stains, staining with the acid fuchsine of Auerbach.

The cytoplasm undergoes changes in structure as secretion progresses, first becoming vacuolar, then slightly granular, still taking plasma stains, and finally densely granular staining with the nuclear dyes. There is evidence to indicate that the cytoplasmic changes are controlled by the nucleus.

The nucleus seems to be the center of metabolic activities participating in the formation of the secretion substance, but playing a passive rôle in the actual process of secretion or extrusion of material from the cell.

The following are the titles of papers presented at a joint meeting of the section and the American Mycological Society:

Some Reasons for Desiring a Better Classification of the Uredinales: J. C. ARTHUR.

Uredineæ of the Gulf States: S. M. TRACY.

North American Gill Fungi: F. S. EARLE.

Lichens and Recent Conceptions of Species: BRUCE FINK. (By title.)

Cultures of Colletotrichum and Glœosporium: P. H. ROLFS.

The Affinities of the Fungus of Lolium temulentum: E. M. FREEMAN.

Peridermium cerebrum Peck and Cronartium Quercum (Berkeley): C. L. SHEAR.

Ramularia: An Illustration of the Present Practise in Mycological Nomenclature: C. L. SHEAR.

Notes on Pachyma cocos: P. H. ROLFS.

Penicillium glaucum on Pineapple Fruits: P. H. ROLFS.

The Occurrence of Fusoma parasiticum Tubuef in this Country: PERLEY SPALDING.

Some Peculiar Fungi New to America: W. G. FARLOW.

FRANCIS E. LLOYD,
Secretary.

SOCIETY OF AMERICAN BACTERIOLOGISTS.

THE seventh annual meeting of the Society of American Bacteriologists was held in the New Medical Building, University of Michigan, December 28 and 29, 1905.

Professor Edwin O. Jordan, president of the society, gave the introductory address on 'Variation in Bacteria.'

The following papers were presented:

Preliminary Communication upon a Spirochætal Infection of White Rats, and Observations upon the Multiplication of the Spirochætes in Fluid Medium: Drs. NORRIS, PAPPENHEIMER and FLOURNEY, Pathological Laboratory, Bellevue Hospital, New York.

With the blood of a case of relapsing fever, the authors were able to inoculate successfully monkeys and white rats. The following is a summary of the results obtained:

1. A subcutaneous inoculation in white rats, with blood containing spirochætes, is followed in the course of two or three days by the presence of more or less numerous spirochætes in the circulating blood. These persist from one to three days.

2. Unlike the spirochætal infection of man and monkeys, no relapses occur.

3. The rats show no obvious symptoms of illness, no local reaction, no visceral lesions of consequence, save turgescence and enlargement of the spleen.

4. In all, a series of about twenty-five generations have been kept alive through rats.

5. Observations show that immunity is conferred by previous infection. Inoculation of spirochætal blood, plus small doses of serum from animals that have gone through a previous infection, retards, or completely inhibits, the development of the spirochætæ in the circulating blood of rats. Subcutaneous inoculation of serum, fol-

lowed several days later by injection of spirochætal blood, has not, in the few experiments made, prevented the development of the infection in rats.

6. There is no evidence that longitudinal division ever occurs. On the other hand, the constant occurrence of organisms showing an extreme attenuation in the central portion, as well as organisms lying end to end, with their pointed extremities in close approximation, strongly indicates transverse fission or possibly fragmentation. Long thread-like forms showing several areas of attenuation are seen at times.

7. No evidence of an enveloping or undulating membrane was seen in specimens stained by Wright's, Giemsa's, Prosca's or Loeffler's methods. Likewise no evidence of distinct cilia was obtained. The spirochætes, therefore, more closely resemble the bacteria than protozoa.

8. In human and rat blood to which has been added sodium citrate to prevent coagulation, there can be seen within twenty-four hours after inoculation with a few drops of rat blood containing spirochætes, a very evident increase in the number of these organisms. The spirochætes are vastly more numerous in the smears from the culture fluid than in control smears taken at the time of inoculation, notwithstanding the dilution of infected blood with approximately thirty to fifty times its volume of medium.

By inoculating several drops of this first generation into a second blood tube, the organism was found in approximately the same numbers in the transplant. A third generation, however, failed to grow.

Multiplication apparently occurs in the undiluted citrate blood from infected rats, kept overnight at room temperature. Moreover, citrated blood, kept at room temperature for six days, retains, unimpaired, its infective properties.

Spirochæte Obermeieri: F. G. Novy and R. S. Knapp, University of Michigan, Ann Arbor, Mich.

The spirochæte studied was obtained through the kindness of Dr. Norris. It has been kept alive by successive passage through white rats for over two months. As a result of intraperitoneal inoculation the parasites appear in the blood in thirty-six to forty-eight hours after inoculation and disappear within twenty-four hours, and do not reappear. The rats are then immune to subsequent inoculation. The disappearance of the spirochætes was shown to be due to the formation of anti-bodies. Spirochætal blood when kept *in vitro* retains its virulence for more than fifteen days.

The blood of rats which have been given repeated injections of spirochætal blood exerts a most marked preventive and curative action. When injections of such blood are made, before inoculation with spirochætes, the latter fail to appear. Similarly, when simultaneous injections of immune and spirochætal blood are made no infection results. Even when the immune blood is injected ten, twenty-five and thirty-six hours after inoculation with spirochætes, that is to say, at any time before the spirochætes actually appear in the blood, they will fail to appear, whereas in the controls they become numerous.

The curative action of the immune blood is equally pronounced. In rats which have from five to ten spirochætes per field of the one-twelfth-inch objective an injection of two cubic centimeters of immune blood is followed within one hour by a total disappearance of the spirochætes from the circulation (as actually demonstrated before the society). After this the parasites do not appear, while in the controls they persist for twenty-four hours. This remarkable curative action of immune blood in the case of the white rat will form without

doubt the basis of curative treatment in relapsing fever and in tick fever of Africa. It is the intention of the authors to work on the practical application of the principle discovered.

