
This organism was taken from the pipes carrying sulphur-containing solutions in an industrial plant. The growth of this bacterium in the pipes produced slimy, gray, algae-like masses which interfered with the process. The solution had a pH of 0.5 and consisted of certain organic and inorganic sulphates. The identification and some of the characteristics of this organism were the subject for investigation in this paper.

Various organic and synthetic inorganic media were used to determine the utilization of differing substrates. Certain compounds were further tested to ascertain their inhibitory effect on the metabolism of this bacterium. Studies were made of the organism at various stages in its growth. Washed cultures of the filaments were qualitatively tested for sulphur.

Several synthetic media containing ammonium salts, carbonates, thiosulfates and sulphates supported growth. The substitution of glucose for carbonates gave better growth. The septate, branching, sheathed filaments with a hold-fast and the presence of sulphur granules seemed to indicate Thiothrix tenuis. Some of the compounds tested inhibited the metabolism of this bacterium.

Discussion of paper brought out the fact that identification of the organism is still in question.

A2. The Morphology of the Butyl-butyric Anaerobes. E. R. L. Gaughran and Stanley Thomas, Bethlehem, Penna., Lehigh University, Department of Biology.

The results of a morphological study of a few strains of the butyl-butyric group under a set of standard conditions are reported. Subsequent reports will deal with the biochemical reactions of the same organisms. Out of more than 75 "species" which have been described in the literature, 9 strains were selected for which various workers have, in differential study, claimed entity. These are: Bacillus granulobacter
(pectinovorum) (Speakman) Donker; Clostridium acetobutylicum (Weizmann) Donker; Bacillus butylicus Fitz; Granulobacter saccharobutyricum Beijerinck; Bacillus saccharobutyricus von Klecki; Bacillus multifermens (tenalbus) Stoddard; Clostridium pasteurianum Winogradsky; Clostridium beijerinckii Donker; and Clostridium felsineum Carbone. Before any observations were made the cultures were grown on Svartz semi-solid agar at 37°C with heat shocking every 5 days for 3 months. Shape, axis, ends, and arrangement of the vegetative cell, sizes of vegetative cell, clostridium and free spore, form of clostridia, sporulation time, type and time of germination, spore capsules, type of flagellation, deposition and disappearance of granulose, behavior to the gram and other special stains with variations in stain pattern and intensity and presence of divergent forms were studied. Numerous technical procedures were employed. All observations were repeated at 3-month intervals for over a year. These studies lead to the conclusion that any differentiation of the bacilli of the butyl-butyric group on the basis of morphology is impossible.


Since Clostridium thermosaccharolyticum and Clostridium sporogenes are important spoilage organisms encountered in the canning industry, it is essential that suitable media be available for their detection.

A comparison of the growth of C. sporogenes in Brewer's medium (pork base), in liver broth with liver particles, and in an experimental liver medium supplied by the Difco Laboratories, revealed good growth of C. sporogenes in all tubes inoculated with suspensions containing from 6 to 60,000 spores per ml. In the same media growth of C. thermosaccharolyticum always was observed in liver broth plus liver particles when inoculated with 650 spores or more, and occasionally when 65 and 6 spores were used as inocula. In the Difco experimental medium growth never was observed in tubes inoculated with less than 650 spores, and in Brewer's medium growth was confined to tubes inoculated with 65,000 and 6,500 spores.

With the addition of 1.5 per cent agar to the various media growth of C. sporogenes was evident in all media and in all dilutions. With C. thermosaccharolyticum growth was observed in all tubes of liver medium...
plus particles and in all tubes of Difco experimental medium. In Brewer's medium, however, growth never was observed if an inoculum of less than 650 spores was used.

Studies on changes of O-R potential revealed little difference among the three media. Sterile media usually had an E.M.F. of $-272$ to $-274$, and during the logarithmic growth phase readings dropped to $-351$ to $-389$. These results indicated that the poorer growth exhibited by Brewer's medium was probably due to the lack of essential nutrients in the pork base, and not to an unsatisfactory O-R potential.


Suspensions of the spores of Clostridium botulinum types A and B, and of putrefactive anaerobe number 3679 were heated for various lengths of time at several temperatures in phosphate solution and in a number of fresh vegetable substrates. They were then subcultured in multiple recovery cultures for a quantitative determination of the survivors as well as for the end point. The subcultures were divided into two or more lots for incubation at different temperatures. Hence, the only variable in any given experiment was the temperature of incubation of the recovery cultures. A substantially greater apparent resistance to heat has been recorded from recovery cultures incubated at 24°C, 27°C or 31°C than from the parallel culture incubated at 37°C. No significant difference has been noted among cultures incubated at the lower temperatures.


A quantitative determination has been made of the bacteria occurring on sardines and equipment at various stages of manufacture, beginning with fish collected at the weir and ending with the processed can. Examinations were made on both viscera and flesh (including skin), sea water, chips from boat holds and salting tanks, brine and material collected from a number of sampling points within the factory. The tests have included total counts of bacteria growing at 37°C, aerobic and anaerobic spore counts at 37°C and 55°C, and with a number of
samples cultures for halophilic bacteria. Some data on the inhibitory effect of salt on the natural bacterial flora of sardines have been accumulated.

The results obtained indicate that few bacteria occur in or on the fish or any part of the equipment until the fish are handled incidentally to packing in the can. After trimming and packing, a substantial percentage increase in the total count per gram of tissue was found. The absolute count, however, was not high. No thermophils and no significant number of heat resistant spores were found at any sampling point. Results from the tests for halophils were negative or of no significance.

A6. *Serratia marcescens* as a Cause of Pink Sauerkraut. D. Frank Holtman, Columbus, Ohio, The Ohio State University, Department of Bacteriology.

