequent growth has a much higher apparent activation energy at pH 4.9 than that of controls at pH 6.9.

The rate of disinfection at temperatures above the growth optimum is increased by lowering the pH. The growth-inhibitory and disinfection-promoting effects of 0.0007 M quinine at pH 6.9 are practically eliminated at pH 5.9. At pH 4.9 quinine apparently protects against the initial growth inhibition or disinfection accompanying the transfer of cells from a medium of neutral reaction to one of acid pH. The rate of disinfection at 46.1 C, although accelerated by 0.0007 M quinine at pH 6.9 is retarded by quinine at pH 4.9. The growth-inhibitory and disinfection-promoting actions of the drug are evidently due to the free alkaloid base. The protective effects of quinine at acid pH values occur in other phenomena as well as growth, by a mechanism that is not clear.

A36. The Rate of Growth and Disinfection of Escherichia coli in Relation to Temperature, Hydrostatic Pressure, and Quinine. Frank H. Johnson and Isaac Lewin, Princeton University, Department of Biology, Princeton, N. J.

The growth rate of Escherichia coli during the early logarithmic phase in a synthetic medium at neutral pH is limited by a single system in which the activity
increases with rise in temperature, but decreases beyond an optimum at 37-39 C by a reversible denaturation of the protein catalyst. Bacteriostasis without disinfection occurs during brief exposures to 45 C, and growth is resumed at once on cooling to 37 C. Hydrostatic pressures of 1,000 lb/in² retard growth below 35 C but accelerate it above; 5,000 lb cause slight disinfection at low temperatures but greatly retard disinfection at temperatures above 45 C.

The net effect of quinine depends on concentration, temperature, hydrostatic pressure, coenzyme, pH, and oxidizable substrate. Growth inhibition increases with temperature, and is reversible on dilution or cooling. Relatively high concentrations at low temperatures or lower concentrations at higher temperatures cause disinfection. Small amounts of bacterial extracts of co-dehydrogenase I oppose the growth-inhibitory and disinfecting action. Pressure is additive to the effects of quinine at low temperatures but strongly opposes its action at high temperatures. Quinine apparently acts in two ways: (1) promoting a reversible and an irreversible protein denaturation, and (2) specifically blocking hydrogen transfer through the co-dehydrogenase system. The pressure effects indicate large molecular volume increases of reaction or activation, typical of reactions involving proteins.

A37. The Evaluation of Germicidal Agents by an Infection-Prevention Toxicity Method. EARLE H. SPAULDING AND AMEDEO BONDI, JR., Temple University School of Medicine, Department of Bacteriology and Immunology, Philadelphia, Pa.

The infection-prevention (IP) technique of Nungester and Kempf is a valuable procedure for the study of skin disinfectants. The usefulness of this procedure, however, may be increased by including toxicity determinations, which, except for the omission of the culture, are carried out in the same manner as the infection-prevention tests. The highest dilution of disinfectant which prevents pneumococcus infection in one-half of the mice is designated as IP/50. The toxicity end point (T/50) is then determined by finding the lowest dilution which fails to kill one-half of the test mice. IP/50 divided by T/50 yields a number which has been termed the infection-prevention toxicity (IP-T) index. Compounds which are highly bactericidal and relatively nontoxic possess indices of high values.

A chlorinated phenol, two cationic detergents, an organic mercurial, iodine, and tyrothricin have been evaluated by this method. The highest index was obtained with tyrothricin. Representative results will be presented and compared with those obtained with in vitro methods and with the toxicity-index procedure of Welch and Hunter. The value and limitations of the infection-prevention toxicity method will be discussed.

A38. Evaluation of Disinfectants by Tests in Living Animals. M. E. PIERCE AND E. B. TILDEN, Northwestern University Dental School, Department of Bacteriology, Chicago, Ill.

