are to all appearances vegetatively alike. They differ apparently only in size. Zygotes were never found in both filaments, but only in the one containing the larger amount of food.

The difference in the number and size of the pyrenoids and the amount of starch present in the chloroplasts and in the staining reaction of the cytoplasm of the gametes, clearly indicate at least that in certain species of *Spirogyra* the male and female gametes are distinctly morphologically as well as physiologically different. Since starch is formed more abundantly in the female gametes than in the male, the female plants evidently possess a greater vegetative activity than the male plants. Blakeslee[1] in his recent studies of *Mucors* concludes that the female plants (+ strains) in dioecious forms are more vegetatively luxuriant than the male plants (— strains).

A more detailed account than is presented here will appear later.

HARLAN H. YORK

DEPARTMENT OF BOTANY,
BROWN UNIVERSITY

THE SOCIETY OF AMERICAN BACTERIOLOGISTS

SYSTEMATIC AND PHYSIOLOGIC BACTERIOLOGY

THE annual meeting of the society was held in New York City, December 31, 1912, and January 1 and 2, 1913, under the presidency of Dr. William H. Park. The sessions were held at the American Museum of Natural History, the University and Bellevue Hospital Medical College and the Rockefeller Institute. The society expressed its indebtedness to these institutions for their courtesy. The annual dinner was held on Wednesday evening, January 1, 1913, at which the president's address was delivered. Dr. Park spoke upon "The Applications of Bacteriology in the Activities of a City."

With this as his text Dr. Park traced the history of the Research Laboratories of the Board of Health of New York City, an institution which easily takes rank with the Pasteur Institute of Paris and other institutions of the kind in Europe. In the original work which has been done under Dr. Park's direction no other American laboratory engaged in public health work can point to so many achievements which have resulted in advancing our knowledge of infectious diseases and methods for controlling them.

The following officers were elected for a term of one year:

President—C.-E. A. Winslow.

Vice-president—Charles E. Marshall.

Secretary-treasurer—A. Parker Hitchens.

Council—W. J. MacNeal, L. F. Rettger, D. H. Bergey, H. A. Harding.

Delegate to Council of A. A. A. S.—S. E. Prescott.

The following papers were read:

The Value of Glycerinated Potato as a Culture Medium: M. R. SMIRNOW, M.D., New Haven, Conn., instructor in bacteriology and pathology, Yale Medical School.

The glycerinated potato culture medium belongs to the class of the so-called media, which as the term implies, are media of various compositions and are used only for special purposes. They may be employed as follows: (1) for the purpose of isolating microorganisms; (2) to furnish a sufficiently favorable medium for the growth of certain organisms; (3) for specific or differentiating tests; (4) to bring out special features of growth. Aside from blood media, the most frequently used of the special media are the glycerinated potato and agar, but even these are practically limited to the cultivation and the study of acid fasts. It has long been the opinion of the writer that if some of our so-called special media were put to a more general use, hitherto unknown biological features in the study of microorganisms would come to light. This was emphasized by the finding of a marked contrasting culture on glycerinated potato of a glanders bacillus, which was being isolated at our laboratories during the last year. This organism was isolated from a human case of glanders. When first obtained it gave but a faint yellow growth on plain potato, by no means the so-called honey-like growth. It was then planted on glycerine potato with more success. On this medium it gave a luxuriant growth of a bright yellow color and typically honey-like in character. It was this peculiar and striking difference in the growth of the glanders bacillus that led up to the work here outlined. The cultural characters of twenty-five microorganisms were studied on glycerine potato, plain potato and broth potato, the

[1] Blakeslee, A. F., "A Possible Means of Identifying the Sex of (+) and (—) Races in the *Mucors*," SCIENCE, N. S., 37: 880–881, 1913.

two latter media being used as controls. The media were freshly made as needed. A number of potatoes were cut into cylinders, washed in running water for about an hour and then allowed to remain in a basin of water over night. One third of the lot was placed into 6 per cent. glycerine broth, and one third into plain broth for about two hours, and the remainder was left in the water. The three batches of potatoes were then tubed and glycerine broth and plain broth were poured into the tubes up to the level of the glycerinated and broth potato, respectively. The plain potato had no fluid added to it. The media were then sterilized in the autoclave at 18 lbs. pressure for 15 minutes, and then stored until used in cold place to prevent drying. The plants were all made of the same stock cultures, at the same time incubated both at 22° and 37.5° C. Each test was carried out a number of times, to assure constant results. The results obtained will for convenience be divided into three groups. The first comprising bacteria that show a striking contrast in their cultures on the different potato media; the second showing a slight difference, and the third those showing no difference. The first group includes the following microorganisms:

Two different strains of *B. mallei* of human source.
One strain of *B. mallei* received from New York.
Two strains of *Actinomyces* received from Washington.
B. pyocyaneus, old stock culture.
B. subtilis, old stock culture.
An unidentified spore-forming bacillus, isolated from the intestinal tract of a rabbit and designated as "B. rabbit spore."