Samples of “pink” sauerkraut have regularly yielded chromogenic torulae (*Torula glutinis*) which, upon inoculation into normal sauerkraut, have produced red discoloration comparable to that occurring naturally. However, one sample investigated microbiologically yielded red-pigmented bacteria, identified as *Serratia marcescens*, as well as torulae. The torulae grew on plate cultures as grayish-white colonies and, when inoculated into normal sauerkraut, were unable to produce a pink or red discoloration, although they utilized practically all of the available acid within a few days at 20°C. *Serratia marcescens*, alone or inoculated simultaneously with the false yeast, was incapable of producing “pink” sauerkraut from a normal product. When the bacteria were inoculated after the torulae had reduced the acidity of normal sauerkraut to a negligible amount, a deep red color developed. Both microorganisms were recovered from this product. The red color was obtained only under aerobic conditions and was comparable to that present in the original sample.


It has been previously reported by us that an organism described as *Phytomonas polycolor* by Clara, isolated from a disease of tobacco, was apparently *Pseudomonas aeruginosa*.

Five cultures of *Pseudomonas aeruginosa*, obtained from human or
animal sources, were fatal to small laboratory animals, rabbits, guinea pigs and mice, on experimental infection. The organisms were always recoverable from the heart, spleen, liver, lung, etc. Blood cultures made of infected animals showed a definite multiplication of the organisms within the animal host.

On tobacco these cultures produced a severe leaf spot and necrosis, by either needle puncture inoculation or spraying. Needle puncture inoculations into the stem of young plants brought about destruction of the surrounding tissues. This was often accompanied by an extensive wet rot adjacent to the site of inoculation. Koch's postulates were always fulfilled in regard to these tests.

It is believed likely that several green fluorescent bacteria which have been described as causing a disease of plants are *Pseudomonas aeruginosa*.


The possibility that a bacteriological study of flue-cured tobacco might throw more light on the mechanism of the ageing process has led to a preliminary study by the authors of the flora of flue-cured tobacco as placed in hogsheads preparatory to ageing. Marked differences in the flora of good and poor quality tobacco have been found.

Good quality flue-cured tobacco is characterized by a flora consisting largely of saccharolytic, yellow, gram-negative rods with peritrichous flagella which may be subdivided into 3 principal groups on the basis of sugar fermentations. The most important numerically of these fermentations without gas the common laboratory sugars with the exception of lactose. The second is very similar but ferments lactose. The third group, which may include organisms not closely related, forms gas from the common sugars.

The bacteria of poor quality flue-cured tobacco are frequently more numerous than those on high quality flue-cured tobacco. Poor quality flue-cured tobacco differs primarily from the latter in the nature of the organisms comprising the major portion of the flora. In the case of poor quality tobacco 3 types of organisms are encountered in large numbers. These 3 types which show little activity on carbohydrates consist of monotrichous, yellow, gram-negative rods; motile, non-spore-forming, pale-pigmented, gram-positive rods; and motile, pink, gram-
negative rods. The dominance of any one of these 3 groups has always been found associated with poor quality as judged by tobacco experts.


The ability to ferment melibiose is given special importance in Stelling-Dekker's classification of the sporogenous yeasts, for this characteristic is the basis upon which the two groups of species containing Saccharomyces cerevisiae and Saccharomyces carlsbergensis are differentiated. In these two species are found the most important industrial strains. S. cerevisiae does not ferment melibiose; S. carlsbergensis does. Both hydrolyze raffinose, which yields melibiose on hydrolysis.

Kluyver, Stelling-Dekker, and Lodder fermented a known amount of raffinose in an apparatus which allowed the quantitative measurement of the carbon dioxide evolved. The species that fermented melibiose produced more gas than the species which could not. Zimmermann inoculated yeasts into raffinose broth and 14 days later titrated the remaining sugar to learn whether the melibiose had been fermented.

The following method for fermentation of melibiose using raffinose gave results that correlated in every case with results of direct fermentation tests on melibiose; this method does not require the use of special apparatus:

Ten-millimeter test tubes containing 3 ml. of 4 per cent raffinose broth and an insert were inoculated with the yeasts under study. Incubation was at 30°C and recordings made daily of the amount of gas. When the volume of gas showed a decrease, the tube was again inoculated with a young culture of a strong melibiose fermenter, S. carlsbergensis var. mandschuricus strain NRRL no. 379. Gas production following this second inoculation indicated that the organism under test did not ferment melibiose; continued decrease in the gas volume indicated that the organism under test did ferment melibiose.

The standard practice for the production of distillery yeast involves the anaerobic growth of yeast in sour-grain mash. This procedure requires 40 to 60 hours for the production of a single culture, which is not pure. In addition comparatively large volumes of culture are required to inoculate each fermenter. A process was desired that would be strictly pure-culture, continuous, require but a few hours for yeast production, and which would reduce extensively the size of the equipment.

These requirements were fulfilled by producing yeast under pure-culture aerobic conditions. The conditions necessary for this type of propagation such as pH, temperature, nitrogen content, medium, and aeration were determined by using the quality and quantity of the yeast produced as the essential criteria. A system was devised for operation on an industrial basis under strictly pure-culture conditions. The completed process, operated on a production basis, was tested to determine the quality of this yeast as compared with sour-mash yeast.

The concentration of yeast produced was great enough so that only one-third the previous volume was required for fermenter inoculation. Consistent operation of industrial equipment without contamination was accomplished. The time required for yeast production was reduced to 3 or 4 hours as a result of continuous operation. Finally, it was proven both by laboratory and plant-scale fermentations that the yeast was not only fully equal to old-process yeast, but slightly superior on the basis of alcohol production.


The blue crab (Callinectes sapidus) in its soft-shell stage is considered a great delicacy but is available only during the "moulting" season. Soft-shell crabs were obtained from commercial dealers and placed in freezing storage. It was found that those frozen alive maintained firmer texture and turgidity upon thawing than those dressed before freezing. Most of the studies were on dressed ready-to-cook crabs. There was very little loss in flavor and texture during 15 months of freezing storage and no development of off flavor or odors.

