INFLUENCE OF DRYING UPON SOIL USED AS A MEDIUM FOR BACTERIA. H. J. Conn and Mary Darrow, New York State Agricultural Experiment Station, Geneva, New York.

Bacterium radiobacter has been inoculated into sterilized soil, after bringing it to optimum moisture content and adding nutrients sufficient to assure good growth of the organism. Its numbers have then been determined by use of the microscope. Under optimum conditions the counts reach about two billions per gram. In some soil samples, however, it was found that no matter what nutrients were added the organism was not able to multiply to much over one billion, if it even reached that figure. Some unfavorable condition seemed to be holding it in check.

As the soils in which its members remained low had all been for some time in air-dry condition, the effect of varying moisture content was studied. This study has now been made on three different soils, and it has been found that keeping them moist for about two weeks before adding the nutrients and sterilizing does improve them as a medium for the growth of this organism. Best results were obtained when the soil was kept for this period moistened to about 30 to 35 per cent of its water-holding capacity. After two weeks at this moisture content, however, these soils become less favorable for the organism. No explanation of the phenomenon is yet offered.

PAPER MILL SANITATION IN RELATION TO THE MANUFACTURE OF FOOD WRAPS AND CONTAINERS. J. R. Sanborn, New York State Agricultural Experiment Station, Geneva, New York.

The making of paper containers for such a perishable and easily contaminated product as milk gives paper manufacture increased public health significance. Certain types of container board are heavily contaminated with coliform organisms; in other cases, spore-bearing bacteria, micrococci, or filamentous fungi may predominate.

Uncontrolled development of microorganisms in pulp and paper mills presents serious obstacles to quality in paper wrappers and containers. Accumulations of growth as stringy or gummy masses interfere with production and result in spotting and in lack of uniformity and strength. Discoloration and decomposition processes in stored pulp or stock definitely affect quality, as well as the presence of objectionable odors, which are sometimes transferred from pulp to finished paper.

Strict microbiological control of pulp and paper operations is successful in preventing such difficulties and also sets a sanitary standard for plants engaged in fabrication and handling of food wraps and containers. Programs
for control include general sanitary practices, systematic slime prevention measures, chemical treatment of process water designed to reduce bacterial numbers in pulp, and handling of sheets so as to render them suitable for direct contact with such foods as milk and meat. Wrapper and container board for foods should, upon leaving a mill, conform to sanitary and bacteriological standards.

Prevalence of Human Infection with Trichinella Spiralis. O. R. McCoy, Department of Bacteriology, School of Medicine and Dentistry, University of Rochester, Rochester, New York.


Recent Attempts to Stabilize Bacteriological Nomenclature. R. S. Breed, New York State Agricultural Experiment Station, Geneva, New York.


Six hundred fifty-five samples of pasteurized milk from various parts of New York State during different seasons have been tested in formate-ricinoleate broth for the presence of members of the Escherichia-Aerobacter genera. Of these, 147 gave positive presumptive results, 144 of which confirmed according to Standard Methods. Two of the three unconfirmed samples yielded slow lactose-fermenting members of the Escherichia genus, and the third a related species which belonged to the Escherichia or Salmonella genus.

In comparative tests on 221 samples of pasteurized milk, formate-ricinoleate broth, brilliant green 2 per cent bile broth, enrichment in standard lactose broth (24 hours' incubation) followed by inoculation into formate-ricinoleate broth, enrichment in buffered lactose broth (24 hours' incubation) followed by inoculation into formate-ricinoleate broth, gave approximately the same number of positive presumptive tests. A slightly higher yield of positive presumptive tests was indicated for the brilliant green bile broth, but some of these were due to anaerobic spore producing rods.

Of 99 samples of raw milk tested in formate-ricinoleate broth, 56 gave positive presumptive tests. Of these, 47 confirmed according to Standard Methods. From five of the nine sample which did not confirm slow lactose-fermenting Aerobacter organisms were isolated; from the other four samples Proteus organisms which were able to produce gas from formate, were isolated.

Fifty-three organisms which, according to Standard Methods, yield false presumptive tests were found to belong to the Proteus Aerobacter and Bacterchia and related groups.


The composition of the citrate-ricinoleate agar used was: 0.5 per cent peptone; 0.1 per cent sodium ricino-
leate; 0.4 per cent sodium citrate (Na₃C₆H₅O₇·5H₂O); 0.2 per cent sodium nitrate; 1.5 per cent agar; neutral red 1/20,000; and brom thymol blue 1/20,000. The pH was 7.0. The 1 cc. inoculations of milk added to the citrate ricinoleate agar approximately 0.5 per cent lactose.

