

stage at which barred chicks usually exhibit distinct barring in their first feathers. Such barring is absent in these F_2 chicks.

**THE TENTH ANNUAL MEETING OF THE
SOCIETY OF AMERICAN BACTERIOLOGISTS
HELD AT BALTIMORE, MD.,
DECEMBER 29-31, 1908**

THE tenth annual meeting of the Society of American Bacteriologists was held in the rooms of the laboratories of pathology and of physiology of the Johns Hopkins University and Hospital, Baltimore, Md., on December 29, 30 and 31, 1908.

Professor H. L. Russell, of the University of Wisconsin, president of the society, occupied the chair.

The scientific program consisted of thirty-two papers, all of which aroused much interest; certain of them are reproduced in abstract below. The society also met in joint session with Section K of the American Association for the Advancement of Science on December 30, when a paper on "Anaphylaxis" was read by one of its members, Dr. M. J. Rosenau, of Washington, D. C.

About fifty-two persons were in daily attendance at the several sessions of the society.

During the sessions the following matters of business were transacted: Dr. William H. Welch, of Baltimore, was reelected to represent the society on the council of the American Association for the Advancement of Science. To fill the vacancy on the Committee on Methods and Identification of Species, caused by the absence of Professor F. D. Chester, Professor C.-E. A. Winslow, of Boston, was duly elected. Professor Erwin F. Smith, of Washington, D. C., was delegated to represent the society at the approaching meetings of the International Botanical Congress at Brussels in 1910. The question of the society withdrawing its affiliation with the American Association for the Advancement of Science and transferring the same to the American Society of Naturalists and agreeing to meet with the latter body in the future was warmly discussed. It was decided to sever the present relations and join meetings with the naturalists should they decide to meet apart from the American Association for the Advancement of Science.

The following are the names of the officers of the society elected for the year 1909:

President—Dr. J. J. Kinyoun, Washington, D. C.

Vice-president—Dr. Veranus A. Moore, Ithaca, N. Y.

Secretary and Treasurer—Dr. N. MacL. Harris, Chicago, Ill.

Council—Dr. W. W. Ford, Baltimore, Md.; Dr. F. C. Harrison, Macdonald College, Quebec; Dr. H. W. Hill, Minneapolis, Minn.; Mr. Lore A. Rogers, Washington, D. C.

By the election of the following gentlemen, the limit to active membership in the society, as defined by the constitution, has now been reached, namely, 125:

Dr. Burdett L. Arms, assistant director of the bacteriological laboratory of the Board of Health, Boston, Mass.

Dr. John W. Connaway, professor of comparative medicine, and veterinarian in the College of Agriculture, the University of Missouri, Columbia, Mo.

Mr. George E. Gage, assistant in bacteriology, Yale University, New Haven, Conn.

Mr. Daniel D. Jackson, director of the laboratories, Department of Water Supply, Gas and Electricity, New York City.

Dr. Harry T. Marshall, professor of pathology and bacteriology, University of Virginia, Charlottesville, Va.

Dr. Otto Rahn, assistant professor of bacteriology and hygiene, Michigan Agricultural College, East Lansing, Mich.

Mr. James C. Temple, soil bacteriologist, Georgia Agricultural Experiment Station, Experiment, Ga.

ABSTRACTS OF CERTAIN PAPERS

Acid Fermentations of Milk: E. G. HASTINGS and B. W. HAMMER, University of Wisconsin, Madison, Wis.

In milk, butter and cheese are constantly found organisms identical in all important points with those supposed to be characteristic of certain fermented milks, especially the Bulgarian yoghurt. Production of 3-4 per cent. of acid in milk, growth at high temperature characterize the organism. The therapeutic value which has been ascribed by Metchnikoff and others to the fermented milks, such as yoghurt, is probably due to the composition of the milk, rather than to the presence of the peculiar organism. Since opportunity is constantly offered for the alimentary tract to become seeded with the organism, if it finds favorable conditions for growth in the alimentary tract, it should establish itself, no matter how slight the seeding may be, and we should find it in the feces constantly. Massive seedings can only temporarily establish the organism unless the environment is favorable.

Some Factors concerned in the Fixation of Nitrogen by Azotobacter: CONRAD HOFFMANN and B. W. HAMMER, Bacteriological Laboratories, University of Wisconsin, Madison, Wis.

