jelly-fishes as he has in his other writings, and it is not a matter of surprise that he should be quoted[1] as saying that the forms with which he worked became paralyzed, when the marginal organs were excised, when one reads[2] that he found "in all the species I have come across that excision of the margins of the umbrellas produces an effect analogous to that which is produced by excision of the margins of the Hydromedusæ" where such an operation results in the total paralysis of the bell. However, when one reads farther, he says, with much verbosity, that

> There is an important difference, however, between the two cases in that the paralyzing effect of the operation on the umbrellas (of the Scyphomedusans) is neither so certain nor so complete as it is on swimming bells (of hydromedusæ). That is to say, although in the majority of experiments such mutilation of umbrellas is followed by immediate paralysis, this is not invariably the case.

Romanes found that *Aurelia aurita* showed "instantaneous and complete paralysis of the gonocalyx" on excision of the marginal organs, while *Cyanea capillata* was less marked in this respect.[3] Eimer's observations were practically the reverse of this.

There can be no question that Romanes was entirely correct in his observations, for he repeated them during several summers, specifically examining the point in question in the light of Eimer's work. It is fair to assume, too, that Eimer made no mistake. Hence, it seems that *Aurelia aurita* reacted differently on Cromarty Firth, Scotland, from what it did in the North Sea with respect to the matter at issue. Romanes probably used a different species of *Cyanea* (*Cyanea capillata*) from Eimer's form (which was probably *Cyanea lamarckii*) and I have used a third species, *Cyanea arctica*, which seems to be recognized by systematists as a good species. My species of *Aurelia*, *Aurelia flavidula*, is recognized by some as distinct from *Aurelia aurita*, but both Louis and Alexander Agassiz did not so regard it.

From my observations, *Aurelia flavidula* very rarely is paralyzed completely and, indeed, I have but an impression that I have seen *Aurelia* absolutely quiescent after the marginal organs have been removed. Unfortunately, I did not examine the question critically until last year and my previous observations were not recorded. During the past year, however, I found no specimen which did not regain pulsation after a longer or shorter period after the marginal organs were removed. The case of *Cyanea* is directly the reverse, for this form becomes totally paralyzed when the organs are removed. Reference to the statements from Eimer and Romanes, given above will make it clear how these observations correspond to theirs. They agree closely with those of the former and are totally at variance with those of the latter.

The matter is of importance from the point of view of the physiologist who wishes to use some primitive form of contractile substance with which to experiment and these observations are especially directed to them. *Cyanea arctica* will remain quiescent after the marginal organs are removed and respond only to mechanical, chemical and other external stimuli supplied by the operator. In fact, *Cyanea* rivals the classic *Cassiopea* for experimental work. *Dactylometra* reacts like *Aurelia flavidula*.

MAX MORSE

NEW YORK,
March 8, 1910

[1] For instance, Parker in his *Popular Science Monthly* articles on the nervous system makes such a statement and while giving no references, yet he has written me that he was impressed that Romanes's observations led to such conclusions.

[2] "Jelly-fishes, Starfishes and Sea Urchins," Appleton.

[3] *Phil. Trans.*, Vol. 167.

THE SOCIETY OF AMERICAN BACTERIOLOGISTS

THE eleventh annual meeting of the Society of American Bacteriologists took place on December 28, 29 and 30, 1909, in the administration building of the Harvard Medical School, Boston, in conjunction with the annual meeting of the American Association for the Advancement of Science. It can be confidently asserted that the society has never held a more successful and profitable meet-

ing, both as regards the numbers in attendance and the quality of the papers presented. The president of the society, Professor J. J. Kinyoun, occupied the chair at all of the sessions. The reports of the secretary and treasurer showed that the affairs of the society were in a healthful condition.

The nominating committee placed before the society the names of the following for election to the offices for the ensuing year, and they were unanimously declared elected:

President—Professor V. A. Moore, Cornell University.

Vice-president—Professor F. P. Gorham, Brown University.

Secretary and Treasurer—Professor C. E. Marshall, Michigan Agricultural College.

Councillors—Messrs. Prescott, Amyot, Stevens and Harris.

Delegate to the Council of the American Association for the Advancement of Science—Professor Erwin F. Smith.

The report of the committee on the identification of bacterial species was presented and adopted. As it is of an important nature, it is given here in full: (1) The standard card for the description of species has proved highly satisfactory in the hands of those who have given it a thorough trial. The maximum benefit from the card can only be realized, however, as its use becomes still more general. The committee, therefore, wishes again to urge upon the members of the society the great value of this method of recording bacterial characters. Advantage will accrue not only to the individual investigator, but in still higher degree to other workers on account of the comparability of the data thus obtained. (2) The numerical system of recording bacterial characters, while valuable for cataloguing cultures, must necessarily fail to approximate the natural classification of species. The method which at present seems most promising for determining the true relationships of these microorganisms is the statistical or biometric method. Bacterial species may most satisfactorily be defined by the quantitative study of measurable characters in a considerable series of cultures, the modal points or centers of frequency being given specific names, and larger groups having a number of common characters receiving the rank of genera or families. The committee urges upon the members of the society the importance of further systematic investigation along this general line. (3) In view of the great value of biometric classification to all workers in bacteriology the committee suggests the following resolution:

Resolved, That the Society of American Bacteriologists, recognizing the importance of a systematic study of bacterial species by the statistical method and the necessity for financial assistance in carrying out work which involves so large a proportion of routine, authorizes the committee on identification of bacterial species to present this need to any persons or institutions having charge of the distribution of funds for assisting scientific research.

(4) Finally, the committee believes that it would be of great advantage if descriptions and cultures of all new species or varieties could be submitted to some central bureau where they might be studied and compared and kept in such condition that bacteriologists could at any time obtain duplicate descriptions and subcultures for their own use. The committee therefore recommends the following resolution:

Resolved, That the Society of American Bacteriologists believes that the establishment of a central bureau for the preservation and distribution of descriptions and type specimens of the bacteria would be of great value to all workers in science.