The bacterial counts from several lots of fresh crabs were fairly uniform. The bacteria were mainly on the surface of the body and relatively few were found in the intestinal tract or body fat, even in
crabs which had been dead for a number of hours. Blanching in hot water lowered the bacterial count, but caused the crabs to be rather soggy when fried and did not enhance the keeping quality. *Escherichia coli* were found on a few fresh crabs but not on those in freezing storage. The total bacterial count decreased slowly during storage. There was no clear-cut correlation between the quality of the crabs before freezing and the bacterial content of the product after storage in the frozen state.


The pasteurization of crab meat was undertaken for the purpose of extending its keeping quality in order that it may be shipped farther than is possible at the present time, as well as to destroy any pathogenic organisms that may contaminate the meat while it is being handled.

The meat was bought in 1-pound cans from a wholesale dealer at the Washington waterfront. It was tested for *Escherichia coli*, total number of organisms as found by the plate method, and pH value.

The crab meat from each can was divided into two portions, packed, sealed, and pasteurized at one of the following temperature ranges; 145°F for 30 minutes; 150°F for 20 minutes; 155°F for 15 minutes; 160°F for 10 minutes; and 170°F for 1 minute.

Pasteurized lots of crab meat were opened at weekly intervals to find out the length of time they would keep in the refrigerator at 41°F to 43°F. These were compared with freshly purchased crab meat as to color, odor, and taste.

It was found that the pasteurized crab meat kept as long as 5 weeks at 41°F to 43°F, was free from *E. coli*, contained a small number of bacteria, and the color, odor, and taste were not impaired.


Due to the rapid spoilage of crab meat, any test which decreases the time element in testing for pollution would materially benefit the product.

A modified Frost "little-plate" method incorporating differential media was used. The plates were incubated in a warm moist chamber at 37°C, 43°C, and 46°C. Results were read after 8 hours. Of the media
used, MacConkey’s agar (Difco) proved most satisfactory. The temperature adopted was 43°C.

Because the volume was necessarily small, dilutions of crab meat were inoculated directly into tubes of warm MacConkey’s agar. For amounts greater than 1 ml. screw-capped dilution bottles containing 30 ml. of MacConkey’s agar were used. Tubes and bottles were solidified as rapidly as possible after inoculation and placed in a water bath at 43°C for 8 hours. Results were recorded with the aid of a microscopic lamp and a hand lens. Typical Escherichia coli appeared as ruby-red colonies surrounded by a zone of precipitated bile.

Comparative tests with lactose, modified Eijkman and MacConkey’s agar were used. Of 129 crab samples examined, 43 lactose tests showed acid and gas in 24 hours. Of these 19 confirmed. From 124 samples a total of 63 showed gas in Eijkman tubes, of which 48 confirmed. Of the total number (34) of positive MacConkey samples, 20 were picked for confirmation. All 20 proved to be E. coli.

Crab meat dilutions of 1:2 were also run with MacConkey’s agar. Ninety were positive, 53 were picked for confirmation, and 50 proved to be E. coli.

A14. The Bacteria in Brick Cheese During Ripening. E. M. Foster, J. C. Garey and W. C. Frazier, Madison, Wisconsin, University of Wisconsin, Department of Agricultural Bacteriology.

Brick cheese made from raw and pasteurized milk by the conventional and washed-curd methods with Streptococcus lactis and Streptococcus thermophilus starters, alone and in combination, was examined for the bacteria present during ripening. Cultural counts were made on samples taken at weekly intervals and representatives of the predominant organisms isolated at each sampling period.

Identification of over 1,000 cultures from 18 lots of brick cheese showed Streptococcus lactis predominant throughout ripening when it was used as the starter. It was also detected in cheese made with Streptococcus thermophilus alone and, with one or two exceptions, eventually became predominant. Streptococcus thermophilus died out rapidly in cheese in which it was the starter and was seldom detectable in appreciable numbers after 2 or 3 weeks. Lactobacilli developed in all cheese made from raw milk, but were usually absent in pasteurized milk cheese. Lactobillus casei was always the predominant rod form and usually was detectable after the second week of ripening. Lacto-
bacillus brevis and Lactobacillus lactis were found in smaller numbers in several samples.

Streptococci other than the starter organisms frequently developed during ripening: namely, Streptococcus faecalis, Streptococcus bovis and Streptococcus liquefaciens. The first two were particularly noticeable in cheese made from pasteurized milk. There was no regularity in the occurrence of these organisms, hence their presence apparently depended upon the individual milk supply. When they did develop they were usually detectable after the first to second week of ripening. No other organisms were found in significant numbers in any of the cheese.

A15. Microorganisms Associated with Gassy Swiss Cheese. HARRY H. WEISER, Columbus, Ohio, Ohio State University, Bacteriology.

Gassy or split-rind Swiss cheese constitutes a serious economic loss in the cheese industry. This defect makes for a poor physical appearance and is associated with a flat, tasteless and bitter cheese.

A bacteriological examination of samples taken from the abnormal cheese showed two kinds of microorganisms: anaerobic bacteria and lactose-fermenting yeasts. These microorganisms were conspicuously absent in samples of normal cheese, indicating that they might play an important role in initiating the abnormal condition. They were classified as Clostridium perfringens and Torula cremoris.

In extremely gassy cheese, C. perfringens and T. cremoris were present in significant numbers, while the latter were not isolated from cheese only mildly defective.

An examination of fresh milk showed the presence of C. perfringens and T. cremoris in those instances when unsanitary practices were followed on the farm.

An improvement in the sanitary conditions under which milk is produced, as well as the use of a good active starter in the cheese plant, materially improves the quality of Swiss cheese.