The possibility of employing azotobacter for artificial soil inoculation, as well as the importance of its existence in soil as a nitrogen increaser, are factors which can not be ignored. With these as a basis, the work reported in the paper of the above title was performed. It appears that some fermentable carbohydrate is essential for nitrogen fixation; the same is true with reference to the presence of phosphorus and some base. The influence of (1) varying amounts and kinds of carbohydrates; (2) of varying kinds of phosphates (mono-, di and tri-forms), and (3) of the period of incubation, were all factors which were considered. The employment of the sand slope and the large Petrie dish cultures proved most efficient in securing the maximum development of azotobacter in pure culture.

Persistence of Anthrax Spores in Raw Water: E. G. HASTINGS, University of Wisconsin, Madison, Wis.

Anthrax spores have been found to persist for eight years in raw pond water. The longest period of persistence in raw water noted previously was five months; in sterile distilled water thirty months. The water was infected by tannery refuse and growth of the anthrax bacillus had taken place before or after the sample was taken from a stagnant pond. It would seem that the supposition expressed by many that the anthrax organism can grow in nature is true.

Synthetic Media for the Isolation of B. coli from Water: MAURICE L. DOLT, Brown University, Providence, R. I.

It was found possible to grow *B. coli* on one per cent. solutions of asparagin if 0.2 per cent. of sodium or ammonium phosphate is added. No other of the soluble inorganic salts seemed to serve.

Substances having an asymmetric carbon atom in their molecule and a CHOH group, such as glycerin, ammonium lactate, malic acid, can be substituted for the asparagin. These substances seem to favor the growth of the *B. coli* and to inhibit the growth of other water organisms. It is very likely the presence of this asymmetric carbon atom and the CHOH group in the cholic acid of bile which gives it these same properties.

Every red colony developing on litmus-lactose-agar made up with glycerin, ammonium lactate, or malic acid in addition to ammonium or sodium phosphate was a colony of *B. coli*. On the malic

acid plates only red colonies developed and they were all *B. coli*.

The use of these media in water analysis seems most promising. The presence of *B. coli* even in small numbers can be detected by plating large amounts of the water without preliminary enrichment.

The work is at present being extended to the detection and differentiation of *B. typhosus*.

The complete paper is in the *Journal of Infectious Diseases*, 5, 1908, p. 616.

A Synthetic Medium as a Substitute for Loeffler's Blood Serum in the Diagnosis of Diphtheria: F. P. GORHAM, Brown University, Providence, R. I.

The proteid-free medium which in the liquid form had been found by Hadley to be favorable for the growth and toxin production of *B. diphtheriae* was used in combination with agar as a substitute for Loeffler's blood serum. It was as follows:

Glycerin	3.40 parts.
Sodium chloride	0.60 "
Calcium chloride	0.08 "
Magnesium sulphate	0.32 "
Dipotassium phosphate	0.23 "
Ammonium lactate	0.75 "
Ferric phosphate	0.08 "
Glycocoll	0.10 "

Distilled water to make 100 parts, 1.5 per cent. agar, reaction made + 1 with sodium hydrate.

It was found possible to make a diagnosis from swabbings on this medium in less than fifteen hours.

The organisms were of about the same types as on the Loeffler's blood serum, though there was some indication that the granular types were more common on the synthetic medium than on the blood serum.

Note on Roup in Fowls: PHILIP B. HADLEY, Division of Biology, Rhode Island Agricultural Experiment Station.

The present report is based upon the examination of five fowls which died with the characteristic symptoms of "roup." The symptoms and macroscopical pathological appearances were as follows: Onset of disease like simple catarrh; watery exudate from nose and eyes; accumulation of thick mucus or soft, cheesy exudate in orbital sinus, naso-lachrymal canals and palatine space. Walls of mouth cavity and anterior esophagus whitened and necrotic, sometimes punctated with firm, yellowish-white nodules from 1 to 12 mm.

in diameter, and rising from 0.5 to 3 mm. from the surrounding surface; nodules also present in dorsal wall of crop. Esophagus inflamed from crop to proventriculus. Small intestines inflamed and walls thickened, containing mucus mixed with blood. Large intestines usually normal. Ceca inflamed once at necks and once at tips. Liver in two cases contained small white necrotic areas; bile-ducts inflamed and thickened. Kidneys enlarged in one case. Ureters invariably packed with urates, which also fill cloaca. Spleen enlarged in one instance.