F. P. Gorham
C.-E. A. Winslow

The council declared four vacancies to exist in the membership of the society and the following were elected to fill those vacancies: Dr. G. W. Stiles, Department of Agriculture, Washington, D. C.; Professor W. E. King, Kansas State College of Agriculture, Manhattan, Kans.; Professor R. E. Buchanan, Iowa State College of Agriculture, Ames, Iowa; Professor Oscar Klotz, department of pathology, University of Pittsburgh, Pittsburgh, Pa. The council also recommended that inasmuch as by the election of the foregoing persons the active list of membership was filled up, the wording of the constitution be altered to admit of an active membership of 150 instead of the present number of 125. This recommendation was favorably received and will be acted upon by the society at the next annual meeting.

On the evening of the twenty-ninth the society was the guest of the Boston Bacteriological Club at a "smoker" held in the rooms of the Technology Union, where the members thoroughly enjoyed themselves in an informal manner.

The program, as shown by the following titles and abstracts, was carried out and evoked much

interest and discussion, making the meeting one of the most successful that the society has yet experienced:

Some Observations on the Immune Body: J. J. KINYOUN, Health Department, Washington, D. C. (President's address.)

The president in the address delivered before the Society of American Bacteriologists gave a résumé of his observations on the several immune bodies in connection with the production of antisera and other substances associated with the phenomena of immunity. He claims that a distinction should be drawn between the specific anti-bodies as for example, the anti-toxin of diphtheria or of tetanus and other bodies which are also present in such sera. The claim is advanced that there is present in all such sera containing specific anti-bodies, others which may be termed common immune bodies. These have the property of increasing the resistance of the cells against many substances which are harmful to them and are of diverse origin. These common immune bodies are intimately associated with the leucocyte and it is believed that the leucocyte gives them origin. The statement is also made that the curative value of all anti-bacterial sera is due not so much to the specific anti-bodies, but to the common immune bodies which are always present.

An Improved Method of Employing "Antiformin" and Ligroin, in the Examination of Sputum, etc., for the Tubercle Bacilli: J. J. KINYOUN, Health Department, Washington, D. C.

The improvement of the method is in the simplification of the process both as to time and in manipulation. In the case of sputum, a small quantity of the "antiformin" forms one to three cubic centimeters, and about one cubic centimeter of ligroin (spec. grav. 0.715 to 0.720) is added at the same time. The sputum is placed in a shaker and shaken for about fifteen minutes, at which time the cellular contents and mucus are dissolved together with a greater proportion of the bacteria. A small quantity of this is placed in a centrifuge tube and spun at moderate speed for a minute or so to bring the ligroin to the top. The layer of saponified material lying at the juncture of the sputum and ligroin will contain nearly, if not all, the tubercle bacilli. Tissues can also be examined in the same way; small bits of the suspected tissue are placed in the antiformin together with ligroin and shaken in the same manner as for sputum, then centrifugalized and examined. This method is also well adapted for the examination of feces. The main advantage is the saving of time.

On the Production of Agglutinating Sera for Diagnostic Purposes: J. J. KINYOUN, Health Department, Washington, D. C.

The writer states that there is always an element of uncertainty in the production of antisera when the smaller laboratory animals are employed. And particularly is this true with the rabbit and guinea pig. It occurred to him to use medium-sized pigs (or shoats) for this purpose. These animals were found to be well adapted for the purpose as they withstood large quantities of living cultures of *B. typhoid, B. paratyphoid, "a"* and *"b"* and *B. coli* without the least discomfort. After two or three injections of any of these organisms above referred to, an agglutinating serum was obtained which reacted in high dilutions. The bleeding was from the tail; the quantity taken varied as to the size not less than 100 c.c. nor more than 400 c.c. at a bleeding. The animals are easy to handle, their maintenance is as cheap as smaller animals, and, moreover, they can always be depended upon not to die just before you complete the immunization.

Some Observations on the Fermentation of Silage: W. M. ESTEN, Storrs Agricultural Experiment Station.

The prevailing opinion of investigators in silage fermentation is that respiration and enzymes are the real agents in the process. These conclusions are maintained on account of finding high temperatures in fermenting silage and the forming of ensilage in the presence of chloroform and so forth. High temperatures are found in silage only when the surface is exposed to the air and where an alkaline fermentation is in progress, or when the per cent. of moisture is relatively low. Inside the silo, where acids are produced to preserve the silage, no high temperatures are found. When silage is formed by sterilizing with chloroform and so forth no acid is produced.

In cutting corn for ensilage each piece is covered with a film of sweet juice. In the subsequent filling of the silo the several tons of pressure forces out more juice, so that every piece and fragment of the silage is saturated and covered with a sugar fermentable substance, mostly dextrose. As is well known, all sweet fruit and plant juices undergo two types of fermentation, acid or alcoholic, and in some cases both occur together. The most common change of sugar to acid is by

lactic-acid bacteria. Some fruit juices, like apple cider, contain so much acid that nearly all kinds of bacteria are unable to grow in them. Apple juice contains about .72 per cent. of total acid. Corn juice has about .25 per cent. of unknown acid and some gallic acid (?). In corn juice lactic acid bacteria grow profusely till about .35 to .45 per cent. of lactic acid is formed, when they cease to grow. But yeasts are tolerant to much larger amounts of acid and therefore continue to grow in the corn juice till practically all the sugar is used up. The alcohol formed is mostly changed into acetic acid. In fresh silage large numbers of yeasts and acid bacteria are found. During the first twelve days of fermentation nearly all of the biochemical changes are completed. The maximum growth of acid bacteria is on the fourth day and the maximum growth of yeasts is on the twelfth day. The highest temperature of 29° C. was noted in the first 36 hours. The samples were taken from a hole in the silo five feet from the bottom, and from one to two feet from the edge.

Further Studies in the Acidity of Fresh Milk: W. M. Esten, Storrs Agricultural Experiment Station.

The entire range of variation of the acidity of the milk in a year for a herd of cows numbering more than twenty-five was from .155 to .187 per cent. The law of variation is that the acid of the milk varies inversely as the temperature. Approximately on the first of February the milk of all the cows is at its highest point of acidity. On the first of August it is at its lowest point of acidity. These two dates include the coldest and warmest periods of the year.

The variation during a lactation period proves to be quite remarkable. An acidity of .48 per cent. has been found at the first milking. In from two to three days it falls to about .25 per cent. from this figure, then gradually in about three weeks to the normal, near .17 per cent., which continues until about three weeks before the end of the lactation period, when, at the last milking, it falls to .12 or .13 per cent. The high acidity at the beginning is explained by the fact that the ash and salts are very much in excess of the normal amount. Some of these are probably calcium salts which are necessary for bone production in the young animal.