Spirochætal blood which has been diluted with ten parts of salcitrate solution and filtered through a Berkefeld filter, under a pressure of fifty pounds, yields a filtrate which, when injected into white rats, produces typical spirochætal infection. The spirochætes, as in the case of cultures of *Trypanosoma Lewisi,* are filterable through a Berkefeld filter. Importance of this fact in its bearing upon the so-called ultra-microscopic organisms was pointed out.

All attempts thus far to cultivate the spirochæte on blood agar have failed, but this subject will be followed further. The spirochætes multiply by transverse divisions and show other characteristics which belong to bacteria. On the other hand, the transmission of spirochætal diseases by insects, the persistence of the organisms in such insect hosts for months, and the infection of the eggs of such insects, are the main facts known at present which point to a possible protozoal nature of the parasites.

The persistence of the spirochætes of tick fever in the blood of rats for three to eight days, as shown by Dutton and Todd, would indicate that this organism, though closely related, is, nevertheless, different from that studied by us. It goes to show that tick fever of Africa and the relapsing fever of Europe are due to different species of *Spirochætes.*

This paper will shortly appear in the *Journal of Infectious Diseases.*

Mosquito Trypanosomes: F. G. NOVY, W. J. MACNEAL and H. N. TORREY, University of Michigan, Ann Arbor, Mich.

In a previous paper on bird trypanosomes it was pointed out that these organisms grew readily in the test tube on blood agar and that the resulting forms resembled the flagellates which Schaudinn found in the gut of mosquitoes which had fed on owls infected with *Halteridium* and with *H. Ziemanni.* In other words, the position taken was that the flagellates observed in the mosquitoes did not represent stages in the life history of intracellular parasites, but were actually cultures *in vivo* of trypanosomes present in the blood of the birds used. In confirmation of this position it was desirable to show that trypanosomes could actually grow and multiply in the gut of mosquitoes and that such forms actually did correspond to those which would be obtained *in vitro.*

Accordingly, large numbers of mosquitoes were captured along the river bank and allowed to feed on perfectly clean animals, such as rats, guinea-pigs and pigeons. At varying intervals, thirty-six to seventy-two hours after feeding, the contents of the stomachs of the mosquitoes were examined in living and in stained preparations and cultures on blood agar were made at the same time. Of more than 800 mosquitoes which were examined in this way about 120, or 15 per cent., were found to have a flagellate infection of the intestinal tract. In some this was very marked; large masses of rosettes, flagella inside, completely filling the lumen of the tube.

Several distinct forms of trypanosomes were met with; the most common of these was a *Herpetomonas* (probably *Herpetomonas subulata*) and *Crithidia fasciculata.* Owing to the large numbers of bacteria usually present much difficulty was experienced in obtaining cultures of these flagellates. Eventually, however, the *Herpetomonas* was isolated in mixed culture associated with a minute coccus, while the *Crithidia* was obtained in association with

a yeast. These mixed cultures have now been grown in the laboratory for some six months. Several other cultures were obtained, but these were soon outgrown by the accompanying bacteria.

The cultural forms of these two organisms are exactly the same as that seen in the gut of the mosquito, thus confirming the view expressed that the flagellates found growing in the intestinal tube of insects represent cultural forms *in vivo*, and, as such, correspond to those obtained *in vitro*. In both conditions not only was the form and size the same, but the blepharoplast was anterior to the nucleus. The *Herpetomonas* was characterized by the presence of two diplosomes in the posterior part of the cell. These bodies were found in the parasites within the mosquitoes as well as in those grown in culture. Animals inoculated with the cultures failed to show an infection.

When mosquitoes are allowed to feed on *T. Brucei* or *T. Lewisi* these parasites may be detected in the blood in the intestine of the mosquito twenty-four hours after feeding, and even later, and rats inoculated with such stomach contents develop typical infection.

The trypanosomes which have been met with by various investigators in the stomachs of tsetse-flies, lice, leeches, etc., are distinctly 'cultural forms,' since they show the blepharoplast in a position anterior to the nucleus. This fact indicates that all such forms can be cultivated in the test tube. The *Herpetomonas* forms found in flies and mosquitoes are true cultural trypanosomes, and, without doubt, future studies will reveal the blood parasite from which they are derived. The *Crithidia* show no undulating membrane, in the ordinary truncated form, and on account of their peculiarity for the present at least are to be considered as representing a distinct genus.

Isolation of Trypanosomes from Accompanying Bacteria: F. G. Novy and R. S. Knapp, University of Michigan, Ann Arbor, Mich.

In general, it may be said that bacteria once introduced into a culture of trypanosomes tend to outgrow and check the development of the flagellates. In exceptional instances, however, the bacteria thus introduced exert little or no interference and may be even apparently beneficial. While in the former case the trypanosomes die out, in the latter instance the mixed culture may be kept for six months or longer.

The isolation of the trypanosomes in pure form from such mixed cultures is a matter of some importance, especially when it is desired to study the pathogenic action of the flagellates. The need of some method of separation was particularly felt in connection with the study of the mosquito trypanosomes which, since they are present in the intestinal canal, are always accompanied by various bacteria and yeasts. After many ineffectual attempts the following method was successfully employed for the isolation of pure cultures of *Herpetomonas* and *Crithidia*.

By means of a small glass spatula, made by drawing out the end of a glass rod, a little of the mixed culture was spread in a series of streaks over six Petri dishes containing solidified blood agar. The Petri dish, known as the 'Kriegsministeriums-Modell 2,' made by Greiner and Friedrichs, is particularly adapted for this purpose, inasmuch as it can be sealed effectually by means of a wide rubber band. The sealed dishes are then set aside at room temperature for ten to twelve days. The last plate or two of the series will be found to show isolated colonies of trypanosomes which can be transplanted in the usual way to the test tube. This method will undoubtedly be found useful in future studies of

the flagellates found in the intestinal canal of insects and other sanguivora. The intestinal contents can be spread directly over the plates in the manner indicated.