The microscopical pathological conditions were studied in fresh preparations, smears and sections. The hard exudate from the orbital sinus was shown to be made up of mucus, coagulated serum and disintegrated cell substance. The soft cheesy matter and the mucus contained numerous epithelial cells, many of which contained coccidia in the schizont or macrogamete stage; these were also found in the free state. Similar bodies were also found in great numbers in the mucus from the naso-lachrymal canals, palatine space, walls of mouth cavity; also from walls of pharynx, anterior and posterior esophagus, dorsal wall of crop, proventriculus, duodenum, bile-ducts, liver, small intestine, large intestine, ceca, contents of cloaca and in the excrement of diseased birds. The encysted stage of the coccidium, which is identical with the organism of "blackhead," was found in the intestines and ceca, but not in the head region.

No bacteriological examinations were made, but it was apparent that the factor, coccidiosis, was sufficient alone to produce nearly all the pathological conditions observed. As "blackhead" is a coccidiosis of the ceca and liver of turkeys, and as "white diarrhea" is a coccidiosis of the ceca, intestines, duodenum and sometimes of the lungs, spleen and liver, of young chicks, so the writer believes that many, and perhaps all, cases of genuine "roup" are instances of coccidiosis of the head region, with or without intestinal complications.

White Diarrhea of Chicks: A Study in Avian Coccidiosis: PHILIP B. HADLEY, Division of Biology, Rhode Island Agricultural Experiment Station.

"White diarrhea" of chicks is a disease affecting the duodenum, small intestines, large intestines, ceca, liver, pancreas, kidneys, spleen and lungs, manifesting itself by inflammation and eventual necrosis of the epithelial, mucosal and submucosal tissues, and terminating fatally in

the majority of cases, primarily as the result of damage to the linings of the alimentary tract, and secondarily as the result of bacterial invasion of the tissues together with suspended constructive metabolism. The aim of the present paper is to show the relation of *Coccidium cuniculi* to this disease.

The macroscopical pathological appearances included the following: (1) occasional inflammation of esophagus and proventriculus; (2) inflammation and thickening of walls of duodenum and small intestines; (3) distention and thickening of walls of the cloaca and large intestine; (4) ceca usually inflamed, walls thickened, and containing a hard or soft yellow exudate or "core"; (5) liver usually contained necrotic areas after the thirteenth day of the epidemic; (6) pancreas occasionally greatly enlarged, and having hard cheesy texture; affection of pancreas, liver and duodenum often coincident; (7) lungs frequently congested, containing grayish nodules; (8) heart frequently contained lobular enlargements; (9) kidneys and ovaries enlarged in a few cases; (10) ureters invariably packed with urates (hence the white diarrhea); (11) yolk sac frequently not properly absorbed, yolk stock occasionally inflamed, walls thickened, containing hardened exudate.

These tissues were examined in fresh preparations, smears and sections, and the microscopical pathological appearances were as follows: (1) epithelial cells of the duodenum, intestines and ceca denuded; (2) in these cells, in the mucous cells and also free were many coccidia; (3) coccidia were also found in the large liver cells from necrotic areas, and free in the connective tissue matrix; (4) nodules from lungs revealed marked inflammation and necrosis; epithelium of bronchi and infundibula broken down, and in the cubical and ciliated cells were inclusions which resemble coccidia.

The bodies described above were the schizont or macrogamete stage of *Coccidium cuniculi*, which is also the cause of "blackhead" in turkeys and of some cases of so-called "roup." This stage of the *Coccidium* is probably identical with the *Amœba meleagridis* described by Theobald Smith in 1896. In the present epidemic no permanent cysts were found in birds under one month old. It is thus indicated that the original infection passes at once into the endogenous cycle of development which is maintained for some time before the exogenous cycle appears. The crisis of the

disease is coincident with the formation of the sexual products. The highest daily mortality was reached when the chicks were eleven days old. One month after the beginning of the epidemic 394 chicks had died out of 510. The *Bacterium septemicæ gallinarum* of Rettger was not found in connection with the present epidemic.

Some Experiences Relevant to the Determination of the Bacterial Content of Milk: CHARLES E. MARSHALL, Director, Michigan Agricultural College, East Lansing, Mich.

It is assumed from the observations in the (a) Association Studies conducted by the author and Miss Farrand, in the (b) Butter Studies conducted by W. S. Sayer, Otto Rahn and Miss Bell Farrand and in the (c) systematic work performed by Miss Zae Northrup, all in the bacteriological laboratory of the Michigan Agricultural College, that:

1. Milks vary in their bacterial content as to both (a) numbers and (b) species.

2. This variation is dependent upon the (a) source of the milk, (b) method of milking, (c) cleanliness in handling, (d) temperature maintained, (e) etc.

3. Milks in the light of the above can not develop their germ content (a) with the same rapidity or (b) in the same relative manner, and therefore can not respond *alike* to even ideal or perfect methods of testing.