The quality of the milk varies as the acidity, so that winter milk has more food value than in summer and a higher price in winter is justified by this fact.

The acidity has an important bearing in the inspection of milk. A dairy selling Jersey milk with 5 per cent. of butter fat will sometimes show an acidity of .20 per cent when fresh, and does not then contain a particle of lactic acid. Under these conditions the milk should not be condemned for high acidity, but rather recommended for the high acidity which indicates a high quality. It is therefore requisite that milk inspectors be capable of judging the high acidity of milk which indicates high quality and value, from high acidity caused by growth of acid organisms which produce lactic acid in milk of any quality.

Bacteriological Methods in the Oyster Survey of Virginia: Meade Ferguson, Laboratories of State Board of Health of Virginia. (Read by title.)

Methods of Testing Shellfish for Pollution: Stephen DeM. Gage, Massachusetts State Board of Health, Experiment Station, Lawrence, Mass.

The methods for testing shellfish for pollution in Massachusetts have been devised to facilitate the routine handling of a large number of samples in the easiest and most accurate way.

Collection of Samples.—Twelve to fifteen shellfish are collected from each sampling station, in wide-mouth spring-top glass jars. The sampling stations are distributed well over the area from which shellfish are gathered, and samples of sea water are collected from each station in addition to the shellfish samples.

Transportation of Samples.—Shellfish samples should be delivered at the laboratory within twenty-four hours after collection. Packing samples in ice is probably unnecessary, except during very hot weather.

Technic of Testing.—The individual shellfish is washed with sterile water, opened with a sterile oyster knife and a portion of the shell water transferred to a fermentation tube. The body of the shellfish is then removed from the shell, washed with sterile water, opened with a sterile scalpel and a portion of the alimentary canal transferred to another fermentation tube. Ten individual shellfish from each sampling station are tested in this manner.

B. coli Methods.—Dextrose pepton water is used in the fermentation tubes. The incubation of these tubes and the isolation and confirmation of *B. coli* are in accordance with the standard methods used in water analysis. No systematic search is made for the sewage streptococcus, but

if its presence is noted either on the plates or on the agar streaks, this is recorded.

Interpretation.—Tests of a single shellfish from any location have little diagnostic value. When a sufficient number of shellfish have been tested, the absence of *B. coli*, or of positive fermentations followed by an overgrowth of sewage streptococcus in 80 per cent. of the samples tested, indicates that the location is reasonably free from pollution. If 50 per cent. or more of shellfish from a location show *B. coli*, or fermentation overgrown by sewage streptococcus, the location is dangerously polluted. Between these limits, the interpretation is a question of degree of pollution, based on individual judgment, into which analyses of the sea water from the same source, and a sanitary inspection of the source must enter.

Some Peculiarities in the Counts of Bacteria at 20° C. and at 40° C. from Waters Treated with Disinfectants: STEPHEN DEM. GAGE, Massachusetts State Board of Health, Experiment Station, Lawrence, Mass.

For some years we have been making counts of the bacteria at 40° C. in addition to the usual count at 20° C., and have found that with natural waters and the effluents from good water filters there is an approximately constant ratio between the counts at the two temperatures. For example, effluents from good water filters, and surface and ground waters used as public water supplies in Massachusetts, usually contain less than 100 bacteria per cubic centimeter according to the 20° C., less than 10 per cubic centimeter, as shown by the 40° count, and about half of the latter will produce red colonies on litmus lactose agar.

When dealing with waters, etc., which have been treated with certain disinfectants such as bleaching powder, whose efficiency is produced by oxidation, we have frequently found that while the numbers of bacteria determined at 20° C. might be reduced to less than 100 per cubic centimeter by a small amount of disinfectant, frequently there would be no corresponding decrease in the 40° count, and that considerably more disinfectant must be used to make the 40° count conform to the standard of the good waters, as previously stated.

Furthermore, in a great many instances the 40° count on disinfected waters was as high or higher than the 20° count. This phenomenon has occasionally been observed with natural waters and sewages, but an analysis of the records of many thousand samples shows that the percentage of such samples is not over five per cent. On the other hand, 20 to 25 per cent. of samples of water and 50 to 70 per cent. of samples of sewage and effluents from contact and trickling filters, after treatment with bleaching powder, showed higher counts at 40° than at 20° C. This abnormally high 40° count is seldom found when the 20° count is high, but when the latter count is below 100, these peculiar results are frequent.

These abnormal ratios with disinfected waters are not peculiar to Massachusetts, but have also been noticed elsewhere where bleach disinfection has been tried. In many instances, however, such results appeared to be so erratic that they were considered to be abnormal and were thrown out, and we so considered them at first. When we found that they occurred with a frequency of 20 to 70 per cent., however, we did not feel justified in calling them abnormal or in throwing them out.

It can be stated definitely that this phenomenon of abnormal ratios is not due to spores. A careful study of this point has been made, and the ratio between total colonies and spore formers at both 20° and 40° has been proved to be practically the same before and after disinfection.

Diphtheria Bacillus Carriers in the Public Schools: F. H. SLACK, B. L. ARMS, E. M. WADE and W. S. BLANCHARD, of the Bacteriological Laboratory of the Boston Board of Health.

This paper presents the details and results of an experiment undertaken at the beginning of the school year in the Brighton District of Boston, Mass.

The pupils in this district number over 4,000 and two cultures were taken from each during two successive weeks. All microscopic examinations were made in the bacteriological laboratory of the Boston Board of Health by the regular corps of workers.

Positive results were reported only on those cultures showing the A, C or D types of organisms (Wesbrook).

On the first day, 1,287 cultures were examined; the second, 1,131; the third, 1,029; the fourth, 699—a total of 4,146, and of these 55 or 1.33 per cent. were positive.

These cases were for the most part removed from school.

The second round the following week gave 1,275 cultures the first day; 1,113 the second; 1,029 the third, and 670 the fourth—a total of 4,081, of which 38, or .93 per cent., were positive.

Details concerning these cases and a five-year chart of clinical cases in the district are given.