The Action of So-called Complementoid in Immune Serum: W. H. MANWARING, Indiana University.

Working with goat serum, immunized against sheep corpuscles, the action of so-called complementoid was estimated quantitatively and plotted graphically. From the curves so obtained, the following conclusions are drawn:

1. Hemolytic 'complementoid,' added in increasing amounts to hemolytic serum, or to an artificial hemolytic amboceptor-complement mixture, causes, at first, a rapid increase in hemolytic power.

2. This increase soon reaches an apparent maximum, after which a further increase in 'complementoid' causes: (I.) no change, (II.) a rapid diminution in hemolytic power, or (III.) a slow increase in that power.

3. This variability in the action of 'complementoid' when used in large amounts, depends, at least in part, on the length of time the serum is heated to produce the 'complementoid.'

4. The action of 'complementoid' is so pronounced that quantitative work that does not take its presence into consideration is practically valueless. This applies to such experiments as those forming the basis for the doctrine of 'deviation of complement.'

5. It would be difficult to explain the action of 'complementoid' by means of any of the existing hypotheses regarding the action of immune serum.

6. No conclusion is yet drawn as to whether the so-called complementoid is really a degeneration product of complement, or whether it may not be a mixture of spilt-products of other serum components, or, in part at least, certain thus-far unrecognized thermo-stable components of normal serum.

Abnormal Cheese Troubles due to Lactose Fermenting Yeasts: H. L. RUSSELL and E. G. HASTINGS, University of Wisconsin.

The defective trouble in cheese here described is due to the presence of a milk sugar splitting yeast. This type of micro-organism grows rapidly in milk or whey, especially when the same contains a considerable amount of acid. The milk sugar is decomposed and alcohol and carbonic acid formed in abundance, as well as undesirable flavored products. The organism causing this trouble is destroyed at the temperature of 60° C. in ten minutes, but is capable of resisting the high temperature (55° C.) for thirty-five minutes, which is used in the manufacture of Swiss cheese.

This type of organism is introduced into the milk primarily through the medium of certain customs that commonly prevail in Swiss cheese factories. First, the cold process of recovering the butter fat. Whey is held over from one day to the next in order to permit the fat to rise, and this gives an opportunity for the souring process to go on, and consequently favorable conditions for the development of these yeasts.

Second, soaking the natural rennets in old sour whey and adding this rennet extract solution to the fresh milk. These processes afford ideal conditions for the growth of the yeast germ and consequently permit of the infection of the fresh milk.

Studies of the distribution of this type of organisms show them to be much more abundant in regions where Swiss cheese is made than where the American cheddar system is practised. Yeasts have not heretofore been recognized as important factors in dairy processes, except in a few cases, but where conditions of manufacture

permit of the development of lactic acid the conditions become favorable for the growth of this type of germ life.

The complete paper appeared as Bulletin No. 128 of the University of Wisconsin Agricultural Experiment Station.

Lactic Acid Bacteria: W. M. Esten, Wesleyan University.

Since the publication of the paper on 'Acid Organisms of Milk' in 1896 the investigations as outlined in that article have been continued. The extent of territory then studied was only from Ohio to Maine. Since then samples of milk have been received from nearly every section of the United States and from Canada. There is probably no class of bacteria which has caused so much confusion in regard to names and classification as the lactic acid group. Quite a number of investigators have been studying the same organisms under different names.

In the results of these investigations are found two distinct groups of lactic acid bacteria. First, the gas-forming bacteria and, second, the non-gas-forming bacteria.

The first group is of much less importance than the second. It consists of *Bacillus coli communis,* which is not very generally found in milk, and *Bacterium lactis aerogenes* with all of its varieties. This is the *Bacillus acidi lactici* of Hueppe and also of Eckles. The only difference between *B. coli* and *B. lactis aerogenes* seems to be that of motility. This group is distinctly the aerobic one. This group may be considered as a detrimental contamination to milk and its products.

The second group is the facultative anaerobic one and never produces gas in the sugar media. Their function seems to be principally the production of lactic acid. Although there may be several species of bacteria in this group, the author is of the opinion that only one species of bacterium, with its varieties, belongs to this group or division, namely, *Bacterium lactis acidi,* using the name given to it by Leichman as being the most appropriate. In the publication of 1896 it was called *Bacillus acidi lactici* through an error, also made by Gunther, thinking it was Hueppe's *Bacillus acidi lactici.* Names given to this organism by investigators are as follows: *Streptococcus acidi lactici* (Grotenfelt), *Bacillus acidi lactici* (Gunther), *Bacterium lactis acidi* (Leichman), *Bacillus lactari* (Dinwidie), *Bacillus a* (Freudenrich), and some others. In a former publication *B. lactis acidi* I. and II. were supposed to be recognized. As the result of later investigations the No. II. should be discarded, it probably being a devitalized or degenerate form of No. I.

The characters of *B. lactis acidi* are always distinctive if grown on lactose-litmus-gelatine as a small colony scarcely more than one quarter to one half millimeter in diameter. It avoids growth on the surface almost entirely. Under mica plates it grows more robust and produces more acid. Colonies vary from dark opaque to light-colored ones with dark specks or granules in the central portion. Stumpy spines or processes may or may not be present, a character determined by the thickness and amount of moisture, in the gelatine. In sterile milk at 37° C. it curdles in from twelve to twenty-four hours, after which there is no further change. On lactose-free agar it grows but slightly and lives only a week or two on any kind of agar. The best kind of media for its growth is milk, milk-agar, lactose and dextrose bouillon.

There is probably no organism, with the exception of some soil bacteria, of more benefit to mankind, when we consider that milk which does not contain this organism is a dangerous product if kept for any length of time. Milk free from these lactic bacteria is a good medium for the growth

of all kinds of putrefactive bacteria and disease germs, while milk which contains *B. lactis acidi* soon has all other forms destroyed by the acid or the growth of the lactic bacteria, and, further, when we discover that every properly ripened lot of cream with the most desirable flavor, and every normally ripening cheese, has from 90 to 99 per cent. of this organism present.