To sum up this group: The three strains of the glanders bacillus give lighter colored, moister and more typical honey-like growths on glycerine potato. Their growth on plain potato is more brownish yellow in color and the potato is usually discolored. A metallic luster was noted on broth potato on several occasions with each strain. The *Actinomycetes* give a dry culture made up of isolated colonies, raised and of decided brown color on plain potato, whereas on glycerinated potato they give rise to a luxuriant growth of more conglomerated colonies of a honeycomb-like arrangement and of a light-yellow color. The *B. pyocyaneus* gives a brighter and deeper green pigment on the glycerine medium and a brown or green-brown slimy growth on ordinary potato. The *B. prodigiosus* gives a bright cherry-colored growth on glycerine potato and agar, at 22° C., a slight red or orange on broth potato and a faint pink on ordinary potato. It gives hardly any color at 37.5° C., on any of the media. The *B. subtilis* apparently grows better on ordinary potato, producing a heavy furred culture of brown color. On the glycerine potato it gives a rather delicate lightly furred growth of a light-yellow color. In the second group are included:

B. coli, stock culture.
B. mucosus, stock culture.
Sp. cholera, stock culture.
An unidentified organism isolated from rabbit feces, here designated as "B. rabbit feces."
An unidentified pleomorphic bacillus isolated from a contaminated plate.
A mould.

In general it may be stated that these organisms do not show striking differences in their growths upon the three varieties of potato. Glycerinated potato permits as a rule a much lighter colored growth, less raised, and often more homogeneous in character. In the third group are included the diphtheria, typhoid, dysentery and grass bacillus, the *Streptococcus pyogenes, Staphylococcus aureus* (two strains), *Sarcina aurantia* and a yeast. There are no visible differences in the cultural characters of each of these organisms on the potato media under consideration. The chromogenic organisms (*Sarcina aureus,* yeast) seemed to give brighter and more intense pigment production and at times somewhat more luxuriant growths on the glycerinated potato. In conclusion the writer desires to bring to your attention that with the particular strains used glycerinated potato affords a more favorable medium for most of the twenty-five microorganisms tested. It allows more moist, more homogeneous, less raised growth characteristics, of lighter or brighter color. With the *B. prodigiosus* the color was greatly intensified. Glycerine potato is seldom discolored, whereas both broth and plain potato are frequently discolored, particularly the latter. From the above we may conclude that a more general use of glycerinated potato suggests itself.

The Preservation of Stock Cultures: A. Parker Hitchens, Glenolden, Pa.

For the preparation of stock bacterial vaccines it is necessary to have constantly on hand a large number of cultures of the various pathogenic bacteria. For the preparation of vaccines for the treatment of the various regional mixed infections, it is deemed necessary to collect the various species and types in each region. To keep fresh stock of any culture frequent transplantation is necessary. As the intervals between transplants vary

considerably with the different cultures, a card-catalogue system has been devised. Under this system each culture is represented by a card, upon which are recorded all the dates of transplantation as the fresh cultures are made. The cards are kept in a file, with each card placed under the date at which the next transplant of its culture is necessary. The work upon the cultures each day is carried out in accordance with the cards filed under that date. During the intervals between transplants, all cultures except the *B. influenzæ*, gonococcus and meningococcus are kept cold. Five dates of each culture are kept and the tube of most recent date is unopened. In order to keep a large number of cultures constantly on ice, we have had a refrigerator constructed especially for this purpose. The refrigerator, well insulated, is about seven feet long, six feet high and two feet in depth. It is divided into six large compartments, three above and three below. The middle compartment of the upper row contains the ice, and it is always filled to its capacity, 500 pounds. This quantity of ice maintains a temperature of 10° to 15° C. throughout the entire refrigerator. The refrigerator is well drained and the open framework of the interior allows free circulation of air. Five compartments are devoted to the cultures. These compartments are provided with drawers, which slide in grooves and are easily removed. Each drawer is of such dimensions that two crates of cultures fit end to end within it. The total number of drawers is 63 and the total capacity 1,600 cultures. The front of each drawer is provided with a groove into which a card is fitted designating the contents of the drawer. With this refrigerator and our system of transplanting we are able to keep ready for immediate use a fresh supply of all the cultures necessary for the preparation of bacterial vaccines.

A Refinement of the Technic of Quantitative Bacteriological Analyses: W. D. FROST, Boston, Mass.

It is generally recognized that the measured quantities of water, used for dilution, lose in volume during sterilization and upon standing. The exact amount of this loss or the means of preventing it are apparently not generally understood. In an extended series of experiments it is found that the loss varies from 1 to 8.8 per cent. and that the average is 5.07 per cent. Various types of autoclaves are tested and it is found that there is considerable variation in the different types. The loss is evidently due to the ebullition and escape of steam, especially during cooling. This loss can be prevented by closing up the autoclave cold, as is frequently done in sterilizing blood serum. When closed in this way the autoclave is not always efficient in the time or at the pressure ordinarily used. In order to insure sterilization it will be necessary to extend the time, increase the pressure or sterilize on two consecutive days. The evaporation due to standing a few weeks is equal to the loss in the autoclave. This is not prevented by a thin paper cap. Paraffined paper is recommended, also cork stoppers covered by a thin layer of cotton instead of an ordinary cotton plug. In using the bottles after making the dilution it is suggested that the sterile side of this cap be forced into the mouth of the bottle with the cork. This permits efficient shaking.