The following conclusions are reached:

1. That at least 1 per cent. of all healthy school children are carriers of morphologically typical diphtheria bacilli (Wesbrook's A, C, D types).

2. That such bacilli are communicable from one to another and the condition is usually a transient one.

3. That the organisms are ordinarily of little or no virulence.

4. That while it is possible, by passing through a susceptible individual, their virulence might be raised to cause the disease, this is not a frequent occurrence.

5. That the disease diphtheria is kept alive in a community rather by virulent organisms in immune persons than by these non-virulent bacilli.

6. That where *virulent* diphtheria bacilli are present as shown by outbreaks of the disease, cultural tests of all contacts and isolation of those showing positive cultures is a duty owed to the community.

7. Where the *disease* does not exist, isolation of carriers of probable *non-virulent* bacilli is of no proven benefit, and is a costly and laborious procedure entailing much unnecessary hardship on innocent and probably harmless parties.

8. The attempt to control diphtheria in a city by a round of cultures from all school children at the beginning of the school year does not seem encouraging from this series of tests.

9. The proposition to stamp diphtheria out of a city by cultural tests of all the inhabitants and isolation of all carriers is impossible from any practical standpoint.

The Virulence of Old Cultures and Subcultures of B. mallei: B. L. Arms, M.D., Assistant Director of the Bacteriological Laboratory of the Boston Board of Health.

From work done at the Boston board of health laboratory the following conclusions are drawn:

1. That in glycerine broth, *B. mallei* live and retain their virulence for at least two months, even when kept at body temperature.

2. That a culture of *B. mallei* may be virulent after growing on potato for at least a month.

3. That some stains of *B. mallei* retain their virulence through a great many subcultures on artificial media.

How shall the Value of Disinfectants be Determined? E. M. Houghton, Detroit, Mich.

Some Observations on the Wassermann Reaction: Lawrence T. Clark, Detroit, Mich.

Departures from the principles upon which the original Wassermann method for the serum diagnosis of syphilis is based are fraught with the dangers attending unreliable and, in many cases, entirely erroneous results. In making the test a thorough knowledge of the strength and keeping qualities of the various factors entering into it is very essential. Such facts, enumerated in the conclusions, make it possible to diagnose a high percentage of doubtful cases with a considerable degree of accuracy.

Conclusions.—The complement content of fresh guinea-pig serum varies materially with different pigs.

More uniform and accurate results are obtained when the guinea-pig serum is standardized to known normal and syphilitic sera before doubtful samples are tested.

Hemolytic serum kept at uniform low temperature retains its activity for a relatively long time, although it loses some of its original strength and needs restandardizing from time to time.

Suspensions of thoroughly washed red blood corpuscles (ram) kept at 1.6° C. have been used up to fourteen days after drawing with good results.

Samples of serum inactivated (56° C. one half hour) and kept free from contamination, remain unchanged for several days. This enables one to store samples and run several at one operation.

Practical tests made with properly standardized reagents gave 95 per cent. and 93⅓ per cent. accurate results in known and doubtful cases, respectively.

Negative reactions were obtained after vigorous specific treatment in eight cases which gave positive reactions before treatment. A future publication will deal with this phase more extensively.

It would seem to be indicated by results from the limited number of cases tested that the complement fixation reaction, when carefully carried out and thoroughly controlled, is a reliable means for diagnosing the doubtful case.

The Usefulness of Curves in the Interpretation of Biochemical Processes: Otto Rahn, Michigan Agricultural College.

If a curve of a biochemical process is plotted, taking as abscissa the time elapsed and as ordinate the total amounts of compounds produced, the shape of this curve will in many instances indicate the nature of the change taking place. In a purely chemical or enzymatic change, the active mass does not increase, and therefore the rapidity of the process measured by the angle of elevation of the curve does not increase. (Under enzyme is understood a chemical compound, unable to multiply.) The curve changes with the

time, becoming more and more parallel to the base line. If we are dealing with changes caused by microorganisms, the active mass is increasing as long as microorganisms increase, and consequently the velocity of the process, or the angle of elevation, will rise as long as the increase continues. This elevation of the curve is characteristic for compounds produced by any multiplying organism. From the time the increase ceases, we are dealing with a purely enzymatic curve.

The exact plotting of the curve allows us to make fairly accurate statements about the multiplication and the duration of the increase of bacteria, even if they can not be counted by our present methods. The point of inflection of a curve shows the moment when the organisms producing the substance under study reach their maximum number and can be studied with the greatest convenience.

In some instances, the point of inflection is changed to a straight line, indicating a very resistant strain of bacteria; this seems to take place especially in poor media, as soil extracts. A few experiments indicate that poorly nourished bacteria are able to produce a larger amount of fermentation products than well-nourished bacteria, though they need a much longer time to accomplish it.

The Society Card as a Basis for Classifying the Bacteria producing Soft Rot in Vegetables: H. A. HARDING and W. J. MORSE, New York Agricultural Experiment Station.

This group includes *B. carotovorus* Jones, *B. aroideæ* Townsend, *B. omnivorus* van Hall, *B. oleraceæ* Harrison and some other described forms.

A comparison on the basis of the society card brings out the fact that these described cultures are identical in all cultural characters except the results from the fermentation tube.

Extended study of this point indicates that this difference is more apparent than real, since the normal gas-forming ability of this group lies so near the amount required to saturate the fermentation tube that the appearance of visible gas varies with the fermentative vigor of the particular culture.

These results indicate that in cases where the fermentative ability of a culture is weak there is need of a more accurate instrument than the fermentation tube for accurately detecting gas formation.

(Data to appear as New York Agricultural Experiment Station Technical Bulletin 11, 1909.)

Does the Group Number on the Society Card Carry the Classification far enough to Break up the Species? H. A. HARDING, New York Agricultural Experiment Station.

This point was tested with approximately fifty strains of *P. campestris* (Pam.) Smith. This species was chosen because it is a well-known, chromogenic, plant-pathogen in which the limits of the species can be determined with the minimum chance of error.

Some of the tested strains were freshly isolated from the host while others had been cultivated in various laboratories for many months. The larger part of these cultures were revivified just previous to being tested and were tested on standard media. In some cases these precautions were purposely omitted. Independent observations were made in some cases by three different workers and media prepared by three different persons was used.