It seems important to me to make a clear distinction between cheese defects and cheese diseases. A cheese defect may be caused by false operating during the making and curing. Cheese diseases are caused by specific agents, mostly bacteria. There exist two groups of such causative microorganisms. The first are obligate agents of diseases. If present even in small numbers in an otherwise normal
cheese, they cause the disease. Examples are *Clostridium tyrobutyricum* van Beynum and Pette and *Bacterium proteolyticum*. The second group is the one of the potential agents of diseases. They cause trouble only if certain conditions are fulfilled. Their presence in entirely normal cheese which is handled in a normal way is harmless. They will multiply only if the cheese offers to them favorable conditions which are not present in normal cheese or in normally treated cheese. *Clostridium sporogenes* which causes the disease of Swiss cheese called "white stinker" is the type of this group. It is always present in milk but is harmful only if the cheese has not enough acidity or if, because of too few propionic acid bacteria, the formation of eyes is slow and the cheese is therefore kept for a long time in a warm cellar. This type of cheese diseases takes an intermediary position between the defects and the diseases caused by the agents of the first group.

*A17. The Microbiology of Frozen Eggs. I. Presence and Significance of Coliform Organisms in Frozen Eggs.* FRANCIS E. COLIEN, Omaha, Nebraska, Creighton University School of Medicine, Bacteriology and Preventive Medicine.

A study was made of the bacterial flora of 181 samples of frozen eggs. The samples, egg whites, sugared and plain yolks and whole eggs, were cultured in lactose broth and plated on Endo medium. Representative colony types from each sample, a total of 1,090 colonies, were selected and used for further study. Of these 594 (approximately 50 per cent) were considered as belonging to the coliform group. Two hundred and seventy cultures produced acid and gas in lactose in 24 hours and a characteristic colony on Endo medium; 155 (26 per cent) were imvic + + - - and 125 (21 per cent) imvic - - + +. The remaining cultures belong to other imvic types or are atypical coliforms. These two groups were tested for the fermentation of lactose, inositol, glycerol and cellobiose and for their ability to produce oxidase. These cultures were tested on isolation and at various intervals for a period of 3 years.

It would seem that the presence of the coliform group in frozen eggs should be considered in the sanitary control of egg-breaking, the keeping quality of this product and its usefulness in the manufacture of food products.

Bacteriological analyses were made on samples of fermenting egg white received from commercial drying concerns. The natural fermentation of fresh and frozen samples of egg whites received from Washington, Wisconsin, Iowa, Pennsylvania, Virginia and Maryland were studied in the laboratory by periodically removing samples for bacteriological and chemical analyses. *Aerobacter aerogenes* and *Escherichia freundii* (Citrobacter) were found to be the organisms predominating in the normal fermentations. In abnormal fermentations, species of *Proteus, Pseudomonas* and *Serratia* were usually found to be present in predominate numbers.

Normal fermentations of fresh egg white were characterized by an initial increase in pH from about 7.1 to 8.9 or 9.0 within 6 hours, followed by a decrease in pH to about 6.1 within a 72-hour period. This decrease in pH was accompanied by a corresponding decrease in free sugar. There is no apparent evidence of proteolysis.

Abnormal fermentations in which species of *Proteus, Pseudomonas* or *Serratia* predominated were characterized by evidence of proteolysis and a longer period of time required for the pH to decrease to about 6.1.

The rate and type of fermentation depends upon the numbers and types of bacteria initially present in the egg white.

**A19. A Comparison of Presumptive Tests for Coliform Bacteria in Seawater and Shellfish.** LEslie A. Sandholzer, Norfolk, Virginia, National Institute of Health, Division of Public Health Methods, Craney Island Laboratory.

A comparison was made of standard lactose, formate ricinoleate and Eijkman media on 100 homologous samples of seawater, oyster meats and oyster liquors. Partial confirmation of the positive presumptive tests was accomplished by the use of brilliant-green-lactose-bile broth and eosin-methylene-blue agar. The confirmation was completed for each test. From the results obtained the "most probable number" of coliform bacteria per ml of original sample was computed. These data were then evaluated statistically.

In the case of seawater, there was no significant difference between the 3 presumptive media, but confirmation on eosin-methylene-blue agar yielded a consistently higher m. p. n. than did the identical tests partially confirmed in the brilliant-green medium.
When oyster meats were similarly tested, no valid differences in the m. p. n. were found between the 3 media when partially confirmed in brilliant-green broth. Partial confirmation on eosin-methylene-blue agar yielded no differences between lactose and formate or lactose and Eijkman media, but formate showed significantly higher m. p. n. values than did Eijkman broth. The same thing also held for oyster liquors, except that both lactose and formate yielded higher m. p. n. values than Eijkman broth when the partial confirmation was made on eosin-methylene-blue agar. Both oyster meats and liquors showed greater m. p. n. values when confirmed on the brilliant-green-lactose medium. The mean values of the m. p. n. for oyster meats and liquors were consistently greater than those for seawater regardless of the presumptive or confirmation medium employed.

A20. The Occurrence of Salmonella in Retail Market Meats. W. Bailey Cherry, M. Scherago and R. H. Weaver, Lexington, Kentucky, University of Kentucky, Bacteriology.

Using the tetrathionate enrichment method of Kauffmann and the selenite enrichment method of Leifson, 250 samples of market meat have been examined for the presence of Salmonella. Of 200 samples which were pork products, or probably contained pork, 12 (6 per cent) yielded Salmonella. Of the pork products examined Salmonella were found in 6 of 30 livers, in 1 of 9 brains, in 2 of 23 samples of "hamburger steak", in 2 of 45 samples of fresh pork and in 1 of 44 samples of sausage. None was found in 9 samples of pork and beef loaf, 10 samples of kidney, 4 samples of cooked pork, 5 samples of smoked sausage or 21 samples of cured ham and bacon. One of the 31 samples of beef but none of the 11 samples of lamb or of the 5 calf sweetbreads or of the 3 chicken livers examined yielded Salmonella. Eight types of Salmonella were identified.