4. Methods of testing are only incidental to the real problem; they should be employed to indicate and assist in control.

5. While composition of media, means of dilution, time of plating, temperature for plate development and other factors are highly important in the execution of these tests, the most essential factor at this time is uniformity of methods that the variable minor discordant elements may to a large extent counteract each other through the accumulation of evidence.

6. It follows that the purpose of the Committee on Standard Methods of Bacterial Milk Analysis is justified, even though many of its detailed decisions are arbitrary and perhaps unwarranted should uniformity of methods be established.

7. However, much would be sacrificed if laboratory men forgot the real purport of such analyses and used them simply to estimate the milk per se instead of the conditions which are indicated by such analyses.

It is suggested that perhaps more rapid progress would be made and more enduring results secured were the energies of workers devoted to more

exhaustive elemental studies which will assist in the solution of some of these problems.

Cremating Furnace for Laboratories: CHARLES E. MARSHALL.

It consists of an open brick chimney, lined with fire brick on the inner wall, and with an air space between the inner and outer walls. At the bottom of the chimney are the cremating apartment, the fire box and the ash pit. The essential and valuable feature of the cremating chimney is the using of gas pipes for grates to support the material, thus enabling the placing of a fire below. These pipes are so inserted in collars communicating with the air outside that a cold current of air passes through when heated and passes into the chimney at the inner end of the pipes which rest on a ledge on the inner wall of the chimney.

Published by the Experiment Station Report of the Michigan Agricultural College for 1908.

Preparation of a Standard Solution of Litmus:

CHAS. W. BROWN, Michigan Agricultural College, East Lansing, Mich.

It was found that 2.5 grams of azolitmin dissolved in one hundred cubic centimeters of distilled water would give a solution of such strength that one cubic centimeter when added to one hundred cubic centimeters of milk would give a blue color of the intensity desired for litmus media. With this as a measure, thirteen samples of litmus were standardized, giving the numbers recorded in the table.

Sample	Grams Required to make 100 c.c. of Standard Solution	Per Cent. Insoluble
Azolitmin	2.5	0
Merck's purified	7	2.3
Soluble litmus	8	14.5
Litmus cubes	140	87.1
No. 1	15	44.3
No. 2	32	68.7
No. 3	68	81.4
No. 4	98	89.4
No. 5	128	92.8
No. 6	164	92.8
No. 7	110	91.5
No. 8	154	94.3
No. 9	146	91.7
No. 10	122	91.8

A definite amount of the sample was weighed out and dissolved in a definite volume of distilled water, either by placing in an incubator at 37.5° C. over night or by heating in flowing steam for thirty minutes. The filtrate was compared in Nessler's tubes with the standard solution

(2.5 grs. of azolitmin in 100 c.c. water) diluted one to five thousand—one or two drops of decinormal potassium hydrate was added to each Nessler's tube to bring out the color. From this comparison the number of grams required to make one thousand cubic centimeters of a standard was interpreted.

Variation in the Acidity of Fresh Milk: W. M. ESTEN, Storrs Agricultural Experiment Station, Storrs, Conn.

The extreme variation, of a herd of 26 cows, covering a period of seven months, was from .075 to .23 per cent. The samples of milk were taken about every fortnight. The method of testing the acidity was by titration with $\frac{1}{10}$ normal NaOH, using 17.6 c.c. of milk and dividing the amount of tenth normal by 20. This result gives the per cent. of acidity in terms of the lactic acid molecule. The samples of milk were collected from each cow in the morning from 5 to 6 and were titrated about 10 A.M. In the interval, after a vigorous shaking, 2 c.c. of milk were taken from each sample for the bacteriological test. The experiments commenced on May 16 and extended to December 16. When curves were plotted and drawn for each cow, for different breeds of cows, for averages of breeds and for all the cows collectively, it was discovered that individual cows varied in their own curve and from the curves of the other cows, and that the breeds markedly varied from one another. There was also indicated for every cow a marked decrease in acidity during the summer, and a marked increase during the winter months. The food and seasonal period seemed to be controlling factors in the variation. The eleven Jerseys had an average acidity of .18 per cent., while the Holsteins had an average acidity of .16 per cent. The individual Jerseys showed a larger variation than the individual Holsteins, indicating that the Jerseys are more susceptible to changes and have a more sensitive nervous temperament. The total average of 378 tests on 25 cows was precisely .17 per cent., a value which gives a fairly correct idea of the normal acidity of milk.