The Significance of the Time at which Gas is produced in Lactose Peptone Bile: WILLIAM W. BROWNE, Ph.D., College of the City of New York.

During the summer of 1912 routine bacteriological examinations of oysters of Narragansett Bay were made under the direction of Professor F. P. Gorham, of Brown University, with the hope of determining the extent of the pollution of the oyster beds of Rhode Island by the sewage of the neighboring cities and towns. The examinations were made according to the methods proposed by the American Health Association. Lactose peptone bile was used as a presumptive test to indicate the presence of members of the *Bacillus coli* group and other lactose fermenters of intestinal origin. (1) Lactose peptone bile tubes inoculated with the shell liquor of oysters taken from 119 different beds produce the greater part of their gas by the end of the forty-eighth hour. (2) Lactose peptone bile tubes inoculated with the shell liquor of oysters taken from polluted areas produce almost all their gas by the end of the forty-eighth hour. (3) Lactose peptone bile tubes inoculated with the shell liquor of oysters taken from districts comparatively free from pollution produce the greater part of their gas by the end of the seventy-second hour. (4) Consideration of this temporal factor in the production of gas in lactose peptone bile might aid in the determination of whether the pollution was recent or remote.

A Comparative Study of the Smith Fermentation Tube and the Inverted Vial for the Determination of Sugar Fermentation: WILLIAM W. BROWNE, Ph.D.

During the sanitary survey of Narragansett Bay conducted under the direction of Professor F. P. Gorham, of Brown University, a comparison was made of the efficiency of the Smith fermentation tube and the inverted vial as used in the presumptive test with lactose peptone bile to indicate the presence of members of the *Bacillus coli* group and other lactose fermenters of intestinal origin. Fermentation tubes and inverted vials containing lactose peptone bile were inoculated with the shell liquor of oysters taken from polluted areas and the following results were obtained:

Percentage of Efficiency

	Cubic Centimeter	
	24 hrs.	48 hrs.
Fermentation tube	84.6%	94.3%
Inverted vial	92.3%	96.1%
	One Tenth Cubic Centimeter	
	24 hrs.	48 hrs.
Fermentation tube	86.5%	90.3%
Inverted vial	59.5%	84.6%
	One Hundredth Cubic Centimeter	
	24 hrs.	48 hrs.
Fermentation tube	32.6%	55.7%
Inverted vial	23.0%	42.3%

Resistance of Microorganisms Suspended in Glycerine or Oil to the Sterilizing Action of Heat: C. J. Bartlett and F. B. Kinne.

Dreyer and Walker have recently reported the results of heating spores suspended in glycerine and oil. They show that spores in glycerine were not killed with certainty after heating two hours at 1½ atmospheres, 2 hours at 1¼ atmospheres or one half hour at 2 atmospheres. In our experiments we have worked with the *Staphylococcus aureus,* with the *Bacillus anthracis* and with the *Bacillus subtilis,* and with a bacillus with very resistant spores, apparently the *Bacillus vitalis.* These have been heated in glycerine, water, olive oil, cottonseed oil and paraffin for different periods at the temperature of boiling water and in the autoclave at 7½ lbs. pressure and at 15 lbs. pressure. The *Staphylococcus aureus* is quickly killed in all of these, even at the temperature of boiling water. The spores of the anthrax bacillus and of *B. subtilis* are quickly killed in boiling water, usually in three minutes or less. In glycerine they have been found alive after one and one fourth hour at this temperature and in oil after fifty minutes, and in the autoclave after heating in oil fifteen minutes and in glycerine in ten minutes at 7½ lbs. In water they do not live after five minutes at this pressure. The spores of the *B. vitalis* are killed in about one half of these tests by heating in boiling water for two hours, while in oil and glycerine they resisted this temperature for two hours in every instance. After heating in the autoclave they were found alive in oil at 15 lbs. for two hours and in glycerine after one and a half hour, but not longer. In water they were never found after twenty minutes at 7½ lbs. and after ten minutes at 15 lbs. It is evident that spores are more resistant to the action of hot oil and glycerine than to that of hot water.

The Comparative Viability of Pneumococci on Solid and on Fluid Culture Media: L. J. Gillespie, Hospital of the Rockefeller Institute for Medical Research.