The Microscopic Estimate of Bacteria in Milk: FRANCIS H. SLACK, M.D., First Assistant Bacteriologist, Boston Board of Health Laboratory.

The special apparatus used for centrifugalizing the milk samples consists of an aluminum disc and covers ten inches in diameter and five eighths inch in depth, fitted to hold twenty small glass tubes, arranged radially. These tubes hold about two cubic centimeters each and are closed at both ends with rubber stoppers.

The samples in the tubes are centrifugalized ten minutes at a speed of 2,000 to 3,000 revolutions a minute, thus collecting the whole sediment from each sample on the outer stopper.

The sediment is obtained by breaking up the cream, pouring out the milk and carefully removing the stopper with the adhering sediment, not allowing any milk to run back on the sediment to disturb it. It is then evenly smeared with a drop of sterile water over a space of 4 sq. cm. on a glass slide, dried and stained with methylene blue.

Microscopic examination shows the approximate number and morphology of bacteria present as well as the presence of pus and streptococci.

The number of bacteria found in a representative one twelfth oil immersion field bears a fairly constant relation to the 1–10,000 plate culture (grown for twenty-four hours in a saturated atmosphere at 37° C., 1 per cent. agar being used with a reaction of + 1.5).

Thus, as a rough estimate, each coccus, bacillus, diplococcus or chain in a representative one twelfth oil immersion field represents 10,000 bacteria to a cubic centimeter in the sample of milk examined.

Advantages are: rapidity of examination, accuracy, easily learned technique, lack of costly apparatus.

The writer believes the method can, in experienced hands, safely be used for certifying milk, certifying those samples in which no bacteria are found, the large number of samples which could be examined and the increased efficiency of the supervision more than compensating for a slightly greater accuracy in plate counts.

The Quantitative Determination of Leucocytes in Milk: ARCHIBALD R. WARD, University of California.

The determination of leucocytes in milk has been suggested by several writers as a means for the detection of dairies marketing milk from cows with inflamed udders. A series of duplicate determinations from the same sample of milk were made by the method of Doane and Buckley, of the Maryland Agricultural Experiment Station, College Park, Md., and by the method described by Dr. Stewart, of the Philadelphia Bureau of Health. The Doane-Buckley method gave more satisfactory results with duplicate determinations than did that of Dr. Stewart. The numerical results by the Doane-Buckley method varied from four to forty times higher than those obtained by the Stewart method.

Kinds of Bacteria concerned in Souring of Milk: P. G. HEINEMANN, University of Chicago.

All so-called lactic acid bacteria belong to two groups, the colon aerogenes group and *streptococcus* group. This arrangement is arrived at by a comparative study of culture characteristics of pathogenic, sewage, fecal and milk streptococci. The

coagulative power of pathogenic, sewage and fecal streptococci becomes equal to that of milk streptococci by repeated passages through milk. Streptococci from milk form in long chains in lactose broth, and the chains disappear upon inoculation in litmus milk, and characteristic diplococci and short chains of three to six members appear instead. Artificial lactic acid fermentations, produced by inoculation of pure cultures of lactic acid bacteria of either group or of cow feces in sterilized milk, closely resemble the natural process. Investigations lead to the following conclusions:

1. *Bacillus acidi lactici* is a myth. The ordinary bacteria producing lactic fermentation are *Bacillus aerogenes* var. *lacticus* and *Streptococcus lacticus*. The possibility of *B. coli* participating in lactic fermentation is not excluded.

2. *Streptococcus lacticus* (Kruse) agrees in morphological, cultural and coagulative properties with pathogenic, fecal and sewage streptococci.

3. Souring of milk is caused by cooperation of both groups of bacteria, and is participated in by peptonizing bacteria always present in milk.

4. Gas is produced by *B. aerogenes* var. *lacticus*, but as a rule is held in check and ultimately stopped by the presence and final ascendency of *Streptococcus lacticus* (Kruse).

5. Acid is produced during lactic fermentation by both classes of organisms to a marked degree. *B. aerogenes* var. *lacticus* is more sensitive to the presence of acid than *Streptococcus lacticus* (Kruse). This results in the presence of *B. aerogenes* in large numbers in initial stages of fermentation, *S. lacticus* (Kruse) becoming master of the field in terminal stages.

6. Lactic acid bacteria are of intestinal origin and gain access to milk with particles of cow feces.

7. Artificial lactic acid fermentation in sterilized milk can be produced by inoculation of pure cultures of bacteria of either group or better by the two groups combined.

8. Since *Streptococcus lacticus* (Kruse) is invariably present even in fresh milk collected with good precautions, the sanitary significance of streptococci in market milk will need further investigation.

A Note on the Indol-producing Bacteria in Milk (preliminary communication): S. C. Prescott, Massachusetts Institute of Technology.

The occurrence of indol-producing bacteria in milk suggests the possibility of some connection between these organisms and the intestinal diseases often so prevalent in children fed on raw milk, especially that received in the larger cities, where the milk may be forty-eight hours old before it reaches the consumer.

A large number of samples of fresh milk, collected from about 175 different farms, have been examined to determine if there is any numerical relation between the indol-producing bacteria and the total number present in the milk. The samples were in general about six hours old at the time of the examination. The total numbers were determined in the usual way by plating on agar (reaction + 1) and incubating at 37.5° for twenty-four hours. Dilution of 1–10,000 was employed. Indol was determined by inoculating 1/100 c.c. of milk in a tube of peptone solution, incubating three days at 37.5°, and then testing for indol by adding a minute amount of sodium nitrite and 1 c.c. of 1:1 sulphuric acid.