The evaluation of disinfectants by in-vitro methods has always presented difficulties because of the bacteriostatic action exerted by many substances, especially mercurials. An in-vivo technique is presented in this paper which has been found to give direct evidence concerning the ability of a particular disinfectant to "prevent infection." In brief, this technique consists of contaminating the tail of a
living animal with a given virulent organism, treating with the disinfectant, then inserting the tail in the peritoneal cavity of the animal and observing whether or not infection occurs. A number of widely used disinfectants have been tested in this manner and the results are presented.


The evaluation of germicidal substances by the in-vitro determination of their phenol coefficients has long been known to have certain limitations. Two most important factors in the evaluation of such a substance are its ability to penetrate the system present, and its ability to act in the presence of tissue.

An in-vivo method is presented for the evaluation of germicidal substances in the presence of animal tissue. The method consists of removing a piece of skin, previously infected with Streptococcus pyogenes and subsequently treated with a germicidal substance, from the abdominal wall of a white mouse and inserting it into the peritoneal cavity of that same mouse and then noting whether or not a fatal peritonitis ensues. Simultaneous controls consisted of the identical technique with the omission of the infecting organisms. Tincture of iodine, cetyl pyridinium chloride, Merthiolate, and hexylresorcinol were tested and showed relative germicidal values in the above order, these values not being in accord with the relative values determined by the F.D.A. phenol-coefficient method. The test was repeated using the skin from a dead animal and the relative values of the germicidal substances, with the exception of tincture of iodine, were lower; thus supporting the theory of the germicidal action of living skin.

A23. Standardization of Skin Disinfectants. Leo H. T. Bernstein, Baltimore, Maryland, The Johns Hopkins University, Department of Pathology and Bacteriology.

In this report the determination of the activity coefficient, $k$, of various skin "disinfectants" under actual conditions of use is discussed. Price's quantitative technique for studying the bacterial flora of the skin is employed with an additional mathematical analysis.

The method consists in scrubbing the hands and arms in a standard
manner in one series of basins containing sterile water, applying the disinfectant and then scrubbing in a second series of basins. Each basin is cultured and from the data obtained the total flora of the hands and arms before and after use of the disinfectant is determined. The rate of removal of organisms by mechanical scrubbing is logarithmic and varies for different individuals.

From Price's data the action of ethyl alcohol on the skin can be shown to be logarithmic. By analysis of two published reports and the author's determination, the activity coefficient, \( k = \frac{1}{t_1} - t_4 \log \frac{x_1}{x_2} \), for 70 per cent ethyl alcohol is (1) -0.31744, (2) -0.37684 and (3) -0.38531, averaging -0.35986. The rate of action of iso-propyl alcohol is also logarithmic and increases with increased concentration of the alcohol. Iso-propyl alcohol (98.3 per cent) gives \( k \) values of (1) -0.59078 and (2) -0.67180, averaging -0.63129, for two individuals. Therefore iso-propyl alcohol is 1.75 times as efficient as ethyl alcohol. Using Price's equivalent minutes of scrubbing time value \( E \), \( k \) may also be determined by the formula

\[
k_{\text{disinfectant}} = E k_{\text{mechanical scrubbing}}.
\]

Estimation of a \( k \) value in this manner has shown that results of different investigators are comparable. It is hoped that \( k \) values for other disinfectants will be obtained under actual conditions of use to get a sound basis for comparison.


A modification of the Price method similar to that of Pohle and Stewart was used. Bacterial counts were made from a series of hand washings before and after chemical treatment to determine the effectiveness of the chemical.

Price's caution in interpreting results obtained with mercurial solutions without an antidote can well be applied to other substances. For instance, some chemicals such as ethyl alcohol, and potassium alum, have a hardening action on the skin surface, making subsequent washing less effective. Alum 10 per cent, aqueous, though not germicidal, gives as good a "bacterial destruction" curve as 70 per cent alcohol, since very few bacteria can be washed off after its use. Also, after such treatment, a smaller amount of keratinized epithelial cells and
fragments are removed, which can be demonstrated by electrometric turbidity measurements of washings in clear water.

On the other hand, agents which tend to soften the epithelium, such as ammonium sulfide 5 per cent, and sodium sulfide 0.5 per cent, increase the effectiveness of subsequent washing.

Since many antisepsics have a hardening or a softening effect on the skin, which might influence the results by this method of testing, these factors should be considered in any comparative evaluations.


A. J. Salle and M. Korzenovsky, Los Angeles, California, University of California, Bacteriology.

Tests were conducted to determine the effect of a vacuum on the destruction of bacteria by germicidal agents in solution and in the gaseous state.

The killing dilution of a germicide for bacteria under atmospheric conditions and in a vacuum were the same. The removal of air from the liquid with the possible increase in penetration did not increase the efficiency of the germicide.

Entirely different results were obtained when gaseous germicides were employed. Bacteria placed in the center of rolls of cotton or embedded in fine sand were not killed by the action of the gas under atmospheric conditions but were easily destroyed in the presence of a vacuum. In the absence of a vacuum the gas acted as a surface disinfectant whereas in the presence of a vacuum the material was easily penetrated by the gas.

The organisms tested included Escherichia coli, Eberthella typhosa, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus faecalis, Bacillus mycoides, Bacillus subtilis, and Bacillus anthracis. The organisms were subjected to a high vacuum, then an appropriate amount of the gas introduced and the sterilizer held under a partial vacuum for a period of 30 minutes.

A26. The Determination of the Efficiency of Germicides to be used in Obstetrical and Gynecological Procedures.

Rhett G. Harris, Charleston, South Carolina, Medical College of the State of South Carolina, Department of Bacteriology and Hygiene.

Laboratory investigations of commercial germicides adaptable for intravaginal instillation have shown that many are reduced in activity
when placed under environmental conditions simulating the human vaginal tract. Two factors were found to be largely responsible for this reduction: (1) precipitation of proteins followed by germicide adsorption during contact with a menstruum high in organic content, and (2) the precipitation of the germicide as an insoluble, inactive entity when placed in contact with material having the pH of the normal human vaginal tract. It is believed that the apparent failure of many germicides to meet the clinician's requirements in obstetrics and gynecology may be attributed to these factors.