The following facts have been observed: (1) Broth which is perfectly suited for the growth of copious cultures of the pneumococcus often requires many more organisms (frequently a million times as many) to initiate growth than does agar. (2) Cultures which when fresh from the animal body show a marked effect become on cultivation upon artificial media indifferent in their requirements. (3) Certain cultures (of any strain) show no effect even when fresh from the body. (4) Differences in chemical composition of broth and of agar play little or no part because an imitation "solid" medium, prepared from filter paper and broth, serves nearly as well as agar. (5) The possibility that insufficient aeration in the case of broth plays any rôle is ruled out by comparing agar plates with agar shake cultures. These phenomena may be explained if we suppose that the pneumococcus sometimes requires for its multiplication that substances from the animal body be present in the immediate environment of the cocci, the concentration of which can be too far reduced in the case of broth by diffusion aided by convection. If we suppose rather that the necessary substances are produced by the pneumococci themselves we may assume that such substances are always necessary, and that during acclimatization to artificial media the capacity for such metabolism is increased.

Studies of the Subtilis Group: Karl F. Kellerman and Edna H. Fawcett, Bureau of Plant Industry, Washington, D. C.

The *Subtilis* group includes the spore-forming aerobic and facultative anaerobic bacteria which liquefy gelatine. Numerous cultures of members of this group have been obtained from various sources. Biometrical study of their acid produc-

tion, ammonia production, and the reduction of nitrates, together with careful comparison of their morphology, has shown the necessity for allowing greater range in the description of *Bacillus subtilis, B. cereus, B. mycoides* and *B. megatherium.* No decision has as yet been reached regarding the validity of *B. asterosporus* or *B. ruminatus.* For the first four species named the following synonymy is submitted:

B. subtilis

B. subtilis Cohn (Emend) 1876, (Flugge) 1886, (Zopf) 1883.
mesentericus vulgatus Flugge, 1886.
mesentericus fuscus Flugge, 1886.
liodermus Flugge, 1886.
aerophilus Flugge, 1886.
lævis Frankland, 1887.
mesentericus fuscus Trevisan, 1889.
mucosus Zimmermann, 1894.
destructans Wright, 1895.
mesentericus ruber Globig (Flugge), 1896.
leptosporus Klein, 1900.
sessilis Klein, 1900.
pumilis Gottheil, 1901.
simplex Gottheil, 1901.
mesentericus Chester, 1903.
malariæ Klebs. (Original not consulted.)

B. cereus

B. cereus Frankland, 1887.
ulna Cohn, 1875. (Incomplete description.)
ramosus liquefaciens Flugge, 1886.
subtilis Frankland, 1887.
subtilis Sternberg, 1890.
subtilis Eisenberg, 1891.
petroselina Burchard, 1892.
cursor Burchard, 1892.
loxosus Burchard, 1892.
goniosporus Burchard, 1892.
turgescens Burchard, 1892.
limosus Russel, 1894.
capillaceus Wright, 1895.
crinitum Wright, 1895.
subtilis Wright, 1895.
subtilis Lehman and Neumann, 1896.
ellenbachensis Stutzer and Hartleb, 1898.
fusiformis Gottheil, 1901.
stoloniferus Pohl, 1903.
lutulentus Kern. (Original not consulted.)

B. mycoides

B. mycoides Flugge, 1886.
figurans Crookshank, 1886.
bassicæ Pommer, 1886.
bacterium casei Adametz, 1889.
ramosus Frankland, 1889.
radicosus Eisenberg, 1891.
implexus Zimmermann, 1890.
intricatus Russel, 1892.

B. megatherium

B. megatherium De Bary, 1884.
tumescens Zopf, 1885.
lacteus Lembke, 1897.
petasites Gottheil, 1901.
graveolens Gottheil, 1901.
granulosus Russel, 1892.

Parasites found on Rats in Providence: GEORGE H. ROBINSON, Brown University.

The examination of the rats of Providence for evidence of plague and for the occurrence of parasites has extended over a period of six months, from July to December. During this time 342 rats from different parts of the city were inspected. No evidence of plague was found. The specimens were evenly divided as to sex. As to species there were 333 specimens of *Mus norvegicus,* 2 of *Mus alexandrinus,* 1 of *Mus rattus,* 4 which showed evidences of being a cross between *Mus norvegicus* and *Mus alexandrinus,* 1 apparently a cross between *Mus norvegicus* and *Mus rattus,* and 1 *Mus musculus.* Of these 342 rats, 57 per cent. were infected with fleas, 21 per cent. with mites (*Lælaps echidninus*) and 24 per cent. with lice (*Polyplax spinulosus*). 2,053 fleas were found, consisting of 75 per cent. *Xenopsylla cheopis* Rothschild, 22 per cent. *Ceratophyllus fasciatus* Bosc, 2.5 per cent. *Ctenopsylla musculi* Duges and 0.5 per cent. *Ctenocephalus canis* Curtis. No evidence of a regional distribution of the fleas was observed. A marked seasonal variation was noted, the average flea per rat for July–September being 10.2, while that for October–December was 3.7. The largest number of fleas taken from a single specimen was 300. No relation was found between a filthy habitat and the number of fleas, for the average flea per rat was higher, in general, for the rats from dwelling houses and restaurants than for those from stables and docks. 12 per cent. of the specimens were affected with sores. Parasites, the encysted form of the cat tapeworm, *Tænia crassicollis,* and the ova of some undetermined form were found in the liver of 7 per cent. of the rats. This condition occurred most frequently in the rats obtained from markets.