The Nungester and Kempf infection-prevention test in mice, with pneumo-
coccus as the test organism, was used to evaluate the potency of a number of common disinfectants. Of these, phenol and iodine, each in 2 per cent aqueous solution, and DC 12 (dimethylbenzylauryl ammonium chloride) in 1 per cent solution, aqueous or tincture, were almost completely effective in killing the pneumococcus. If DC 12 was diluted to 0.1 per cent, however, not all pneumococci were killed, the number of mice lost being 42 per cent with 0.1 per cent aqueous, and 22 per cent with 0.1 per cent tincture. Merthiolate in 0.1 per cent aqueous solution was almost completely ineffective, as had been shown by Nungester and Kemp. With the newer “metaphen disinfecting solution,” which contains 4 per cent benzyl alcohol in 0.04 per cent metaphen, the mortality in the mice was 33 per cent, as compared with 86 per cent for the older 0.1 per cent aqueous metaphen. One per cent aqueous metaphen was quite effective, almost as good as 3 per cent saponated cresol. The alcohols, ethyl in 70 per cent concentration and isopropyl (concentrated), were more effective than expected, the mortality in the mice being less than 20 per cent.

Preliminary tests were carried out with some of these disinfectants against Mycobacterium tuberculosis, the mixtures used in a standard phenol coefficient test being injected intraperitoneally into guinea pigs instead of being inoculated into a culture medium. Phenol in 1 per cent aqueous solution, saponated cresol in 2 per cent (by volume), and DC 12 in 1 per cent tincture were effective in killing this organism.


A study of the in vitro bacteriostatic properties of combinations of sulfanilamide and di-phenthane 70 (2,2′-dihydroxy-5,5′-dichlorodiphenylmethane) suggested the existence of a synergism between sulfonamides and certain closely related substituted diphenylalkyl compounds.

Potentiation studies were conducted on a strain of Streptococcus mastitidis in infusion broth pH 7.6. An equivalent of 12.5 per cent of the minimum bacteriostatic concentration (m.b.c.) of one compound was added to each tube in a serial dilution of the other compound. All tubes, including controls, were incubated for sterility, then seeded with 0.1 ml of a 24-hr culture diluted to yield 1,500 to 2,000 organisms per ml of test substance. Tests were incubated for 96 hours at 37 C. Minimum bacteriostatic concentration recorded was the lowest concentration showing no visible growth.

Addition of 0.08 mg per cent di-phenthane 70 (8 m.b.c.) to sulfanilamide, sulfapyridine, and sulfadiazine reduced the bacteriostatic end points from an average of 256 mg per cent to 1 mg per cent; sulfadiazine from 400 mg per cent to 6.25. Concentrations as low as 1/4 m.b.c. potentiated sulfanilamide 32-fold. Reversely, sulfonamides potentiated di-phenthane 70 two-to eightfold. In proteose peptone no. 3 medium no marked potentiation occurred; however, the m.b.c. of sulfanilamide and sulfathiazole averaged 2 mg per cent.
compared to 256 mg per cent in infusion broth. Addition of 0.032 mg per cent PABA raised the bacteriostatic end point to 128 mg per cent. Diphenthane 70 did not neutralize the antisulfanilamide action of PABA. Studies are now in progress on the action of certain closely related substituted diphenylalkyl compounds, also on the bacteriostatic action on other organisms.

A40. The Effects of Pus on Sulfonamide Activity. L. H. SCHMIDT and CLARA L. SESLER, Christ Hospital, Institute of Medical Research, Cincinnati, Ohio.

This study dealt with the effects of various lots of pus on the in vitro activities of sulfathiazole and sulfanilamide against several bacterial species. The pus was obtained from ten patients and represented a variety of lesions and infecting organisms. Various methods of preparation of the pus were used including filtration through muslin, autolysis, and extraction with heat, acids, alkalis, or ether. The preparations were divided and portions sterilized by boiling, autoclaving, or Seitz filtration. The effects of these preparations upon sulfonamide activity were determined both in artificial media (simple and complex) and in human blood.