Some of the most marked variations in acidity were found among the Jerseys and one Guernsey. One Jersey had an acidity of .13 per cent. at the close of her lactation period and commenced the next with an acidity of .2275 per cent. Three other Jersey cows had acidities of .22, .225 and .23, respectively, as their highest extremes. These cows were apparently in perfect health. It was found that pathological conditions had an effect

on the variation of acidity instanced during the summer by a cow with a sore foot. This cow's milk showed a drop to .125 on July 8, a rise to .18 on July 27 and a drop to .135 on August 7. After her recovery her milk acidity rose to .19 and varied afterwards but little. All these results lead to the conclusion that certain factors of food, conditions of health and the change of seasons have their effects, which are shown in the changes of acidity in the milk. A Guernsey had the most remarkable variation of all. Her average was only .108 with variations from .075 to .13. This is the lowest extreme in acidity that has, to the writer's knowledge, been recorded.

The question arises, What are the neutralization elements which we call acidity of milk? Is it the caseinogen alone, or caseinogen combined with other compounds, that causes the reactions? It has been suggested that the amount of acidity is an indirect indicator of the amount of caseinogen present. If this be true the acidity test would be a simple and rapid determination for the approximate amount of caseinogen present. It is to the chemist that we must submit the problem for solution.

Cost of Heating an Incubator with Electricity:

W. M. ESTEN, Storrs Agricultural Experiment Station, Storrs, Conn.

There are two important factors which determine the economic application of electricity in the heating of incubators for constant temperatures, namely, the insulation and the heating and regulation devices.

Copper is universally used in constructing incubators for laboratory use. With the exception of silver, copper is the best conductor of heat known. The insulating material used with copper is imperfect and not properly applied for preventing loss of heat. Copper incubators are generally water-jacketed and leaks often arise, causing disagreeable annoyances. The duration of the copper is somewhat limited. If such materials as asbestos, hair felt, wood and cork board are selected and used in combination there can be constructed an incubator, fire proof, with almost perfect insulation, and practically indestructible, at about one quarter the cost of a copper incubator. Moreover, the diminished cost of operation will pay for its construction cost in a few years. The writer has constructed one of these incubators out of asbestos, wood, hair felt and building paper with an inside capacity of three and a half cubic feet, which costs less than one dollar a year to heat to a constant temperature of 37° C.

The thermostat is constructed in the form of a V out of strips of brass and hard rubber firmly fastened together. The coefficients of expansion and contraction of brass and hard rubber are very different. Any change of temperature either opens or closes the electrical contact on the right-hand side of the V. The most important item in the economy of this new style of incubator is that the heat is applied to the interior, where it is used and needed. Incubators heated by gas utilize only about half the heat applied, and there is, besides, the danger of fire in operation.

The most satisfactory heater is made of No. 29 30 per cent. alloy german-silver resistance wire wound on a frame which fits into one end of the incubator. Two frames, one in each end, with 58 feet of wire on each frame and connected as one coil, afford the most uniform method of heating. The wire can be obtained from the American Electrical Works, Providence, R. I. The cotton-wound wire is preferable, for it affords considerable insulation. When the heating coils are completed a coat of shellac on the wire and frames serves as an added insulation. The resistance of the wire is 229.9 ohms per 100 feet. The only current that can be used with satisfaction is the alternating or incandescent type with large volume and low intensity. The voltage should be 110 for the length of wire indicated, which will produce about .4 ampere of current.

Keeping Qualities of Butter—Additional Data:

OTTO RAHN, Michigan Agricultural College, East Lansing, Mich.

Former experiments have proved that rancid cold-storage butter does not necessarily have free acids—indeed the majority of samples had no increased acidity. It seemed probable that the substances causing the taste and odor of rancid butter come from a decomposition of protein. The organisms which in pure culture are able to make butter rancid, such as *Bacillus fluorescens liquefaciens*, *Bacillus prodigiosus*, *Oidium lactis*, etc., break down proteids easily. The analytical determination of soluble nitrogenous substances, not precipitated by tannin, copper sulphate nor phosphotungstic acid, gave the *highest* increase in the butter sample which scored *lowest*.

Experiments have been carried on to ascertain if unsalted butter did not keep better in cold storage than salted butter. The supposition was that the water and buttermilk of the unsalted samples would freeze, thus preventing any action of microorganisms, while the concentrated brine

of salted butter has a very low freezing point. The result was that the unsalted samples did *not* keep, probably because not all the water was frozen. The unsalted samples scored lower and showed a greater increase of soluble nitrogen.