Comparison of Two Methods for Bacterial Analysis of Air: G. L. RUEHLE and H. A. HARDING.

Report of progress in the comparison of the Rettger method with the official sand filtration method. An exact comparison was found to be difficult to obtain and the relative value hard to estimate. Study to be continued.

A Biometric Study of the Streptococci from Milk and from the Human Throat: E. C. STOWELL, C. M. HILLARD, M. J. SCHLESINGER.

Two hundred and forty pure strains of strepto-

cocci isolated from milk and from the human throat have been compared as to their morphology, Gram stain and gentian violet reaction by the plate method, and their quantitative acid production in seven carbohydrates and related organic media. Hemolysis was studied with 92 strains. We have been able to make no correlation between the length of chain and the relation to violet stain with any other character. Seventeen out of 92 cultures gave hemolysis when streaked on blood agar plates. Five of these cultures came from normal milk, five—the most vigorous hemolizers—were from milk where udder trouble was indicated in the cow, and seven were normal throat forms. The seven substances tested showed a definite order of availability for the acid production. This order ("metabolic gradient") and the per cent. of culture yielding 1.2 per cent. or more of acid when grown at 37° C. for three days is shown in the following table:

	Per Cent.
Glucose (monosaccharide)	98.0
Lactose (disaccharide)	76.0
Saccharose (disaccharide)	65.5
Salicin (glucoside)	42.7
Raffinose (trisaccharide)	37.5
Inulin (starch)	9.0
Mannite (hexahydria alcohol)	1.5

It will be noted that the degree of availability is closely associated with the size and complexity of the substance. According to the positive reaction —over 1.2 per cent. acid—in the test substances 88 per cent. of the cultures may be placed in eight groups. The following features separate milk from throat streptococci: (1) milk organisms yield over 2.5 per cent. acid in lactose and saccharose at 37° C.; (2) they seldom ferment substances higher in the metabolic series than saccharose; (3) they readily ferment dextrose, lactose and saccharose at 20° C. On the other hand, throat streptococci (1) seldom yield over 2.5 per cent. acid in any substance; (2) over 40 per cent. of the cultures yield over 1.2 per cent. acid in either salicin or raffinose; (3) at 20° C. they almost never attack any of the seven test substances.

A Systematic Study of the Coccaceæ in the American Museum of Natural History Collection: I. J. Kligler, Department of Public Health, American Museum of Natural History.

A biometric study of 54 strains of cocci in the museum collection was made in order to test the classification proposed by the Winslows in their book on the "Systematic Relationship of the Coccaceæ." Twelve morphological and physiological tests were applied and the results recorded quantitatively whenever possible. The results corroborate the work done by the Winslows. The cocci—other than streptococci—group themselves into five distinct classes according to the pigment produced as follows: (*a*) *White pigment—Albococcus;* (*b*) *orange pigment—Aurococcus;* (*c*) *yellow pigment—Micrococcus;* (*d*) *yellow pigment and packets—Sarcina;* (*e*) *red pigment—Rhodococcus.* The other properties correlate remarkably with that of pigment production and prove that this generic division is a fundamental one. The definition of species is also based on real differences. The species recognized by Winslow were found to be valid, but the number was incomplete. Three new species were recognized (*Alb. urea, M. melitensis* and *S. aurantiaca*) and the possible existence of a few others suggested. Further study is necessary. The application of the principles of biometry to the systematic study of the Coccaceæ has yielded very successful results. It is hoped that new workers will apply this principle to the systematic study of this and other groups of bacteria.

Bacteriological Collection and Bureau for the Distribution of Bacterial Cultures at the American Museum of Natural History, New York: C.-E. A. Winslow.

In January, 1911, a prospectus, from which the following sentences are quoted, was sent out from the American Museum to the leading laboratories of the country. "The Department of Public Health at the American Museum of Natural History has equipped a laboratory to serve as a central bureau for the preservation and distribution of bacterial cultures of both pathogenic and non-pathogenic organisms, and particularly of types of new forms and varieties. It is hoped that the laboratories of medical schools, colleges, boards of health, agricultural experiment stations, etc., and those engaged in biochemical work of all sorts, will furnish the museum with cultures at present in their possession, and the laboratory is now ready to receive and care for any such cultures. Types of new species and varieties are particularly desired at the present time and as they may be isolated in the future. The laboratory, of course, can not undertake to keep on hand bacteria difficult of cultivation, such as can be maintained only for a few weeks after isolation from the body; neither can it at present supply virulent cultures which rapidly lose their virulence under laboratory conditions. It should, however, be able to furnish

cultures of organisms of all the ordinary types which can be maintained under cultivation. Pathogenic forms will be sent only to properly qualified persons.'' The value of the proposed collection was quickly appreciated. Cultures from all over the United States and Canada have been contributed freely. In all, 45 different laboratories have sent in cultures, and arrangements have been made for exchange with Professor Kraus, of Vienna, who now has charge of the famous Kral collection. On December 1, 1912, the collection included 578 strains representing 374 different named types, and in the list, which has been printed and may be obtained on application, are most of the important pathogenic and non-pathogenic species which have been definitely described.