Germicides to be used for intravaginal use should, therefore, in addition to meeting the requirements of dilution, surface tension, penetration and low tissue toxicity, be free from the defects noted above.


The germicidal values of the acetates of a series of primary aliphatic amines was determined by the F. D. A. method at 37°C. Aqueous dilutions of the acetates containing 10, 11, 12, 13, 14, 16 and 18 carbon atoms in the alkyl chain (decyl, undecyl, dodecyl, tridecyl, tetradecyl, hexadecyl and octadecyl amine acetate, respectively) were included in tests without serum and all but the C₁₈ were included in tests with serum. The compounds were germicidal (killing in 10 minutes but not in 5) on the 3 test organisms used. Staphylococcus aureus was killed by different members of the series in dilutions ranging from approximately 1:1,000 to 1:14,000, Escherichia coli by dilutions ranging from 1:1,000 to 1:19,000 and Saccharomyces cerevisiae by dilutions ranging from 1:1,000 to 1:7,000. As the numbers of carbon atoms increased the germicidal values first increased and then decreased, highest potency appearing with the intermediate members of the series. Serum reduced the germicidal potency of the compounds, especially on Staphylococcus aureus and Escherichia coli.


Bacillus metiens spores (170 × 10⁶ per ml) suspended in Butterfield's
formula-C water and stored at 10°C showed no appreciable change in resistance to chlorine or chloramine solutions over a period of 2 years. The times required to kill 99 per cent of the spores exposed at 20°C (pH 7) to 25 p.p.m. available chlorine as hypochlorite and chloramine, were 3.0 minutes and 89 minutes, respectively, when a freshly prepared spore suspension was employed. The killing times for the same suspension 2 years later were 3.2 minutes with hypochlorite and 91 minutes with chloramine. The difference in killing times of the fresh and old suspensions was well within the limits of error of duplicate determinations.


This investigation reports the effect of tallow soap, the sodium sulphate detergents, and sodium alkyl sulphate itself on the toxins of Shigella dysenteriae, Salmonella aertryke, Salmonella enteritidis, Staphylococcus aureus, Streptococcus pyogenes, Pasteurella tularensis and sheep cells by means of the Shwartzman response.

The bacteria were grown in special broth for 48 hours, centrifuged and the supernatant removed. The concentrated bacteria were then heated at 60°C for 1 hour and 1.0 ml. of this vaccine added to each gram of 1 per cent soap tested. The soap-toxin mixtures were kept at room temperature at varying intervals from 1 day to a month.

The standard controls were (1) necrosis of sufficient size; (2) all untreated soap controls had to exhibit no typical Shwartzman necrosis; (3) untreated bacterial antigens were able to produce typical necrosis. Positive Shwartzman sites were removed, ground in mortar with 10 ml. saline added, then centrifuged until clear and routine agglutination tests performed. Positive agglutination results ranged from 1:20 to 1:640. The control agglutinations were as high. When necrosis due to soap appeared, the zone of necrosis was usually outside the inoculated area and did not exhibit purplish discoloration.

The results obtained show that the Shwartzman response is very useful in determining the detoxifying power of soaps.

Antigens removed from soap mixtures which were not significantly altered were sheep cells and Salmonella enteritidis, while Shigella dysenteriae gave irregular reactions.

Bacillus aterrimus produces a black pigment only on media containing carbohydrates. This distinguishes it from Bacillus niger which produces a black pigment only on media which contain tyrosine. Both of these bacilli are separated from Bacillus subtilis by this one characteristic of black pigmentation.

By making serial transfers in glucose broth, plating on a glucose-nitrate agar, selecting the colonies producing the least pigmentation, testing on potato and replating several times, dissociants of Bacillus aterrimus were finally developed which would not produce the black pigment and have remained stable. The intermediate variants were gray and various shades of orange and red. The ease of dissociation varied greatly with individual cultures.

Bacillus niger was also dissociated by selective plating on appropriate media. The white dissociants of both Bacillus aterrimus and Bacillus niger cannot be distinguished from Bacillus subtilis. Certain strains of the latter are known to produce pink to brown colorations especially on potato which further points to the close relationship of these 3 organisms.

It is, therefore, concluded that Bacillus aterrimus and Bacillus niger are black varieties of Bacillus subtilis.


In concentrations above 30 per cent, sugar has a significantly inhibitory effect on the growth of microorganisms. A study was made of the effect of 30, 40, and 50 per cent solutions of sucrose, glucose, and mixtures of these two sugars on Saccharomyces cerevisiae and Aspergillus niger. At equal weights glucose inhibited the growth of Saccharomyces cerevisiae and Aspergillus niger to a much greater extent than did sucrose. In general, mixtures of the two sugars used in the same concentration inhibited growth to a degree intermediate to that of either sugar alone. Representative data indicate that after 48 hours incubation of the 40
per cent series, the sucrose, glucose, and sucrose-glucose syrups mixture showed, respectively, 5,700,000; 25,000; and 100,000 yeast cells per ml. by direct count. Similarly, the dry weights of the mold mats after 6 days in this series were 186 mgs. in the sucrose solution, 121 mgs. in the glucose solution, and 161 mgs. in the 40 percent sucrose-glucose solution. Pasteurization temperatures altered only slightly the relative toxic action of the two sugars. A slight difference in the hydrogen-ion concentration of the various sugar solutions was found to be not significant in accounting for the difference in inhibition by sucrose and glucose. Half of the sucrose content of 65 per cent grapefruit and pineapple syrups could be replaced by glucose with excellent results in their preservation. Preliminary data also show that the growth of bacteria is inhibited by glucose to a greater extent than by equal concentrations of sucrose.

A32. The Characterization of Species of the Genus Lactobacillus. CARL S. PEDERSON, Geneva, New York, New York State Experiment Station.

The identification of lactobacilli, like the identification of other organisms, is a difficult problem for anyone unacquainted with the group and the more important identifying characters.