With two exceptions none of the lots of pus, irrespective of source or method of preparation, affected the activities of sulfathiazole or sulfanilamide against strains of *Diplococcus pneumoniae*, *Streptococcus hemolyticus*, or *Staphylococcus aureus*. The two exceptions were cases in which the pus had been contaminated with novocaine. These preparations did antagonize the activities of the sulfonamides against the above organisms. In one of these cases pus obtained on subsequent days without the use of novocaine did not have this antagonizing action.

All the preparations, however, appeared to antagonize the activities of these sulfonamides against *Escherichia coli*. This effect may have been due to growth stimulation since the tests with *Escherichia coli* were carried out in a simple medium which afforded much better growth upon the addition of pus.

The above findings provide little support for the conception that pus and tissue debris contain substances which antagonize the common sulfonamides.

A41. The in Vitro Potentiating Action of Sulfonamides and para-Aminobenzoic Acid on Penicillin Against Pathogenic Bacteria of Recent Isolation. JORGE VIGOUROUX and GRACIELA LEYTON, Bacteriological Institute of Chile, Santiago, Chile.

The antibiotic action of penicillin for a number of strains of pathogenic bacteria is reinforced over a wide range by the addition of minute amounts of either sulfathiazole, sulfapyridine, sulfadiazine, or para-aminobenzoic acid (PABA). The incorporation of one of the sulfonamides and PABA in the same solution with the penicillin nullifies the potentiation. Penicillin-sulfonamide mixtures have a more pronounced action on penicillin-sensitive strains, however, when PABA is substituted for the sulfonamide. The sensitivity of the organisms to
penicillin plays no part in the potentiation. The extent to which the antibiotic action of the penicillin is increased is directly related to the intrinsic antibacterial action of the substance under investigation. Occasionally organisms are encountered which are susceptible to mixtures of a sulfonamide and penicillin but resistant to the compounds when applied singly.

In general sulfapyridine has the highest potentiating action. The outstanding exception is with Brucella, where sulfadiazine is most active. On the basis of these findings and certain other preliminary studies not to be reported at this time the therapeutic use of combinations of sulfonamides and penicillin in certain infections is indicated.


Streptomycin is an antibacterial agent predominantly effective against gram-negative organisms. Impure preparations are bacteriostatic in action, causing either a decrease in the number of organisms present or at least an inhibition of multiplication of the organisms. True bactericidal action is seldom observed, especially among the enteric organisms.

The sensitivity of a large number of strains of bacteria, belonging to several species, has been tested. The amount of streptomycin necessary to inhibit a given strain may vary from day to day. This difference is due at least in part to slight variations in the number of organisms present, the age and density of the culture, and to the species of organisms involved. Difference in buffer concentration, concentration of enrichment substances present, pH, and other factors influence the sensitivity of an organism to streptomycin to an even greater extent. A statement of the sensitivity of any organism to streptomycin is therefore significant only if expressed in relation to a standard strain.

M2. Studies on the Bacteriostatic and Bactericidal Action of Streptomycin on Bacterium tularense. S. S. Chapman, Lt. (Jg), USNR; Cora M. Downs; and S. F. Kowal, M/Sgt., USA; Camp Detrick, B Division, Frederick, Md.

Bacterium tularense was grown in a peptone medium, described by Snyder and others at Camp Detrick, and the bacteriostatic and bactericidal concentrations of streptomycin were determined. The presence of bacterial growth was determined by turbidity readings, plate counts, and mouse titrations. For a broth inoculum of 5,000,000 organisms per ml, the bacteriostatic concentration of streptomycin was between 0.2 and 0.4 units per ml. One unit per ml sterilized the cultures within 24 hours, while 10,000 units per ml sterilized the cultures in less than 30 minutes. Organisms which grew in the presence of 0.1 and 0.2 units per ml did not appear abnormal, but colonies from tubes containing 0.4 and 0.8 units of streptomycin per ml grew slowly and showed a preponderance of unusually large pleomorphic forms.