The only microorganism which multiplied without doubt in cold-storage butter and which occurs in almost every butter sample, is a small *Torula*. This *Torula* is the only microorganism of our butter samples which can develop on agar with 15–30 per cent. salt. A small *Torula* has been found by Rogers in fishy butter. A *Torula* is present almost in pure culture in salted herring and may be the cause of its characteristic taste and odor. The description of the *Torula* is incomplete, however, they do not disagree.

Bacillus lactimorbi, Jordan and Harris (*nova species*)—*Its Relation to Milk-sickness and Trembles*: E. O. JORDAN and N. MACL. HARRIS, University of Chicago, Chicago Ill.

This organism appears to be a hitherto undescribed bacterium and was isolated by the writers from several cases of trembles in cattle, from one case of the disease in a horse, from two lambs and from four cases of milk-sickness in the human subject. It would seem that the disease in man is incurred through the ingestion of infected milk, milk-products or of meat; in animals by the eating of infected pasturage or by drinking infected water, the contamination of these being from the soil in which the bacterium has its abode.

By means of pure cultures the writers have succeeded in reproducing the essential features of the naturally acquired disease in young rabbits, dogs, calves and one horse; cats and lambs have been infected with the production of pathological lesions, but without any well-defined clinical symptoms.

The organism causing the disease is a strict aerobic, flagellated, sporing, liquefying bacillus about the size of the anthrax bacillus, and very strongly resembling the tetanus bacillus in its most characteristic form. It is, however, very prone to undergo considerable variation in morphology due to methods of cultivation, temperature and fluctuations in reaction of the media being chiefly responsible. Stained with methylene blue the typical tetanus-like bacillus shows well-marked polar metachromatic granules, with at times a central one. It grows either very vigorously or delicately on agar, depending upon whether it is incubated at 25°, 30° or 37° C. Colonies on agar are of a streptococcus type accompanied by a film growth of a delicate nature,

either on the top or on the bottom of the agar surfaces. In glucose agar stab tube no gas is formed and the stab growth is smooth, delicate and whitish. On potato no growth has been obtained, but on Heinemann's synthetic potato medium a so-called "invisible" growth occurs. Broth is moderately clouded and occasionally a delicate surface film is formed. In gelatin the stab extends nearly all the way down the needle-track in a smooth, even manner and of a grayish color; the surface growth is scant, delicate and pearly gray; a saucer-shaped zone of liquefaction makes its appearance upon the surface about the third to the tenth day, and progressively extends outwards and downwards until the whole of the gelatin is liquefied. Occasionally where liquefaction has been delayed feathery outgrowths from the stab have been observed. In litmus milk growth does not readily occur unless the medium has been heavily seeded, then a slow-appearing alkaline reaction makes itself noticeable about the third day at the surface of the medium, which later on extends throughout the tube, eventually rendering the milk semi-translucent; no clotting or proteolytic changes have ever been seen. On Jordan's asparagin medium a well-marked surface film growth takes place with an ultimate slight clouding of the whole medium.

Note on a Lactic Acid Forming Bacillus Closely Resembling *B. bulgaricus* Isolated from Cornmeal: P. G. HEINEMANN and MARY HEFFERAN, University of Chicago, Chicago, Ill.

During an investigation of yeasts for the preparation of so-called salt-rising bread a bacillus was isolated which, as far as the examination has proceeded, agrees in morphology, staining properties, cultural characteristics and the ability to produce large amounts of lactic acid from milk with the characteristics of *B. bulgaricus*, the organism which has recently received considerable attention and has been recommended by Metchnikoff for the preparation of sour milk. A stain from the mixture of cornmeal, milk, sodium bicarbonate and salt showed a large number of bacilli with the characteristic granular staining of Metchnikoff's bacillus. After cultivation in milk the bacilli stained uniformly. When granules appear the picture recalls somewhat the appearance of small spores and seems to agree with Kuntze's description of his "Körnchen" bacillus. Attempts to obtain these bacilli in pure culture by plating on the ordinary laboratory media failed utterly, but they were readily isolated if cultivated in milk at 37° C. or if plated on milk-agar. The colonies

after a few days have peculiar root-like ramifications on milk-agar and may be mistaken for small colonies of *B. subtilis*. In pure culture the milk is coagulated slowly, rarely in less than two or three days. The coagulum is soft and creamy, without the separation of whey, and unlike the more solid coagulum formed by *Streptococcus lacticus*. The amount of lactic acid produced in milk is much higher than the amount produced by *S. lacticus*. After fourteen days 1.65 per cent. acid was determined. By addition of glucose or lactose to ordinary agar a moderate growth appeared after two or three days. If this bacillus is sowed into sterile milk with a culture of *S. lacticus*, acid is formed more rapidly than by either of the two organisms alone and the coagulation of the casein is also more rapid.