In all 524 samples were examined, ranging in total numbers from less than 5,000 to 121,000,000 bacteria per cubic centimeter. Of these but 38 samples exceeded 1,000,000 in total count; 132 samples, or almost exactly 25 per cent., gave strong

indol reaction; 278 samples in which the determination was carried out quantitatively showed the following relation between total numbers and occurrence of indol:

	No. Samples.	No. showing Indol.	Per Cent.
Above 1,000,000.....	13	9	70
Between 500,000 and 1,000,000.....	2	1	50
Between 100,000 and 500,000.....	34	14	41
Below 100,000.....	229	32	14
25,000 or below.....	133	17	12

Absorbent Cotton as a Medium for Distributing Pseudomonas radicicola: H. A. HARDING and M. J. PRUCHA, Agricultural Experiment Station, Geneva, N. Y.

Absorbent cotton wrapped in paper and tin-foil is now widely used as a means of distributing *P. radicicola.*

The large number of failures to get results with this method contrasted with the high percentage of success when the germs were shipped in soil, lead to an examination of the packages of inoculated cotton.

In these examinations the directions on the packages were followed as closely as possible except that sterile solutions were used in order to confine the resulting growth to the germs actually upon the cotton.

Repeated examinations of twenty-five separate packages of cotton gave only an occasional colony resembling *P. radicicola* and in most cases not a single suspicious colony was found.

As a check upon the accuracy of these examinations duplicate samples from six packages were examined in the laboratories of Professor F. D. Chester and Drs. C. E. Marshall, E. M. Houghton and J. G. Lipman.

An explanation of the absence of *P. radicicola* from the inoculated cotton was found in the inability of the germs to withstand the accompanying desiccation.

Two separate laboratory trials with bouillon cultures of *P. radicicola,* placed upon sterile absorbent cotton showed that all but an occasional germ died within a few days.

Under farm conditions the contamination which enters the fluid usually represses the few surviving *P. radicicola.*

The details of the work are given in the New York Agricultural Experiment Station Bulletin 270.

The Bacteria of the Root Nodules of the Leguminoseæ: KARL F. KELLERMAN and T. D. BECKWITH, Bureau of Plant Industry, Washington, D. C.

For the present preliminary work organisms from four species of legumes have been studied: velvet bean, soy bean, garden pea and alfalfa.

Our laboratory results are summarized briefly as follows:

Beef agar (made according to formula adopted by the American Public Health Association): surface colonies, circular in outline, somewhat convex, rather wet, shining, tinged with straw color, 1 to 6 mm. in diameter. Submerged colonies, lenticular in outline, convex after reaching surface, 3.5 by 5 to 0.25 by 0.5 in diameter.

Synthetic agar, low in nitrogen (agar flour, 10 g.; magnesium sulphate, 0.2 g.; potassium phosphate (monobasic), 1 g.; cane sugar, 10 g.; filtered tap water, 1,000 c.c.): surface colonies, circular, translucent, convex, wet, shining, 1.5 to 4 mm. in diameter. Submerged colonies, lenticular, convex after reaching surface, 0.5 by 1 to 2.5 by 5.5 mm. in diameter.

Synthetic agar (same as above plus 4 g. dibasic ammonium phosphate): surface colonies, circular, somewhat convex, wet, shining, faintly tinged with cream, 1 to 5 mm. in diameter. Submerged colonies, lenticular, convex after reaching surface, 0.25 by 0.5 to 2.5 by 5 mm. in diameter.

Does not liquefy beef or synthetic gelatin; does not form indol. Aerobic; does not form nitrites or nitrates; does not form gas.

Litmus milk: with velvet bean there is apparently no change in seven days at 28° C.; after sixteen days the litmus is almost decolorized and some acid has been produced. Soy bean is similar, except the milk becomes scarcely acid, and subsequently a very slow precipitation of the casein takes place. Alfalfa, on the other hand, produces alkali very distinctly, and forms a viscous pellicle.

Potato cylinders: the velvet bean organism produces a colorless to grayish-white, even growth. The soy bean has a very spreading growth, between clay and cream buff. Alfalfa, colorless to grayish-white; the colorless areas separated from the whitish ones, giving a coagulated appearance.

Variations in Gas Production by Bacteria-producing Soft Rot in Vegetables: H. A. Harding and M. J. Prucha, New York Agricultural Experiment Station.

During the past five years the group of organisms connected with the soft rot of vegetables has been studied jointly by the Botanical Department of the University of Vermont and the Bacteriological and Botanical Departments of the New York Agricultural Experiment Station. In this study about forty-five cultures, including six which have been described in literature as distinct species, have been studied in detail. A comparative study of their points of difference has been repeated ten times in most cases.

This group lies just on the border line of gas formation from dextrose, lactose and saccharose in Smith tubes. In all cases there is growth in the closed arm and production of acid. A majority of the cultures produce gas, ranging in amount from a small bubble to a c.c. These determinations have been made each time in duplicate or in triplicate.

As optimum conditions are obtained an increasing number of cultures produce gas from all of these different sugars. A culture known as Vermont XLVIII. which has long been considered as a type of the class fermenting only lactose was recently induced to produce gas from dextrose at the Vermont Laboratory.

Some striking differences still remain. A culture known as 0.2e was studied in the laboratory about a year and then inoculated into a plant in the greenhouse. It there produced the typical soft rot. A culture isolated from this experimental plant was called 0.2f. The second culture agrees with the first in pathogenicity and in all other cultural characteristics except that of gas formation. While 0.2f ferments all three sugars 0.2e forms gas only from lactose and saccharose. This notwithstanding that these two cultures have now been studied together for some years.

Other similar instances have been observed but this will suffice to indicate that there are cases where a single routine test of fermentation may lead to errors in classification.

The Employment of Glycerin as a Differentiating Medium for Certain Bacteria: Eduardo Andrade, Florida Board of Health.

It has been determined by previous investigations of the writer that the addition of glycerin to nutrient media increases the acid-producing power of some intestinal bacteria. As an indicator for this change, acid fuchsin Grübler is neutralized to the point of decolorization with caustic potash. Both inorganic and organic acids react on the indicator, changing it to red. Alkalies decolorize it and change it to a light yellow color. The indicator is extremely sensi-

tive; 0.00003 of a gram will indicate 0.001 of a gram of hydrochloric acid.