During the period of somewhat less than two years, from January 1, 1911, to December 1, 1912, the laboratory distributed to 122 different colleges and research laboratories of the United States and Canada 1,700 different cultures, in every case without charge. It is the policy of the department to send cultures free to all teaching laboratories of college and university grade, and to all research laboratories, whether cultures are sent to us in return or not. Many cultures have been called for by teaching laboratories for use in their class work. The most important service the laboratory has been able to render, however, has been in furnishing authentic cultures to investigators who have been making a study of certain special groups, and the published papers which have resulted, in which various detailed characters of the museum types are described, of course greatly increase the value of the collection.

DAIRY BACTERIOLOGY

Transportation of Milk: M. C. SCHROEDER, M.D., assistant director, Research Laboratory, Department of Health, City of New York.

The problem of the transportation of milk is influenced chiefly by the necessity of subjecting it to the long or the short haul. Most of the smaller cities and towns receive milk from a distance of ten miles, so that milk is transported in wagons only and is delivered to the customer quite fresh. Here, icing during the warm, and protection during the cold, together with frequent inspection of the delivery wagons, and the taking of samples for bacterial tests solve the problem fairly well. New York receives about 30,000 quarts a day from about 145 such outlying farms. The greater bulk of the milk, about 1,800,000 quarts, is brought from distances of 50 to 300 miles. This milk is first drawn to the receiving station, mixed in tanks, simply aerated, or pasteurized and cooled, bottled and canned, and shipped in refrigerator cars holding 272 to 375 40-quart cans, or from 450 to 700 boxes of 12 quarts each. The most important question in the long haul is the refrigeration. Two methods of icing have been utilized. Direct (crushed ice being placed upon cans and bottles), second ''indirect'' (the ice being placed in boxes called bunkers at the end of the car). The bunkers are found to be too small for the ice necessary to keep the milk cold if the weather is hot or the journey long. Milk comes over 15 railroads and enters New York through eight terminals. Trains start in the country from 7 A.M. on, and arrive at the terminal from 9 P.M. to 2 A.M. if not delayed. At the terminal it is loaded in large trucks and is drawn one or more miles to pasteurizing or distributing centers. Here it is handled and sorted and finally loaded into smaller wagons for delivery. The milk supply of New York is safeguarded from bacterial contamination as follows: by annual sanitary inspection of the farms on which the milk is produced, and the more frequent reports of the farmers delivering the milk as to the conditions existing of production and care, by inspecting the icing of the milk and the conditions of the cans and bottles being shipped back to the creamery; by inspecting the conditions under which it is sold; it also seeks to detect the condition of production, transportation and sale by taking bacteriological samples of milk from creameries, at the railroad terminals, from wagons, pasteurizing plants, hospitals, stores, etc. Thus last year the number of samples taken and analyzed was 61,142. For the control of the milk supply, the Department of Health has only 24 inspectors for about 44,000 farms, 30 inspectors for the five boroughs and 4 inspectors taking bacteriological samples for both city and country.

Problems in Sanitary Milk Classification, with special reference to the Experience in New York City: ERNST J. LEDERLE, Ph.D., Commissioner of Health, City of New York.

In contradistinction to most other large municipalities, New York City undertakes practically the entire supervision of its milk supply from the cow to the consumer, notwithstanding that nearly all the 45,000 farms on which this milk supply is produced are located outside the city, and more than 6,000 of them outside the state. The milk

supply may become a source of danger to the public health by being infected with the germ of bovine tuberculosis, the germs of typhoid fever, scarlet fever, diphtheria and tonsillitis, by having in general an excessive bacterial growth and by not having a proper nutritive value. In view of these sources of danger, the means to be employed to make public milk supplies safe are as follows: (1) the prevention of adulteration; (2) the production of a clean milk of low bacterial count. This involves cleanliness of the cows and milkers, clean barns, clean vessels, the exclusion of dust, immediate reduction of temperature after milking, icing during transportation, the sale in sanitary stores; (3) the production of milk free from pathogenic organisms, involving the prevention of the introduction of infectious disease through human agencies, flies and dust. The general milk supply of every large city is unfit for use in infant feeding, and as the attempt to bring the general market milk to the degree of purity required for infant feeding can never be successful, the only way in which sanitary authorities can meet existing conditions is by requiring the pasteurization of all milk which is not of special grades. The official classification of milk in New York City is as follows:

Grade A:
1. Certified.
   Guaranteed.
2. Inspected milk (raw).
3. Selected milk (pasteurized).

Grade B:
1. Selected milk (raw).
2. Pasteurized milk.

Grade C:
For cooking.

The following changes are under consideration: (1) the elimination of Grade *B* (raw) entirely, and requiring it to be pasteurized; (2) the elimination entirely of Grade *C* from the retail trade; (3) an increase in the requirements for milk intended for pasteurization.

Problems in Sanitary Dairy Inspection: H. A. Harding.