Salt-rising bread is started by mixing cornmeal, milk, sodium bicarbonate and salt. It seems that milk is a necessary part, without which probably the bacillus is unable to grow and form the necessary lactic acid for liberating carbon dioxide from sodium bicarbonate. By inoculation of sterilized milk with cornmeal it was ascertained that the bacillus originated from the cornmeal and not from the milk. It is, therefore, quite possible that this bacillus is widely distributed and an investigation is now under way to determine the distribution. We are attempting to isolate this bacillus from a large variety of sources, principally flours, dry fodder, silage, feces of man and domestic animals, sourkrout and other similar substances. The study has extended over a period of a few weeks and we hope to be able to make a more complete report in the near future.

The Gas Production of *B. coli*: FREDERICK G. KEYES, Brown University, Providence, R. I.

All previous work on gas production has been of little value because of imperfect methods of collecting and analyzing the gases produced.

The ordinary fermentation tube does not give the exact amounts of gas produced because of the solution of the gases in the medium and its diffusion therefrom.

The methods ordinarily used by the bacteriologist for analyzing the gases produced are imperfect because they give merely the CO₂ content and tell little about the other gases which may be present. Also, standard media, even at best, are sufficiently variable to influence materially the amount and composition of the gases produced.

Therefore the writer set about to devise an apparatus which would enable him to collect and

analyze all the gases produced by an organism on a synthetic medium containing substance of known chemical constitution. The results of the use of this apparatus for studying the gas production of *B. coli* are as follows:

	Time in Hours at 37° C.	Total Gas in Per Cent.	CO ₂ Per Cent.	H Per Cent.	N Per Cent.
On a synthetic medium in vacuum.	24	28.6	63.36	36.6	0.05
	72	45.7	63.40	36.15	0.45
	115	96.3	63.56	35.94	0.50
On standard medium in vacuum.	48	197.1	55.87	43.50	0.65

The Utility of the Society's Card in Classifying the Cheese Flora: H. A. HARDING and M. J. PRUCHA, New York Agricultural Experiment Station, Geneva, N. Y.

During the past four years the flora of American cheddar cheese has been intensively studied at the New York Agricultural Experiment Station. In connection with this study an attempt has been made to classify the organisms which were found in nine normal cheeses and so characterize them that they may be recognized by succeeding students.

During the progress of this study there appeared a classification of the bacteria of milk by Conn, Esten and Stocking. This work was of material assistance to us and covered the milk flora so completely that all but one of the organisms found by us in cheese is evidently included under the types described by them. This classification as arranged by Conn is an adaptation to bacteria of the conventional botanical system of classification.

The society's card has also appeared during this interval and the cheese flora has also been arranged in accordance with its system of group numbers.

As the result of this double classification the germs found represent 22 types according to Conn or 33 groups according to the society's card. The classification of the germs found in nine normal cheddar cheeses according to each of these systems is given in the accompanying table. This table is taken from Technical Bulletin No. 8, New York Agricultural Experiment Station, in which are given the details of this study.

The amount of data required to determine the group number and the type name is practically the same, but a careful comparison of the utility of these two systems in connection with this study is strongly in favor of the society's card.

This advantage lies in the increased accuracy with which different workers assign germs to like groups, in the quickness with which such assignment can be made and in the ease with which duplicates can be detected or the accumulated stock of records be consulted.

By the use of the card the results of one worker are made immediately available to succeeding workers and each can recognize the forms which have been already described and build upon the foundation already laid. By accumulating results in this way, it will soon be possible to have as exact a knowledge of the bacterial flora of any given class of objects as we now have of the higher flora of a region.

It is believed that the introduction of the society's card will prove the most important addition to laboratory and classification technique since Robert Koch brought out the gelatin plate.

Autolysis of the Gonococcus: C. T. MCCLINTOCK, M.D., Ph.D., and L. T. CLARK, B.S., from the Research Laboratories of Parke, Davis & Co., Detroit, Mich.

Autolysis of gonococcus cultures has been observed and reported by numerous workers.

The purpose of the present study is: (1) to confirm the results obtained by others; (2) to determine the amount of ferment present, the method by which it breaks up the cells and the extent to which the action can be carried; (3) to determine the effect of the resulting autolysate on the growth of the gonococcus, and other organisms in vitro, and (4) to study the effect of the autolysate on the organisms in vivo and its possible therapeutic application.