The ability of sodium ricinoleate to inhibit the growth of organisms associated with false tests was demonstrated in this medium. Sodium nitrate is used to prevent the production of gas which would cause explosion of the agar and make accurate counting of colonies difficult. *Aerobacter* and proteolytic organisms produce an alkaline reaction from sodium nitrate, but this salt is not used as the sole source of carbon. On this medium containing neutral red, *Escherichia* organisms, able to produce acid from lactose, but unable to attack citrate, appear as large red colonies. *Aerobacter* organisms, able to attack citrate, show an alkaline reaction, signified in the presence of brom thymol blue by the production of a blue green color. Proteolytic organisms can be detected on this medium, containing 10 per cent milk, by the presence of a clear zone around the colonies. These zones may be made more conspicuous by flooding the plates with an acid solution of bichloride of mercury or another similar substance.

Plating milk samples of citrate-ricinoleate agar makes possible the differentiation and enumeration of *Escherichia, Aerobacter*, and proteolytic Gram-negative rods.

A Comparison of Brilliant Green Lactose Bile and Formate Ricinoleate Media for the Detection of the Escherichia-Aerobacter Group in Milk and Ice Cream.


The relative effectiveness of brilliant green lactose bile broth 2 per cent and of formate-ricinoleate broth for the routine detection of the *Escherichia-Aerobacter* group in pasteurized milk and ice cream has been studied. Of 542 samples (369 milk and 173 ice cream), 219 gave positive presumptive tests in each medium; of these, 197 were confirmed from brilliant green lactose bile broth and 192 from formate-ricinoleate broth. The per cent confirmation, therefore, was 90 per cent and 88 per cent respectively. An analysis of these data was made by an adaption of Halvorson and Ziegler's method. It showed that the two media were equally useful for the routine detection of the *Escherichia-Aerobacter* group in pasteurized milk and ice cream.

**Rabbit Fibroma to Myxoma Transformation with Heat-Inactivated Myxoma Elementary Bodies.**

George P. Berry, Department of Bacteriology, University of Rochester, School of Medicine and Dentistry, Rochester, New York.

Experiments from our laboratory have been reported as showing that the virus of Rabbit Fibroma (Shope) can be changed into that of Infectious Myxomatosis (Sanarelli). In these transformation experiments, rabbits were inoculated with suitable mixtures composed of active fibroma virus and heat-inactivated myxoma virus, the latter being present in heavy suspensions of myxomatous skin lesions. Besides myxoma virus, these crude suspensions obviously contained a host of other things. It was possible, therefore, that the "transforming agent" might not have come from the virus, but from the infected host. The work
described below not only indicates that the "transforming agent" is derived from myxoma virus, but also adds strong confirmation to our interpretation that fibroma virus is actually changed into myxoma virus. Through the kindness of Dr. T. M. Rivers, we have been able to work with suspensions of washed elementary bodies of myxoma, prepared in his laboratory at the Rockefeller Institute. These suspensions are essentially free of rabbit tissue and produce myxomatosis in a dilution of 10⁴. Heating them at 65°C. for 30 minutes renders them completely non-infectious. In 5 experiments with 3 different myxoma elementary body suspensions, inactivated at 65°C. and at 75°C., we have transformed fibroma virus into myxoma virus.

**Factors Influencing the Rate of Lactic Fermentation.** Otto Rahn, C. P. Hegarty and E. P. Deuel, The Laboratory of Bacteriology, Cornell University, Ithaca, New York.

**Eastern Pennsylvania Chapter**

**One Hundred and Twenty-Fifth Meeting, Philadelphia County Medical Society Building, Philadelphia, Pennsylvania, May 25, 1937**

**Bacterial Type Transformation.** Hobart A. Reimann, Jefferson Hospital, Philadelphia.

Bacterial type transformation has been discussed for years in regard to many bacteria and viruses. The theoretical importance of type transformation in regard to infectious disease and to epidemiology is obvious.

In 1934 a strain of Micrococcus tetragenus was obtained from the blood of a patient with meningitis and arthritis. The organism produced white colonies which, upon aging, developed yellow daughter colonies and a third or translucent form. Subsequently pink, pink-yellow and brown colonies appeared, each of which bred true. They were immunologically distinct types. Spontaneous transformation from one type into another was repeatedly observed, it being an apparent chance phenomenon rather than forced variation. Each type was dissociated into its M, S and R culture-phases; 15 variants of the original white form being isolated and studied.

The various forms exhibited constant differential cultural preferences for different ranges of temperature and hydrogen-ion concentration of media, and different degrees of resistance to various bactericidal influences. The white form, as derived from the patient, grew best at temperature, CO₂ tension and pH ranges similar to those found in vivo. It appeared that type transformation and culture-phase variation were phenomena which permitted a bacterium to exist in a wider range of environmental conditions.

**The Effect of Dietary Minerals upon Host Resistance.** Charles F. Church, Department of Pediatrics, School of Medicine, University of Pennsylvania and Children's Hospital of Philadelphia.