With the exception of the reduction of nitrate there was no variation in the group number as determined from these cultures.

The variation in nitrate reduction, as determined by the official method for nitrite, was apparent rather than real since it was not shown by the nitrite test with the starch, KI, H_2SO_4 test. The faint reactions obtained with the official test were undoubtedly due to absorbed nitrite. Nitrite is not absorbed equally by all tubes and a large number of check tubes must be held to insure accurate comparisons in faint reactions.

A New and Improved Method of Enumerating Air Bacteria: LEO F. RETTGER, Yale University.

Studies on Bacterial Mutation: LEO F. RETTGER, Yale University.

A Comparative Study of Intestinal Streptococci from the Horse, the Cow and Man: C.-E. A. WINSLOW and G. T. PALMER, Massachusetts Institute of Technology.

Andrewes and Horder's statistical study of the streptococci has for the first time made it possible to classify the principal types of this complex group in a fairly satisfactory manner. One of the most interesting points about Andrewes and Horder's classification, and the earlier observations of Gordon and Houston on which it was founded, was the apparent difference between streptococci from the intestines of the horse, the cow and man. In the present investigation we have tested this point by isolating one hundred strains of streptococci from fæces of each of the three animals; we have cultivated them in broth containing four different fermentable media (dextrose, lactose,

raffinose and mannite), and determined by titration the amount of acidity produced by each strain in each medium. An examination of the results obtained confirms and harmonizes the work of the English observers in all particulars. The commonest streptococci in human fæces are *S. mitis* (acidifying dextrose and lactose), *S. fæcalis* (dextrose, lactose and mannit) and *S. equinus* (dextrose alone). In the fæces of the cow *S. equinus* and *S. mitis* are present; but *S. fæcalis* is absent and a form rare in human fæces, *S. salivarius* (dextrose, lactose and raffinose), is fairly abundant. In the fæces of the horse practically all the streptococci present are of the *S. equinus* type. (Full paper, *Journal of Infectious Diseases*, VII., 1.)

The Determination of the Number of Leucocytes in Milk by a Direct Method: S. C. Prescott and R. S. Breed, Boston, Mass.

The methods in general use for determining the number of leucocytes present in milk are all based on the use of the centrifuge. The assumption is that all but a small fraction of the leucocytes are precipitated and also that this fraction is a fairly constant proportion of the whole and can safely be neglected. An investigation carried on in the Boston Biochemical Laboratory during the past summer has shown both of these assumptions to be incorrect. By the use of a new method, it has been found that the distribution of the leucocytes in a given sample of milk after centrifuging varies greatly in different samples of milk, although their distribution is approximately the same in different samples of the same milk. Usually more than half are present in the cream, one fourth or less in the precipitated slime, and the remainder in the skim milk.

The variation in position of leucocytes in different samples is apparently due to the variable percentages of cream present. The distribution of the leucocytes in a centrifuged sample corresponds closely to the previously known distribution of bacteria in similar samples.

The new method by which these facts have been ascertained is as follows: a measured drop (.01 c.c.) of milk to be examined is spread evenly over a measured area (1 sq. cm.) on a glass slide, dried with gentle heat, the fat dissolved out with xylol, fixed with alcohol for a few minutes, the slide again dried and over-stained with methylene blue and partially decolored with alcohol. The number of leucocytes present is then determined by examination with the microscope. Results done in duplicate show a small percentage variation proving that the practical error is not a large one.

A series of tests of milk show that much larger numbers of leucocytes are normally present in milk than has been supposed. The average number of leucocytes present in the samples examined is approximately 1,500,000 per cubic centimeter, while numbers less than 100,000 per c.c. are uncommon.

The Bacteriology of Condensed and Evaporated Milks: S. C. Prescott and R. N. Hoyt, Massachusetts Institute of Technology.

Some Problems of Sanitary Milk Production: P. G. Heinemann, A. B. Luckhardt and A. C. Hicks, The University of Chicago.

A series of experiments was made during the month of September at a sanitary dairy to throw light on the following points: (1) the bacterial content of separator milk and cream, (2) the value of narrow top pails with and without strainers, (3) the bacterial content of milk after straining through layers of absorbent cotton, (4) a study of body cells in separator slime and an attempt at classification.

It was found that the bacterial content of the separator cream was very small, the average of 48 tests being 132 bacteria per cubic centimeter of 40 per cent. separator cream. The separator milk in the same number of tests contained 2,130 bacteria per cubic centimeter and the original milk contained 738 bacteria per cubic centimeter. We conclude from these experiments that the action of the separator tends to break up clumps, chains and imperfectly divided forms so as to increase the colony count.

The experiments with the narrow top pail with and without strainer showed that the count was 620 bacteria per cubic centimeter with the strainer and 674 without the strainer. These figures are the averages of 108 tests. The small difference in favor of the strainer may possibly be due to experimental error and of little significance. Still we think that the strainer should not be omitted, since the milk needs straining at some point or other of production to remove foreign material which is bound to gain access even in the most carefully managed dairies.

As a result of 240 consecutive tests we conclude that straining milk through thick layers of absorbent cotton, as is customary in many dairies, is decidedly disadvantageous. The force of the milk being poured on top of the strainer seems to break up bacterial aggregates so as to increase the colony count in the strained milk.

Our study of the body cells in the separator slime has led to the following conclusions:

1. Polymorphonuclear leucocytes of the neurophile type, large mononuclear leucocytes, and small lymphocytes appear normally in the separator slime of the milk of healthy cows, and as far as we can see they bear no relation to the number of microorganisms present, including streptococci.

2. Eosinophiles may occur in the slime of the separator. The cause and significance of their presence remains problematical.

3. The white corpuscles in milk of normal and diseased cows, and in the blood of the same animals, ought to be studied, differentiated and classified. Such a study will put the subject of leucocytes in milk on a more exact scientific basis than heretofore, and further our knowledge on the significance of the relative number of the various corpuscles in milk in normal and diseased conditions of the cow in general, and in pathological processes of the mammary glands and the udder in particular.

The details of our experiments and a critical discussion of previous work will appear in the January number of the *Journal of Infectious Diseases*.

A Bacterial Disease of Alfalfa caused by Pseudomonas medicaginis (Sackett) n. sp.: WALTER G. SACKETT, Agricultural Experiment Station of Colorado.