Characters found especially useful in species differentiation in this group are end products of fermentation, temperature range of growth, action on litmus milk, morphology, and fermentation of various sugars. Fermentation of certain sugars and related compounds is particularly significant in characterization but need not necessarily be important in separating species.

Before a true picture of the ability of any species to ferment sugars and related compounds can be obtained, it is necessary to study a series of cultures and plot frequency curves to show the true ability to ferment sugars. This has been done for some species but further studies are needed before even the person familiar with the group may identify certain cultures. With complete knowledge of the group, even the aberrant strains can be placed.

Considerable confusion exists concerning the nomenclature of the particular strain of *Lactobacillus* (Amer. Type Culture Coll. no. 7469) which is employed extensively for the microbiological assay of riboflavin. It has been named *Lactobacillus casei* (it is probably identical with *Bacillus casei e* of Freudenreich), *Lactobacillus casei e*, *Lactobacillus helveticus* (the *Bacterium casei* (epsilon) of Von Freudenreich), *Lactobacillus helveticus*, and *Lactobacillus casei*. Its characteristics, however, have not been recorded in the literature.

The results of a comparative study of various members of the genus *Lactobacillus* show that strain no. 7469 possesses the characteristics of *Lactobacillus casei* (Bergey), not those of *Lactobacillus helveticus* (*Bacillus casei e* of von Freudenreich). In common with numerous strains of *Lactobacillus casei*, it forms dextro lactic acid; grows at low temperatures (between 6° and 18°C), in media containing 5 per cent of sodium chloride, in media adjusted to pH 7.5, and in relatively simple media; and forms relatively large, smooth, lens-shaped colonies. The cells are small. *Lactobacillus helveticus* strains, on the contrary, form inactive lactic acid; fail to grow below 18° to 20°C, in media containing 2.5 per cent of sodium chloride, in media adjusted to pH 7.5, and in relatively simple media; and form filamentous colonies. The cells are large. No. 7469 forms less titratable acid and more carbon dioxide than do the *Lactobacillus helveticus* strains.

Furthermore, members of the *Lactobacillus acidophilus-bulgaricus-helveticus-lactis* group fail to grow in the medium employed for the assay of riboflavin regardless of the presence of riboflavin.

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Five-hour serum broth cultures of a strain of *Erysipelothrix rhusio-pathiae* regularly containing ten million lethal doses per ml. for 18- to 20-gram mice have been used in a mouse-protection potency test of immune horse serum. Simultaneous injections of culture and serum are made into the peritoneums of the mice. Animals are observed for 7 days and results are expressed in terms of protection obtained with control serum which is used in each test. The test dose of organisms is usually 100,000 lethal doses of the above-described strain. With this test it is possible to detect a protective potency in immune horse sera.
from horses which have been under immunization for as short a period as 2 months. It is possible to detect between twofold and up to four hundredfold increases in potency. Probable protective endpoints may be predicted by using precipitin tests with an antigen which consists of an acetone-soluble extract of the organisms.


Each pair of rabbits (16) and one chicken (8) were injected with one of 8 antigens which consisted of 3 strains of Salmonella pullorum and 5 serologically related organisms: Shigella gallinarum, and Salmonella typhimurium, S. anatum, S. enteritidis, and S. cholerae suis. Before immunization all animals were tested with all 8 antigens by the spot-plate method and found negative. After immunization serum was collected and used for testing.

Tube titers were determined for all sera against all antigens and agglutinin absorption tests were run proving all titers resulted from inoculations. All sera were tested by the spot-plate method using the crystal-violet antigen described in U. S. patent 1,816,026 and the recently advocated thiosulphate-glycerol antigen. One drop of serum was mixed with one drop of stained antigen and rotated 2 minutes at an average temperature of 27°C.

The T-G antigen is less sensitive than the crystal violet but is not enough so to eliminate cross reactions.

The spot plate will show positive reactions with Salmonella pullorum at titers of 1:10 or above and will occasionally show titers of 1:2.

Inoculations with any one of the 8 antigens gave demonstrable titers with Salmonella pullorum.

Inoculation with Salmonella pullorum gave demonstrable titers with all 8 antigens. These were detected by the spot plate in all except 4 instances.

In this experiment there is little qualitative difference in the antigenic response of chickens and rabbits; the chickens commonly show a higher titer for chicken pathogens.

“Sandy reactions” may be due to low titers of group agglutinins.
A86. The Use of Dissociation Studies in the Identification of a Mucoid Streptococcus from a Trickling Filter as Streptococcus salivarius. JAMES MANDIA, R. H. WEAVER, AND M. SCHERAGO, Lexington, Kentucky, University of Kentucky, Bacteriology.

In order to identify a formate-utilizing organism which had been isolated from a trickling filter it was found to be necessary to resort to dissociation studies. The original culture appeared to be a mucoid gram-negative Streptococcus which could not be demonstrated to attack sugars. Its characteristics would not permit of its classification in any genus, as described in Bergey's Manual of Determinative Bacteriology. Dissociation produced a tiny-colony phase with large yeast-like cells, a typical diphtheroid phase, a rough phase with vacuolate organisms and a smooth phase by which it could readily be identified as a strain of Streptococcus salivarius. It is postulated that the extreme mucoid character of the original culture prevented its staining gram-positively and, by the slowing up of diffusion into and out of the cell, prevented demonstrable sugar fermentation.

Our experience with this organism substantiates Hadley's contention that no species is completely described until all of its phases have been described. Without such descriptions and without the use of dissociation techniques an organism cannot be identified unless it happens to be found in the same phase from which the official description of the species has been prepared.

Streptococcus salivarius, customarily associated with the human body, appears to have adapted itself in its mucoid phase to growth in the trickling filter.