The addition of the indicator to the ordinary culture media does not influence the growth of bacteria. The delicate changes in reaction are best obtained by Dunham's peptone solution, containing six per cent. glycerin and two per cent. of the acid fuchsin. In the ordinary culture media, such as beef peptone bouillon and beef peptone gelatin and agar, the results are not delicate or constant. Their differences are probably due to the changes in the reaction in sterilization.

The dysentery group shows quite a range in acid production, as to both amount and the time it occurs:

B. dysenteriæ Shiga, acid slight, four to five days, neutral after three weeks.

B. dysenteriæ Kruse, acid slight, four to six days.

B. dysenteriæ Flexner, acid slight, four to six days.

B. dysenteriæ New Haven, considerable, four to six days.

B. "*Y*" Hiss and Russell, none, alkaline in ten days.

Paracolon Group.

B. paracolon Kurth, none, alkaline in fourteen days.

B. paracolon Strong, none, alkaline in fourteen days.

B. paracolon Badash, none, alkaline in fourteen days.

B. paracolon Gwynn, considerable in four days.

B. paracolon Miller, considerable in four days.

B. paracolon Buxton, considerable in four days.

B. paracolon Cushing, considerable in four days.

Hog Cholera Group.

B. cholera suis, none, alkaline in ten days.

B. icteroides Sanarelli, considerable in four days.

The study and growth of the above organisms in media containing glycerin, and the acid fuchsin indicator, shows that they, so far as acid production is concerned, arrange themselves in groups, the *Bacillus* 'Y' of Hiss and Russell, *B. paracolon* Strong and Kurth, forming a distinct group.

Agglutination and Biological Relationship in the Prodigiosus Group: MARY HEFFERAN, University of Chicago.

A series of organisms with cultural characteristics like those of *B. prodigiosus* were examined for agglutinative activity. This series had been under the writer's observation for five years and the biological relationship of the twenty-two different members of the group had been fairly well determined.[1] Agglutination tests showed:

1. A high degree of interaction among those members of the group which were classed together by the sugar fermentation test.

2. Identity of reaction of races known to have been derived from the same culture eight or ten years previously, and kept in different laboratories.

3. Agglutinative reaction among those members of the group which tend to lose the power of pigment production, including one race which produces only a soluble red pigment. No reaction was obtained in this case with *B. fluorescens liquefaciens* or *B. lactis erythrogenes.*

4. Much confusion and inequality of interaction among other members of the group closely related biologically.

The difference between agglutinogenic power and agglutinability was clearly due, in some cases, to a viscid capsular condition of the bacilli. On the other hand, readily agglutinable cultures did not possess correspondingly high agglutinogenic power.

Experiments made to determine the optimum temperature for the agglutination process showed that better results were obtained at either 0° or 55° C. than at room temperature or at 37° C. The action of convection currents in the tubes of serum dilution and bacilli at high and low temperatures was suggested as an explanation.

[1] *Centralbl. f. Bakt.*, 1904, 11, pp. 311, 397, 456, 520.

It was found that the addition of one per cent. formalin to salt solution suspensions of cultures made no difference in the agglutination results, if the cultures thus formalinized were allowed to stand for some time. Freshly added formalin seemed to inhibit agglutination.

Further experiments are under way to determine more exactly the action of formalin in the agglutination process.

Note on the Thermal Death Point of B. dysenteriæ Shiga: W. D. Frost and Mary W. Swenson, University of Wisconsin.

Four different strains of *B. dysenteriæ* were tested; one of the Shiga type and three of the 'Flexner-Harris' type. The method used was that suggested in the 'Procedures Recommended by the Bacteriological Committee of the American Public Health Association,' except that the reaction of the medium was 0.0 on Fuller's scale instead of 1.5 + and also in some cases only 5 c.c. of bouillon was used instead of 10 c.c. In the latter cases the 5 c.c. of bouillon after exposure was mixed with an equal amount of double-strength agar and plated. Exposures were made at temperatures ranging from 55° to 72°. It was found that the majority of the cells were killed between 55° and 60°, but that frequently a relatively small number, possibly one individual in a hundred thousand or a million, may persist at much higher temperatures, even 70°. The cause for this wide variation in resistance to heat among the different cells is apparently due not to variation in the reaction of the culture medium, for both an alkaline and an acid medium were used, nor to variations in the composition of the medium, since the same batch of medium was used throughout, but to some undetermined cause or causes.

A Study of the Laws Governing the Resistance of B. coli to Heat: Stephen DeM. Gage and Grace Van E. Stoughton, Experiment Station, Lawrence, Mass.

Experiments were made in which it was determined that the great majority of the bacteria in any *B. coli* culture are destroyed by five minutes' exposure to some temperature between 50° and 60° C. A few individuals, however, in each culture will survive much higher temperatures, in some cases remaining alive after exposure to 90° C. The very close range (about 10° C.) of temperature at which the destruction of the majority of the individual bacteria occurred, as compared with the considerable range (about 35° C.) in the temperatures at which complete sterilization was effected, would indicate that the determination of this majority death point would be of more value in species identification than is the determination of the absolute thermal death point as at present employed.

Using thermal death point tests alone, this culture of *B. coli* would be included among the sporulating bacteria, although there was no morphological evidence that true spores (endospores) were produced.

Experiments were also made to determine whether, by successively selecting cultures originating from individual organisms which had survived temperatures above the majority death point and submitting these cultures to the death point tests, a race of organisms could be propagated in which the majority of the individuals would be able to resist higher temperatures than was the case with the original culture. The experiments failed not only to produce such a race, but the results indicated the tendency toward the production of a degenerate race whose majority death point remained the same as for the original culture, and whose absolute thermal death point was reduced toward the

majority death point as the number of successive generations was increased.

To be published in the *Technology Quarterly*.