The disease has been known in Colorado since 1904, where, in some localities, it has caused the loss of practically 80 per cent. of the first cutting.

In the earliest stages, the stems have a yellowish, olive-green color and appear watery and semi-transparent; soon the color changes to an amber, due to the appearance and subsequent drying of a thick, clear exudate. This dried excretion gives the stem a shiny, varnished appearance, and a slightly rough feel to the touch. These stems blacken in six to eight weeks, become very brittle and are easily broken, which fact makes it almost impossible to handle the crop without an immense amount of shattering.

So far as our observations go, the disease is confined principally to the stem and lower leaves; it appears to run its course with the first cutting, and those plants which have sufficient vitality throw out a good growth for the second and third cuttings.

The disease has been shown to be due to a bacterium which lives in the soil, presumably, and this infected soil enters the plants through cracks in the epidermis which are caused by freezing.

Brief characterization: The causal organism is a short rod with rounded ends, size $1.2 \times .7\ \mu$, actively motile by 1–4 bipolar flagella, non-spore-forming and to which the writer has given the name *Pseudomonas medicaginis*, n. sp. The organism forms filament but no capsules; Gram negative. Surface pellicle in broth; shining grayish white on agar, fluorescent green after three days; gelatin colonies round, gelatin stab-surface growth only, no liquefaction; potato discolored, moderate growth, orange yellow, starch not destroyed; no growth 37.5° C.; no growth in Cohn's solution; growth in Uschinsky's solution. No liquefaction; rennet curd 40 days, no peptonization 25 days; no indol or hydrogen sulphide; nitrates not reduced; ammonia from peptone and asparagin; fluorescent. Habitat—soil. Pathogenic for alfalfa. Classification, Ps. 212.3332133.

This paper, in full, is now in press as a bulletin of the Colorado Agricultural Experiment Station.

A Comparative Test of Several Synthetic Media for the Isolation of B. coli: H. W. LYALL, Brown University.

The three media studied were Harrison and Vanderleck's esculin medium,[1] Dolt's asparagin medium[2] and Dolt's malic acid medium.[3]

The total count, the number of red or black colonies, and the per cent. of these which proved to be *B. coli* were determined.

The results are given in the following table:

Medium.	Source of Sample	Total Count	Average Number of Red or Black Colonies	Per cent. of Colon
Standard. Lactose agar.	A	527	20	50
	B	84	2	
Esculin.	A	105	26	64
	B	30	2	
Asparagin.	A	90	7	88
	B	24	1	
Malic acid.	A	23	5	94
	B	7	2	

A = Pettaconsett intake—Pawtuxet River.

B = East Providence intake—Ten Mile River.

The conclusions drawn were that the esculin medium is of about the same value as the standard litmus lactose agar over which it has no advantages, the asparagin agar gives a much

[1] *C. f. B.*, I., Orig. 51, 1909, 607.

[2] *Jour. Inf. Dis.*, 5, 1908, 616.

[3] *Ibid.*

higher percentage of colon colonies than either the standard litmus lactose agar or the esculin medium, and is a very favorable medium for colon isolation, the malic acid agar gives a still higher percentage of colon colonies, combined with a very low total count, and is a very satisfactory medium for colon isolation when only the active forms indicative of recent pollution are desired.

Studies in Soil Bacteriology, IV.: The Inhibition of Nitrification by Organic Matter, Compared in Soils and in Solutions: F. L. STEVENS and W. A. WITHERS, assisted by P. L. GAINEY, J. K. PLUMMER and F. W. SHERWOOD, North Carolina College of Agriculture and Mechanic Arts.

In experiments regarding nitrification it was demonstrated that:

In Liquid Medium

Peptone 0.8 per cent. inhibited at 4 weeks.

Cottonseed meal, nitrogen equivalent, 0.1 per cent. inhibited at 4 weeks.

Cottonseed meal, nitrogen equivalent, 0.1 per cent. inhibited at 16 weeks.

Peptone 0.8 per cent. inhibited at 16 weeks.

In Liquid Medium absorbed by Soil

Peptone 1.25 per cent. retarded at 4 weeks.

Peptone 5 per cent. inhibited at 4 weeks.

Peptone 5 per cent. did not retard at 16 weeks.

Cottonseed meal, nitrogen equivalent, 0.4 per cent. retarded at 4 weeks.

Cottonseed meal, nitrogen equivalent, 0.5 per cent. retarded at 16 weeks.

Cottonseed meal, nitrogen equivalent, 0.1 per cent. did not retard at 4 weeks.

Cow manure in quantities equivalent to 1, 5, 10, 20, 30, 40, 80, 160 tons per acre in 2-week, 8-week and 12-week periods, did not retard nitrification in soil at 8 weeks but rather favored it.

Nitrification occurred in pure cow manure at 8 weeks and at 12 weeks.

A Simple Low-temperature Incubator: KARL F. KELLERMAN, Bureau of Plant Industry, Washington, D. C.

During the winter of 1905 the writer found it necessary to improvise a low-temperature incubator, and since that time has had similar ones in almost constant operation. The incubator is fundamentally a four-compartment refrigerator carrying ice in the upper right-hand compartment and a heater, consisting of a single incandescent electric-light globe and a thermo-regulator, in the lower left-hand compartment. The thermo-regulator may be operated on a separate circuit using a storage battery or Edison-Leland cell, or from a shunt circuit from the main feed wire. Such incubators may be installed readily and may be used for either temporary or permanent purposes.

Flagella Staining of Pseudomonas radicicola (B.) Moore: KARL F. KELLERMAN, Bureau of Plant Industry, Washington, D. C.

Staining unfixed smears of *Pseudomonas radicicola* with saturated alcoholic stains according to the method of Edwards and Barlow has occasionally given the figures described by these authors as indicating the polar flagella or "giant whips." I have been able to duplicate these appearances almost exactly by mixing bacteria which had no polar flagella with artificial slime or gum and preparing and staining the slides according to the method of Edwards and Barlow. Therefore, while this method of staining may have a diagnostic value for the peculiar slime secreted by *Pseudomonas radicicola*, I do not believe that the flagella themselves are indicated.