The effect of diet upon host resistance has been tested in 2000 mice of three inbred lines. The animals were inoculated by stomach tube with 0.005 cc. of an 18-hour broth culture of Salmonella enteritidis (3 to 5 million organisms). The stock culture used throughout the work has been kept on agar slants in the ice-box and has shown no change in virulence.

Groups of 20 mice of the same genetic and dietary background were compared.
on each experimental diet. The survival per cent at four weeks after inoculation was taken as the index of host resistance.

Five groups of Line A (Rockefeller Institute Resistant) mice on the standard purified diet showed a mean survival of 91.0 per cent ± 4.0 (standard deviation). When the mineral content of the diet was reduced to one-fourth that of the standard, leaving all other factors unchanged, the survival in five groups of A-line mice was 67.5 per cent ± 4.6. The odds against this difference being the result of chance are 20,000:1.

When calcium only was reduced in the diet (from 149 mgm. per 100 calories to 28 mgm.), the survival was diminished from 90 to 64 per cent. The odds against this being a chance result are 1300:1.

These low-mineral and low-calcium diets were satisfactory for maintenance and health of uninoculated adult mice over periods of months.

It is concluded that calcium is a limiting factor in the host resistance of Line-A mice to Salmonella enteritidis infection.

Staphylococcus Studies. I. Toxin Production. E. P. Casman, Abington Memorial Hospital, Abington, Montgomery County, Pa.

A study of the conditions for the production of staphylococcus toxin was made. It was found that a veal infusion medium containing 2 per cent Difco proteose peptone, 0.7 per cent sodium acetate, 0.5 per cent sodium chloride and 0.3 per cent agar and sterilized in the autoclave was superior to a veal-infusion-free medium containing 2 per cent proteose peptone, 0.7 per cent sodium acetate, 0.5 per cent sodium chloride, 0.1 per cent potassium phosphate (dibasic), 0.1 per cent potassium phosphate (diacid), 0.02 per cent magnesium sulfate, 0.01 per cent calcium chloride and 0.3 per cent agar. Sterilization of veal infusion media by filtration did not result in an increase of toxin production. When the medium was adjusted to pH 6.8, a gaseous atmosphere of 80 per cent oxygen and 20 per cent carbon dioxide and an incubation period from 48 to 60 hours gave the best toxin yields.

Dialysis of a veal infusion medium through cellophane removed most of the substances that could be precipitated by saturation with ammonium sulfate and made possible the preparation of a relatively pure toxin by means of ammonium sulfate precipitation. Yeast extract enriched with dialyzed proteose peptone and 0.7 per cent sodium acetate was equally efficient in the production of a potent toxin that could be purified by precipitation with ammonium sulfate.

Concentration of Staphylococcus Toxoid by the Kidneys. E. P. Casman, Abington Memorial Hospital, Abington, Montgomery County, Pa.

After intravenous injections of staphylococcus toxoid into rabbits whose sera contained no appreciable staphylococcus antitoxin, heart blood and bladder urine specimens were taken and these titrated for their toxoid contents. The concentration of toxoid in the urine specimens after the toxoid injection was always higher than that in the blood serum samples obtained after injection of the toxoid. The degree to which the toxoid was concentrated by the kidneys varied inversely with the amount of urine excreted. When, for example, 0.015 cc. of urine was excreted per minute, the urine contained 60 times more toxoid per cubic centimeter than did the blood.
serum. When 0.35 cc. of urine was excreted per minute, the degree of concentration effected by the kidneys was only 2.5 fold.

Degenerative Changes of the Neutrophiles in Clinical and Experimental Observations. Max M. Strumia, The Bryn Mawr Hospital, Bryn Mawr, Pa.

Critical study of results of blood examination of 136 cases of lobar pneumonia in adults shows that very valuable data for the prognosis may be obtained. The elements essential to the prognosis in increasing order of importance are absolute number of lymphocytes, monocytes and eosinophiles, and percentage of cells showing cytoplasmic or nuclear degenerative changes. The percentage of young cells (nuclear shift or Schilling's index) is too variable to be of much importance. It may generally be stated that if the percentage of young neutrophiles is constantly low the case will probably have a favorable outcome but if the percentage of young neutrophiles is high then the outcome may be almost anything.

Lymphopenia, monopenia, eosinopenia and a high number of degenerated cells are very definitely unfavorable signs. It is essential that the following points be thoroughly covered: first, judgment should be based not on a single count but on a series of counts taking into consideration the variations of the various groups of cells from day to day. Second, the preparations must be technically uniform and as good as can be obtained. Third, correlation must be established between the blood picture and the clinical course.

The prognostic value of the blood examination is particularly important because changes in the blood picture usually take place a considerable period of time before a corresponding change in the clinical picture.