Bird Plague (a preliminary note): J. J. KINYOUN, Glenolden, Pa.

Beginning in May, 1905, the writer examined several dead birds, received from a dealer in Washington, D. C. On examination all these presented certain definite lesions. The organs notably affected were the liver and spleen. A provisional diagnosis was first made of tubercule, but on examination it was negative. The lesions found in the liver and spleen were yellowish nodules of varying size, which projected from the surface of the organs. The majority of the nodules were surrounded with a well-marked zone of inflammatory tissue. There were also spots of coagulation necrosis interspersed between the nodules. There was also found a catarrhal exudate affecting the upper air passage. In a few instances there was enteritis. Direct microscopical examination showed a small bacillus with rounded ends and morphological and tinctorial propensities resembling *Bacillus pestis*. The organisms were present in enormous numbers in the nodules, particularly in those of the spleen. It could also be identified and easily isolated from the heart's blood and all other organs. The cultural characters are: it grows rather slightly on ordinary peptone bouillon agar, it does not liquefy gelatine, nor does it ferment any of the sugars except mannit. Grown on Hankin's salt agar it assumes pleomorphinism; closely resembles the organism of bubonic plague. It grows best in peptone bouillon or agar containing a small amount of sterilized horse or calf serum. Calf serum agar containing two per cent. is coagulated. It is pathogenic to rabbits, guinea-pigs, white mice, pigeons, sparrows, canaries, finches, mocking-birds, thrushes, parakeets. Chickens are immune.

Notes on Class-room and Laboratory Work: F. C. HARRISON, Agricultural College, Guelph, Ontario.

1. Method of keeping lecture notes.
2. Material for table tops.
3. Demonstration of gas production—(*a*) with absorbent cotton, (*b*) with small tube inside test tubes, (*c*) modified Dunham tube.
4. Prevention of moisture in agar plates.
5. Method of preparing gelatine plates for demonstration and museum purposes.
6. Various forms of colony counters.
7. Method of keeping stock cultures.
8. Test-tube containers for sterilizing.
9. Flagella staining for class purposes.
10. Ink for writing on glass.

How Shall the Potency of Antitetanic Serum be Determined? E. M. HOUGHTON, E. C. L. MILLER and F. O. NORTHEY, Detroit, Mich.

Experience has shown that the Ehrlich test for determining the strength of antidiphtheric serum is very reliable, and has been adopted by the U. S. Department of Public Health and Marine Hospital Service as a method of standardizing this serum. Many methods are employed for measuring the strength of antitetanic serum, but none have been generally adopted in this country. The results of laboratory tests indicate that the sera found on the market vary enormously in strength, as tested by the modified Behring method. It would seem desirable that a method be adopted for testing antitetanic serum similar to that in use for determining the strength of antidiphtheric serum, but it seems to the writers that the test animals should be guinea-pigs and that the units of strength should be such that a curative dose of ten cubic centimeters of antitetanic serum

would contain approximately the same number of units as the curative dose of antidiphtheric serum, as recommended by the U. S. Pharmacopœia.

A Method of Isolating the Pneumococcus in Mixed Cultures, Such as Throat Cultures: Gustav F. Ruediger, Memorial Institute of Infectious Diseases, Chicago.

Starting with Hiss's demonstration that pneumococci ferment inulin while streptococci fail to ferment it, Ruediger has prepared a blue litmus inulin-agar medium in which the pneumococci form red colonies. This medium is composed of sugar-free agar with the addition of litmus and inulin and is prepared as follows:

(*a*) Peptone (Witte), 10; agar, 15; sugar-free beef broth (neutral), 1,000. Dissolve by boiling one hour, adding water from time to time. Heat in the autoclave for fifteen to twenty minutes (to prevent subsequent precipitation while sterilizing), clarify with egg and filter through cotton, making the volume up to 800 c.c. with distilled water.

(*b*) Dissolve 15 grams of pure inulin in 200 c.c. of boiling distilled water and add this solution to (*a*). Now add 20 c.c. of a five-per-cent. solution of litmus (Merck's highest purity) and tube, putting 7 to 8 c.c. of medium into each tube. Sterilize in the autoclave under ten pounds of pressure for fifteen minutes. As some pneumococci do not grow well in this medium it is necessary to add 1 c.c. of heated ascites fluid or serum to each tube of melted agar (which has been cooled to 45°) immediately before using. In this mixture the pneumococci grow well and form red colonies in twenty-four to seventy-two hours.

Ruediger has shown further that pneumococci are practically the only mouth bacteria that ferment inulin. It is not fermented by streptococci (Hiss), staphylococci, pseudodiphtheria bacilli, *Micrococcus catarrhalis, Micrococcus tetragenus* and *Bacillus mucosus.* Among ten cultures of diphtheria bacilli was found one which fermented inulin. Twenty-two cultures which were made from red colonies in plates that had been inoculated with material from throats of pneumonia and scarlet fever patients were studied in detail. All are Gram-positive cocci which grow chiefly in pairs on blood agar slants, but some also form chains in liquid media. All ferment inulin and all but four form green colonies in blood agar plates. Capsules could be demonstrated on more than half of the cultures.

The full paper will be published in the *Journal of Infectious Diseases,* January, 1906.

Observations upon the Phagocytic Power of the Blood of Normal Human Beings: Joseph McFarland and Edward M. L'Engle, Philadelphia.

The blood of fifteen presumably normal individuals was examined by the method devised by Leichman and modified by Wright and Douglas and by ourselves. Twenty-four-hour cultures of *Staphylococcus pyogenes aureus* were used in all the experiments. We found that the phagocytic index varied from 23.125 to 4.35. In two cases in which the counts were repeated at intervals of five days there was a remarkable uniformity in one case and a distinct variation in the other. The experiments were all performed between the hours of three and five in the afternoon. All of our blood preparations were stained by Marino's method, which we have found the most satisfactory for our purposes. We also found that the number of bacteria taken up by the leucocytes varies with great regularity according to the strength of the bacterial suspension used. Hence, we have endeavored to use a uniform sus-

pension in all of our experiments. The clinical bearing of these experiments is indicated by the fact that those individuals whose phagocytic index was lowest had suffered from carbuncles or boils or become easily infected from slight causes.