A series of small silos filled with the same cutting of alfalfa under 6 different treatments was studied with respect to the method of preservation. The treatments were (per ton of silage) 80 pounds of molasses, 40 pounds of molasses, 18 pounds of phosphoric acid, 40 pounds of molasses plus inoculation, 30 pounds of salt, and 30 pounds of salt plus inoculation. The bacterial counts showed no correlation either with
quality of the silage or treatment. The best silages from the standpoint of chemical analysis as well as feeding tests were obtained by the use of 80 pounds of molasses and 18 pounds of acid per ton. The silages preserved with 40 pounds of molasses were slightly inferior to the first two and preservation with salt gave still poorer quality. There was no demonstrable difference due to inoculation.

A comparison of the fermentation in the upper and lower levels of the series showed a contrasting chemical composition with evidence of less desirable changes taking place in the upper part of the silos. After periods of storage ranging from 6 to 10 months, irrespective of treatment, the higher levels had pH values that were generally above 4.2, a relatively small amount of reducing sugar and a high content of acetic acid. The lower levels in the same silos had pH values below 4.2, more reducing sugar and averaged 31 per cent more lactic acid. Feeding experiments indicated a more palatable and better quality silage from the lower than from the higher portions of the silos.

A38. Large Scale Production of Azotobacter Cells for the Preparation of Cell-free Enzymes, SYLVAN B. LEE, ROBERT H. BURRIS, AND P. W. WILSON, Madison, Wisconsin, University of Wisconsin, Agricultural Bacteriology.

Evidence exists that points to a similarity of the mechanisms of biological nitrogen fixation in the symbiotic and nonsymbiotic systems. The nonsymbiotic system of Azotobacter offers certain advantages for studies on the mechanism of the fixation reaction especially those which deal with the nature of the enzyme systems concerned directly or indirectly with the fixation reaction. Large quantities of Azotobacter cells would greatly facilitate such studies.

Azotobacter vinelandii was grown in 250 to 350 liters of a nitrogen-free medium in a 200-gallon fermenter of the type used in the pilot-scale production of baker's yeast. The fermenter was equipped with apparatus for aeration of the medium and for temperature control. After a growth period of 30 to 34 hours at 30°C the cells were removed by centrifuging in the Sharples supercentrifuge. Yields of 4 to 5 pounds of cell paste (85 per cent moisture) have been obtained.

The cells are primarily used for the preparation of cell-free enzymes. Fresh or frozen cell paste is ground with powdered glass, and the latter, together with debris and uninjured cells, removed by centrifugation (4500 r.p.m.). Further clarification is accomplished through use of a
Beam's centrifuge; finally, the clarified material is filtered through a Berkefeld filter.

The following enzymes have been demonstrated active in the cell-free state: hydrogenase, various dehydrogenases, and oxalacetic decarboxylase. Determinations have also been made for components of the cytochrome system. In some instances a portion of the cells was treated with cold acetone. Dry powdered preparations obtained by such treatment contain hydrogenase and oxalacetic decarboxylase.

A89. The Quality of Milk in a Large Section of Virginia as Affected by the Constant Occurrence of Streptococcus lactis Variety tardus and Bacillus albolactis. F. S. Orcutt, Blacksburg, Virginia, Virginia Polytechnic Institute, Biology Department.

Pasteurization is normally considered to favor both the public health and economic value of milk. In the present investigation it has been found to be of certain detrimental value to the quality of milk over a large area of Virginia.

Milk is normally protected from the growth of pathogens and protein decomposers by the rapidity with which Streptococcus lactis grows if any opportunity is given for growth of organisms. The presence of Streptococcus lactis and amounts of lactic acid too small to taste may prevent the initiation of growth of most other organisms, even acid liquefiers if not present in too great numbers.

Occurrence of bitter flavor in fresh and butter milk as well as other dairy products, the occurrence of other "off" flavors from protein decomposition, and acid liquefaction in the souring of milk are all common in samples of milk from the area examined.

It has been found in milk from a number of sources in Virginia over a period of 5 years that the type species of Streptococcus lactis is almost entirely replaced by the variety tardus. The slow growth and acid production of this organism allow the active growth of Bacillus albolactis, an acid liquefer, particularly in pasteurized milk where the relative proportion of the spore former has been increased over that in the raw milk. It has been found that if the number of Bacillus albolactis relative to the total count exceeds 12 to 15 per cent Streptococcus lactis is unable to control the growth of this organism.

A40. A Pink-red Formate-utilizing Organism from a Trickling Filter, Vibrio rubicundus, n. sp. Elizabeth M. Gottron, R. H.
In the course of a study of formate-utilizing organisms which had been isolated, on silica gel plates, from a trickling filter, a pink-red, gram negative, encapsulated, curved, motile organism with a monotrichous flagellum was encountered. It has been found to appear also as a coccoid form. It has been found to attack sodium tartrate, sodium acetate, sodium formate, sodium citrate, asparagin, sodium hippurate, uric acid, cystine, glycine, i-inositol, soluble starch, cellobiose and glycogen. A study has been made of its cultural and biochemical characteristics. Since the organism could not be identified with any previously described species it has been named *Vibrio rubicundus*, n. sp.


A number of the newer methods of testing antiseptic action have been investigated. The experimental approach of workers using the manometric method for determining the effect of antiseptics upon the metabolism of bacteria is discussed in the light of experimental data obtained with a variety of agents and organisms. A new method using infected clots made of blood fibrin has been developed. This simulates much more closely the conditions existing in infected wounds than do such older devices as agar cup plates.

**MEDICAL BACTERIOLOGY, IMMUNOLOGY AND COMPARATIVE PATHOLOGY**

1–10 Pathogenic bacteria, I
11–22 Chemotherapy
23–32 Bacterial toxins
33–43 Viruses, etc.
44–53 Viruses—rickettsiae
54–63 Immunology
64–71 Pathogenic bacteria, II
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**M1. Streptococcus Bacteriophage, Type E.** ALICE C. EVANS AND ELSIE M. SOCKRIDER, Bethesda, Maryland, National Institute of Health, U. S. Public Health Service.