Milk resembles the human race in that its value is determined by two forces, its inheritance and its environment. Inheritance fixes the amount of solids which is normal to the milk. The other elements of its food value are determined by the environment under which it is produced and handled. The problem in sanitary dairy inspection is to provide an inspection which affects the selling price of the milk. This can probably be best accomplished by establishing market grades of milk and by defining these grades in terms of the conditions surrounding the production and transportation of the milk. The value of this financial element in sanitary milk inspection is well illustrated by the Geneva milk supply. In October, 1907, all of the milk coming to this city was graded on the basis of the conditions under which it was produced. It was found that the conditions of the production of 37.5 per cent. were poor, 57.5 per cent. were medium and 5 per cent. were good. The conditions then changed so that the producers were paid on a sliding scale, making it more profitable to produce the better grades of milk. In March, 1911, the milk supply of the city graded on the same basis as above was 87.5 per cent. good and 12.8 per cent. excellent. Conditions again changed so that there was no longer this direct connection between the conditions surrounding production and the price received, and in October, 1912, the city supply on the same basis as the above was 81.5 per cent. medium, 15.7 per cent. good and 2.6 per cent. excellent. Farmers have a better financial sense than is generally supposed and sanitary milk will not be produced on a large scale until its production becomes financially more profitable than that of the dirtier grades. Details are given in *Bulletin of New York Agricultural Experiment Station.*

Notes on Yeast-like Organisms in Whey: S. F. Edwards, Bacteriological Laboratory, Ontario Agricultural College.

During the summer of 1909 some work was begun on the problem of so-called fruity flavor or sweet flavor in cheese in western Ontario. The trouble was supposedly due to yeasts or yeast-like organisms. Samples of whey were secured from twenty-five factories where this flavor was prevalent, and from these samples twelve varieties of yeast-like organisms were isolated. Some of the yeasts (so-called) were found in the whey from more than one factory, and some factories had several varieties in the whey. Three lots of experimental cheese were made up, using a starter of these organisms, and the flavors typical of different factories were produced, whereas no off-flavor was present in normal control cheese. These organisms have been retained in the laboratory and further study has been made as the time permitted. The term yeast is a misnomer, for with but one exception we have been unable to demonstrate spore production. Very little attention has been given to morphology, sole dependence for dif-

ferentiating the varieties having been placed on cultural and biological characters. A summary of these characters is given in the subjoined table.

The Cultural Characters of Whey Yeasts

+ indicates positive results. Blanks indicate no action.
For convenience the organisms are designated by letters.

	Ferments[1]				Produces Acid in						Reducing Sugars from									
	Dextrose	Saccharose	Lactose	Maltose	Dextrose	Saccharose	Lactose	Maltose	Raffinose	Mannite	Saccharose	Raffinose	Starch	No Reduction in Mannite	Peptonizes Milk	Liquefies Wort Gel[2]	Indol in Dunham, 20 Days	Indol in Uschinsky + Peptone	Ammonia in Nitrate Broth	Alkali in Litmus Milk
*A*2					+								+		+	+		+		
*A*5	+		+			+	+				+	+			+	+	+	+		
*B*2	+	+			+	+			+		+	+			+	+	+	+	+	+
*B*4	+	+			+	+			+	+	+	+			+	+	+	+	+	
*D*1															+		+	+	+	+
*J*1	+	+	+		+	+	+					+				+	+	+	+	
*J*2	+					+		+			+				+	+	+	+	+	+
*K*1	+	+		+		+		+	+			+			+	+		+	+	
*K*3	+	+			+	+	+				+	+						+		+
*O*1	+				+		+		+		+	+				+		+		+
*P*1	+	+	+		+	+	+					+			+	+		+		
*X*2											+	+				+			+	
Control																				

All of the organisms made scanty growth in Cohn. Yeasts *D*1, *J*2, *P*1, *X*2 made scanty growth in Uschinsky; the others none. Organisms *A*2, *J*1, *P*1 produced a marked pineapple flavor in wort agar plates, and *A*5 and *J*2, marked strawberry flavor in the same medium. Further work with these organisms is planned.

The Action of Bacillus Bulgaricus in Suppressing Gassy Fermentations in Cheese-making: C. F. DOANE, Dairy Division, U. S. Department of Agriculture.

It was found that pure cultures of *bulgaricus* could be used with perfect results in suppressing the undesirable fermentations, principally gas, which have worried Swiss cheesemakers in the past. There seems to be a difference in the efficiency of different strains of *bulgaricus* for this purpose without respect to their activity in forming acid. One per cent. of a whey starter made from one culture was sufficient, while it requires three per cent. of another. The *bulgaricus* starters could not be seen to have any effect on the formation of the eyes or interfere with the flavor or texture. It is believed that the proper use of *bulgaricus* starters will go far towards making a more uniform cheese during the summer months and will make it possible to produce good Swiss cheese during the entire year.

[1] Does not ferment raffinose, glycerine, mannite, inulin, starch.

[2] Liquefaction was slow, in some cases occurring only after a number of months.

The Preparation of Dried Cultures: L. A. ROGERS, Dairy Division, U. S. Department of Agriculture.