Nitrification Studies in Nevada and Utah: KARL F. KELLERMAN, Bureau of Plant Industry, Washington, D. C.

In the arid and semi-arid regions of the west there are two areas which in many ways should be comparable; these areas are the beds of the prehistoric Lake Bonneville in Utah and Lake La Hontan in Nevada. The former, containing the Mormon settlements, has long been famous for the crop-producing power of its soil; the latter has furnished some valuable farms to early ranchers, and recently considerable areas have been brought under irrigation as the Truckee-Carson project.

During the past year nitrification studies have been carried on in Utah by Mr. I. G. McBeth and in Nevada by Mr. E. R. Allen. These studies have indicated that nitrification proceeds in Utah soils following the same general rates of activity in the different layers that occur in eastern soils, nitrate formation decreasing very rapidly below the surface, although it persists to much greater depth than has usually been described. Azotobacter is very frequent and occurs in appreciable numbers even as far below the surface as the tenth foot. In the Nevada soils nitrification is very erratic, the surface layers in many cases nitrifying much less rapidly than deeper layers, while some regions seem to lack the nitrifying flora almost completely. The deficiency in nitrifying bacteria seems correlated with poor crop production in many cases, although the prevalence of alkali is also a source of crop injury. When not present in excessive quantities the white

alkali does not seem to be the controlling element in the nitrification processes, and judging by the innocuous action of gypsum it seems evident that in these regions black alkali is not sufficiently abundant to be an important factor. In the deeper layers of the most unproductive soils a peculiar fungus is very prevalent and is probably associated with the failure of this soil to produce crops.

Desiccated Culture Media: W. D. FROST, University of Wisconsin.

In order to overcome the generally recognized faults of bacterial culture media, such as variation in the composition of small batches, time consumed in preparation, rapidity with which it deteriorates, and its unavailability in small institutions or private practise, the preparation of culture media in large batches in establishments especially equipped for it and then desiccated, is suggested.

The author's work on this problem, covering nearly a decade of time, is considered and samples are submitted.

There is, apparently, no reason why the different culture media can not be put upon the market in a form which requires merely the addition of water and sterilization to make it ready for use. Not only the ordinary, but probably most of the special media, can be prepared in this way and could be put up, where desired, in the form of tablets, these to be of such a size that they could be put directly into test tubes, and when the proper amount of water is added they would be ready for sterilization and use.

Laboratory Desks for Students in Bacteriology: W. D. FROST, University of Wisconsin.

A laboratory desk is described for use in student laboratories for which it is claimed that the maximum number of students can be accommodated in given quarters with, probably, the minimum of confusion. The desk is similar to those used in chemistry, without the shelf above it. It is provided with a wide trough running the full length, which serves as a sink and over which gas hot plates are placed to be used in cooking and sterilization. Each place is provided with three lockers which can be used by as many different students. The reagent shelves are provided for and a shelf for rough weighing at one end, and at the other end the hot-air sterilizer and autoclave. Microscopical work can be done at each place by using artificial light, or at small window desks, provided for separately.

An Inexpensive Incubator Room: W. D. FROST, University of Wisconsin.

A small room is used and maintained at a satisfactorily constant temperature without other change than the attachment to the steam radiator of a thermo-regulator designed for residences. The cost was about thirty dollars, and, with proper shelving, the room will accommodate several hundred students. An arrangement of lockers is also suggested which largely removes the temptation of students to appropriate cultures not belonging to them.

The Absolute Relation of B. coli to Oxygen: F. G. KEYES, Brown University.

The absolute relation of gaseous oxygen to the growth and gas production of *B. coli* promises to be somewhat complicated.

In a study of the absolute gas production of *B. coli* in vacuo,[4] it was found that the gas evolved from a 1 per cent. asparagin, 0.2 per cent. disodium phosphate, 1 per cent. dextrose medium began to fall off very decidedly after 115 hours, but the gas evolved was constant in composition.

In the presence of pure oxygen, no other gas being present, it is found that the rate of evolution of gas is much smaller, but gas production continues for a much greater length of time. The composition of the evolved gas is different when the organism is grown in the presence of oxygen from what it is in vacuo. The composition of the gas depends to a certain extent upon whether the medium is neutralized or not. Some oxygen is absorbed by the growth of the organism in the medium.

The tables below summarize the results of the experiments.

The Absolute Gas Production of B. Typhosus: L. J. GILLESPIE, Brown University.

While attempts were being made to find a synthetic medium suitable for the growth of *B. typhosus* and such as to facilitate a comparison with the gas production of *B. coli*, preliminary experiments were made using a medium containing Witte's peptone. *B. typhosus* was grown on a neutral medium containing 1 per cent. dextrose and 1 per cent. peptone; and the gas evolved, obtained by the procedure given by Dr. F. G. Keyes, was analyzed. The amount of gas found was so small that analyses with the ordinary 100 c.cm. gas burette and gas pipettes were carried out with difficulty. The analyses indicated, however, the following results for 48 hours' growth:

[4] *Jour. Med. Res.*, 16, 1909, 69.

ABSOLUTE GAS PRODUCTION

In Vacuo (Medium not neutralized)					In Oxygen (Medium neutralized with NaOH)					
Hours in Incubator	Total Per Cent. of Gas	Composition of Gas			Hours in Incubator	Total Per Cent. of Gas	Composition of Gas			Oxygen Absorbed in c.cm.
		CO_2	H_2	N_2			CO_2	H_2	N_2	Dry 20° C. 760 mm.
24	26.7	63.23	36.61	0.15	25	15.17	98.83	0.18	0.99	1.57
48	45.6	63.27	36.05	0.37	127	18.56	97.90	0.52	1.58	2.55
115	99.9	63.49	35.81	0.70	167	27.18	98.21	1.06	0.73	5.52

Total gas per 100 c.cm. medium ..... 1.4 c.cm.
CO_2 ..... 92%
H_2 ..... 3%
N_2 (by difference) 5%

By means, however, of an improved gas analysis apparatus devised by Dr. Keyes it was found possible to analyze accurately such small amounts. The results for 15 days' growth were as follows:

	Exper. I.	Exper. II.
Total gas obtained ...	3.49 c.cm.	5.89 c.cm.
Total gas per 100 c.cm. medium	2.55 c.cm.	2.32 c.cm.
CO_2	93.25%	97.10%
H_2	2.03%	2.12%
N_2 (by measurement)	4.44%	.77%
	99.72%	99.99%

Experiment II. probably approaches nearest the truth, as the leakage of only a trace of air into the bulb during incubation would easily suffice to falsify the figure for nitrogen.