The results obtained so far are shown in the following conclusions:

Disintegration or autolysis occurs in gonococcus cultures to a marked degree.

This autolysis can be completely prevented by heat alone at 70° C. for one hour, by heat in the presence of trikrisol at 60° C. for one hour, by 50 per cent. and 95 per cent. alcohol in salt solution and partially by 4 per cent. trikrisol alone.

Specimens stained at certain stages of disintegration show numerous fragmentary cell walls still capable of taking the stain, indicating that the cell wall is not completely disintegrated.

The presence of shadow forms in which the cell wall only is stained indicates that the cell contents are either so changed as to be incapable of taking the stain or have escaped from within the wall.

The presence of soluble proteid substances (as shown by their precipitation with acetic acid) in the surrounding liquid indicates that the contents have escaped from within the cell.

Hence it would seem that autolysis of the gonococcus is effected by rupture of the cell wall and escape of the contents.

The products of this autolytic process markedly inhibit the growth of the gonococcus on artificial culture media. Their use in combating the disease in man will form the subject of a future communication.

A Case of Non-inheritance of Fluctuating Variations among the Bacteria: C.-E. A. WINSLOW and L. T. WALKER, Massachusetts Institute of Technology, Boston, Mass.

Aside from its practical importance, the study of variation among the bacteria promises to throw important light upon some of the fundamental biological problems of heredity and evolution. It is important to distinguish two types of possible variations, those which arise entirely from causes operating within the bacterial cell (either mutations or fluctuating variations), and those which are apparently due to the direct or selective effect of the environment. Goodman¹ has recently demonstrated that by selection of variations of the latter sort the acid production of certain members of the diphtheria group of bacilli may be profoundly modified. In the present investigation the authors have attempted to study the inheritance of fluctuating variations in the paratyphoid group, without special selective action. Cultures of Schottmüller's types A and B were plated out and one hundred subcultures of each were inoculated on agar from separate colonies. A dextrose broth tube was inoculated from each streak. The acidities produced were determined by titrating and the results, when plotted, showed two distinct but overlapping curves of frequency with means at 1.4 for type A and 1.6 for type B. The agar streaks of the four extreme cultures (1.1 and 1.6 for type A, 1.4 and 1.8 for type B), were then plated and a series of one hundred streaks made from each. The new curves of frequency for these descendants reverted completely to the original

means of types A and B, showing no inherited effect of the variations exhibited by their more immediate ancestors.

Bacteriology as an Important Non-technical Study: H. W. HILL, M.D., University of Minnesota, Minneapolis, Minn.

Bacteriology is now chiefly taught as an art, of use in some branch of science or industry, and almost wholly for its applications in these. Bacteriology is seldom studied for itself *alone*. But it presents many most important biological lessons, as suitable for the illustration of biological truths as any other biological study now taught; and from the control of conditions possible, some phases of biology can be illustrated best by bacteriology. For sociology, bacteriology permits, as no other biological study does, the appreciation not only of the unit, but of the interrelations of units—and hence furnishes a biological study closely paralleling sociology itself. (As a weapon in the hands of a sociologist dealing with hygienic problems it is of course practically a necessity.) Apart from its academic values its chief practical significance to the non-technical citizen consists in the training it gives concerning the *nature, distribution and life history* of bacteria and in its *technique*, which teaches the fundamentals of *personal and family* defense against disease, as distinguished from the measures of public health or state medicine. For these reasons bacteriology should be taught in the public schools, since diffusion of its teachings through the citizens in general can not be obtained in any other way.

NORMAN MACL. HARRIS,
Secretary

UNIVERSITY OF CHICAGO

SOCIETIES AND ACADEMIES

THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE

THE thirty-third meeting of the society was held at Cornell University Medical College, April 21, 1909, with President Lee in the chair.

Members present: Atkinson, Auer, Burton-Opitz, Elser, Ewing, Flexner, Famulener, Gies, Janeway, Joseph, Kast, Lee, Lamar, Lewis, Lusk, Mandel (J. A.), Meltzer, Meyer, Morse, Noguchi, Norris, Oertel, Park, Pearce, Shaffer, Storey, Terry, Wallace, Wolf.

Members elected: John L. Todd, Peyton Rous, H. S. Jennings, Andrew Hunter, Charles R. Stockard, E. E. Southard, William W. Hale.

¹ *Journal of Infectious Diseases*, V., 421.