The method of Shackell, consisting essentially in holding the frozen material over sulphuric acid in a high vacuum, is adapted for drying cultures of the lactic acid bacteria, *B. bulgaricus* and other organisms. A chamber was devised in which considerable quantities of powder could be made. The best results are obtained by drying cultures grown on milk concentrated to one half its original volume. Fresh lactic cultures dried by this method curdle milk in twenty hours at 30° when one part of powder is added to 1,000,000 parts of milk. Dried cultures of *B. bulgaricus* curdle milk in twenty hours at 37° when added to the milk in the ratio of 1: 100,000. The activity of a dried culture diminishes more or less rapidly, depending on the conditions under which it is held. The deterioration is less rapid if the moisture content is very low; it is less rapid as the temperature of storage is diminished and is much more rapid in air or oxygen than in an inert gas or in a vacuum.

The Normal Bacteria of Swiss Cheese: E. E. ELDRIDGE and L. A. ROGERS, Dairy Division, U. S. Department of Agriculture.

Special media were devised which gave high

counts comparing in a general way with those obtained by dilution in milk. Numerous examinations were made of various cheeses and three domestic cheeses of the Emmenthal type were followed through a nearly complete ripening period. About 1,000 cultures isolated from these cheeses were studied in detail, particularly in relation to their fermentative abilities. It was observed that many of these cultures gave considerable quantities of gas in a sugar-free concentrated whey. It was not possible, however, to separate these cultures beyond three morphological groups, one of which was a long rod, one a short rod and the third a coccus. At the beginning of the ripening the bacterial flora consisted almost entirely of the short rods. The long rods appeared in the early stages of the ripening and increased steadily. The short rods decreased and in each of the three cheeses made up about 50 per cent. of the bacteria at seven or eight weeks, a period corresponding in a general way with the end of the eye formation. Glycerine fermenting cocci appeared in small numbers in each of the cheeses at an age of five or six weeks. At the end of twenty weeks the bacterial flora was composed almost exclusively of the long rods. The essential bacteria of Emmenthal cheese are evidently not ubiquitous. In two widely separated localities cheeses made without inoculation have invariably failed to give the normal fermentation. Cheese made from milk inoculated with a mixture of a large number of pure cultures, or from special culture media inoculated with good cheese, have given uniformly a normal ripening.

Action of a Few Common Butter Organisms upon Casein: Charles W. Brown, Michigan Agricultural College, East Lansing, Mich.

The action of microorganisms upon proteins is looked upon as an aid in identification. If there is an action visible to the sense of sight, liquefaction by that organism is said to be positive, otherwise it is negative. For example, if an organism growing in milk at room temperature for fifteen to thirty days shows no visible digestion, that organism is said to have no action upon casein. This is a mere supposition and in many cases is incorrect. For milk in which such an organism has been growing for several days, if treated with precipitants to remove the unchanged casein, will be found to contain degradation products such as caseoses and peptones. Especially is this true in old milk cultures where the cells of the organisms have died and undergone autolysis, thus liberating an endo-proteolytic enzyme. The power to liquefy casein by liquefiers is either stimulated or retarded to a greater or less degree by four important factors met with in storage butter—addition of salt, diminished supply of free oxygen, low temperature and association with *Bact. lactis acidi.* Now, if we center our observation upon a number of bacteria, found frequently in samples of storage butter, which have no visible action—other than a slight change—upon milk in tubes, within thirty days and make litmus milk agar plates thickly seeded with the organism under observation, we will observe several different pictures presenting themselves. (1) Some of the organisms produce a gradual clearing, noticeable after seven to fifteen days, due to a slow digestion of the casein. (2) If after incubating twenty-four hours at 20° C. the plates are inoculated with *Bact. lactis acidi* by making a stroke on the surface, we see in the case of some of the organisms a rather abundant growth of the lactic with acid production, curdling of the milk in immediate vicinity of the lactic, surrounded by a clear zone and, surrounding the clear zone, a more copious growth of the organism. (3) The same picture with the exception that the growth of the organism is not stimulated. (4) Growth of the lactic about normal, no acidity, the milk in the immediate vicinity of the lactic completely dissolved and surrounded by a more copious growth of the organism. (5) The same except no stimulated growth of the organism. (6) Growth of lactic normal, no acidity, no clearing, but a stimulated growth of the organism. (7) The same except the growth of the organism is not stimulated. (8) Retarded or prevented growth of lactic, no acidity, no digestion and no stimulated growth. A different picture may present itself, if the litmus milk agar plate of the organism is incubated for three to five days before stroking the surface with the lactic, in that the growth of the lactic may be inhibited and that no digestion may occur. Again, if the supply of free oxygen is diminished both before and after stroking the surface with lactic, or if salt is added, or if a lower temperature is used for incubation, different results will be obtained. These organisms, generally spoken of as non-liquefiers, influenced in their action upon casein by different factors can not be overlooked as agents in the degradation of casein in both storage butter and ripening cheese.

A. Parker Hitchens,
Secretary

(*To be continued*)