Substitutes for Löffler's Bloodserum for the Diagnosis of Diphtheria: W. W. Browne, Brown University.

Attempts were made to grow the diphtheria bacillus on Hadley's medium,[5] solidified with 5 per cent. agar. Growth on this medium was atypical. Albumen was then substituted for the glycocoll of Hadley's medium, but on sterilization the albumen was coagulated as a flocculent precipitate through the medium. The growth was scanty. Attention was now turned to egg as a source of albumen. Egg was mixed with dextrose broth in the ratio of 1 part of broth to 3 of egg and coagulated. The medium was hard and firm. The slow growth of the bacillus on this medium would prevent its use in the diagnosis of diphtheria.

Alkaline albuminate was then substituted for the albumen. Although the albumen was not coagulated by heat, nevertheless, it was precipitated by the acid produced by the bacillus. The growth on this medium was satisfactory, but the precipitation of the albumen prevented ready diagnosis. Acid albumen, on the other hand, showed scanty growth.

Next, a 6 per cent. solution of commercial albumen was mixed with dextrose broth in the ratio of 1 part of broth to 3 of albumen. This medium when coagulated presented a hard firm surface. The bacillus seemed to grow well upon it and diagnosis from throat cultures was fairly easy. While this medium does not seem to be a perfect substitute for the Löffler's serum, yet, in times of scarcity, it might be used to good advantage.

The Hygiene of the Swimming Pool: John W. M. Bunker, Brown University.

The swimming pool is liable to be a source of contagion if the water is used for any length of time. Cases of ear and nose affections have been traced to this source. Typhoid is apt to enter the pool and spread therefrom, and unless an abundant supply of water is available, the expense of frequent renewal is prohibitive.

Filtration is the method of purification ordinarily employed, but usually yields only partial purification, inasmuch as only a small part of the water of the pool is removed, filtered and returned to the pool. The filter at Brown University has a high efficiency and keeps the water of the pool a good color, but the bacterial content of the pool is always high.

Sterilization by heat is out of the question because of expense. Sterilization by the addition of chemicals has proved effective in the case of

[5] *Journal of Infectious Diseases*, Supp. 3, May, 1907, p. 95.

sewage and water, chlorine being especially effective.

The application of this method to swimming-pool water was tried, with the result that hypochlorite of lime in quantities sufficient to give one part available chlorine to two million of water gave efficient sterilization. The pool when so treated remained practically sterile for four days, during constant use. No odor or taste from the chemicals was noticeable. How often such treatment need be applied must vary with local conditions.

Probably for the ordinary swimming pool, if these experiments are borne out by experience, the addition of hypochlorite of lime, in the proportion of one part available chlorine to two million of water, twice a week, would insure a practically sterile pool.

A New Device for the Isolation of B. coli: W. F. WELLS, Massachusetts Institute of Technology.

A laboratory device was described which combines certain advantages of both dextrose broth and lactose bile. It consists of two Durham tubes (the first containing an enrichment medium, as dextrose, the second a selective medium, as bile) so connected by a capillary that the production of gas in No. 1 immediately causes a flow into the bile tube. As the capillary leads from the upper part of the inverted inner tube in No. 1, further increase in gas lowers the liquid below the mouth of the capillary and the flow cuts itself off.

If water containing *B. coli* is put into the dextrose tube the non-motile and aerobic bacteria remain outside the smaller inner tube, while *B. coli*, swimming continually in search of a better medium, finds its way around; so it is likely that such organisms will reach the portion about the mouth of the capillary very soon. With every advantage they multiply rapidly, and in a few hours the inverted tube contains a seething culture of vigorous *B. coli*, and gas forms quickly. The change in level causes a flow into the bile tube, just at the time of most vigorous growth, and then cuts itself off. The bile now contains an almost definite measure of thriving *B. coli*, probably in pure culture. Under these definite conditions the quantity of gas produced should be regular, and the per cent. formed in a given time after the first tube ferments significant.

The tubes are handled almost as simply as ordinary tubes. They are clamped together; a small test tube is hooked into the short leg of the capillary, while the longer legs straddle into both large tubes. They are made up and sterilized as usual, filling upon cooling.

The double medium secures the advantages of both. It does more; it preserves *B. coli* at its most favorable stage, the moment of gas production, and inoculates the bile under definite conditions with a dose of healthy organisms. It may be reasonably expected that the gas formers which are accustomed or can accustom themselves to the digestive tract will be indicated. Practical results show no unexpected error in the reasoning, and as far as they go promise an efficient test.

NORMAN MACLEOD HARRIS,
Secretary
UNIVERSITY OF CHICAGO

SOCIETIES AND ACADEMIES

THE PHILOSOPHICAL SOCIETY OF WASHINGTON

THE 677th meeting was held on February 26, 1910, Vice-president Abbot in the chair. The following paper was read:

The Recovery and Discussion of the Earliest Magnetic Observations along the Antarctic Continent and in the Approaches to the South Magnetic Pole: Mr. G. W. LITTLEHALES, of the U. S. Hydrographic Office, Navy Department.

The results were chiefly in a chart of terrestrial magnetic lines for the epoch 1840, representing the inclination and the declination of the magnetic needle founded upon the observations of the United States exploring expedition which discovered and traversed the coast of the Antarctic continent in about 66° of south latitude between the 160th and 97th degree of longitude east of Greenwich, in the beginning of the year 1840.

The observations presented have but lately been recovered from among a part of the records of the exploring expedition of which all trace was lost for many years, and they have resulted in the portrayal of a passing state to which we could not otherwise have reascended.

Such magnetic lines from original observations made long ago have a value which increases with the lapse of years on account of their importance in elucidating the changes which time works in altering the magnetic state of the earth.

The interpretation of the results proves that American explorers were the first to point out the region of the south magnetic pole by disclosing its presence, at that epoch, as an area of considerable extent, over which the dipping needle stood vertical or nearly vertical, around a position in 68° 50′ of south latitude and in longitude 135° east of Greenwich.