Conclusions.—1. Leishman's method of determining the phagocytic power of the blood, as modified by Wright and Douglas and by ourselves, is a very simple method adapted to clinical application.

2. Marino's stain is most appropriate for demonstrating the leucocytes and the contained bacteria.

3. There is no uniformity in the phagocytic indices of the bloods of supposedly healthy individuals.

4. The phagocytic index of the same individual may be constant or it may vary upon different days.

5. An exceptionally low phagocytic count usually indicates a present or past predisposition to suppuration (the phagocytic index in this regard being determined by the use of *Staphylococcus pyogenes aureus*).

6. The phagocytic index may not be below the average in all cases in which there has been a tendency to suppuration.

The Value of the Voges-Proskauer Reaction: NORMAN MACL. HARRIS, University of Chicago.

The red coloration at times met with in fermentation tubes after testing the gas composition with KOH solution, was first described by Voges and Proskauer in 1898 as occurring in fermentation tube cultures of certain members of the hemorrhagic septicæmia group of bacteria, and the reaction was advocated by them as a test for differentiating members of this group. However, as proposed by them, the test is without value, because their observations were made on bacilli which we now clearly recognize as belonging to the hog cholera group, not to the hemorrhagic septicæmia group.

The color reaction has since been proposed by Durham and MacConkey as a differential test whereby *B. lactis aerogenes, B. mucosus capsulatus* (Pfeiffer), and *B. cloacæ* and bacteria of such types may be distinguished from certain intestinal and other forms of bacteria. Howe and MacConkey likewise recommend it as possessing value in the bacteriological analysis of water.

Contrary to the views of the foregoing observers, the writer finds that the reaction occurs irregularly with such bacteria as *B. coli, B. lactis aerogenes, B. mucosus capsulatus* (var. Pfeiffer, and Friedländer), *B. choleræ suis, B. enteritidis, B. icteroides, B. paratyphosus* (several races), *B. proteus vulgaris, B. cloacæ, B. fæcalis aligenes* and *B. typhosus* and *Streptococcus pyogenes.*

Conclusions.—1. By Voges and Proskauer the reaction was obtained not with any member of the hemorrhagic septicæmia group of bacteria, but with one of the hog-cholera group.

2. The writer finds that the reaction is not confined to any one particular group of bacteria, as found by Durham and MacConkey, but occurs widespread and irregularly amongst bacterial species.

3. As a differential test, the Voges-Proskauer reaction is of little value.

4. Its nature is at present unknown.

The Protection of Cotton Stoppers during Sterilization: W. H. MANWARING and R. A. AKIN, Indiana University.

The falling of condensation-water in the autoclave is largely prevented by a disc of thinnest sheet copper, suspended about a quarter of an inch below the top of the autoclave, by means of a threaded bolt, placed in the opening to the pressure gauge. This bolt is flattened on two sides,

so as to permit free passage of steam to and from the gauge. The stoppers of flasks are further protected by means of small beakers, inverted over the necks of the flasks, during sterilization. Test tubes are protected, in a similar way, by small pans of thinnest sheet copper, made to fit over the wire baskets containing them.

The Production of Acid and Alkali by Bacteria: E. O. Jordan, University of Chicago.

Since Theobald Smith's work in 1895 it has been known that the acid reaction that develops in the broth cultures of many bacteria is produced by the action of the bacteria upon muscle-sugar. It has not been definitely recognized, however, that there are other substances besides sugar in the ordinary nutrient media which, under the influence of bacterial activity, lead to the production of a strongly acid reaction. One prominent text-book, indeed, affirms that 'the formation of free acid is possible only upon nutrient media containing sugar.' As a matter of fact, I have found the liquefaction of gelatin by bacteria or their sterile enzymes always gives rise to a marked acid reaction which may amount to as much as nine per cent. (*B. subtilis*) on the acid side of the phenolphthalein neutral point. A reaction of plus four per cent. is quite common in cultures of liquefying species. This is not surprising when it is remembered that substances like glycocoll and the amino-acids are conspicuous among the digestion products of gelatin. The simultaneous production of ammonia by bacteria tends to diminish the acidity of liquefied cultures, in some cases (*e. g.*, *B. pyocyaneus* at 20°) the acidity being nearly or quite overcome. The acidity is not confined to the liquefied area, but, as might be supposed, diffuses throughout the medium, altering the reaction of the gelatin at quite distant points. In nutrient agar, on the other hand, the diffusion of alkaline products alters the reaction of the medium in the opposite direction. A standard reaction for culture media, therefore, is valid only as an initial reaction. Gelatin and agar, inoculated with liquefying species, begin to diverge from the start and in a short time may become totally different. Bacteria make their own reaction in broth, gelatin and agar and, useful as a standard reaction may be, it has distinct limitations.

Some writers have attempted to establish a fundamental distinction between acid and alkali production by bacteria. Thus Gotschlich in Kolle and Wassermann's 'Handbuch,' 1, p. 100, expresses himself as follows: "In general it may be said that acid production always depends upon a splitting of sugar (or similar substances, like glycerine, etc.); while alkali formation is a synthetic process and stands in intimate causal relation with the growth and increase of bacteria." Such a view would seem to be eminently artificial, since the ammonia to which an alkaline reaction is due is as truly a decomposition product of nitrogenous bodies as the amino-acids formed in the digestion of gelatin of the lactic acid in the fermentation of sugar. Both processes go on simultaneously, and the reaction of a culture medium in which bacteria are growing depends not only on the ability of the species to attack certain food substances, and on the chemical constitution of those food substances themselves, but also on the precise period of growth at which the reaction is tested.

Experiments on the Staining Properties of Bacteria, with Special Reference to the Gram Method: D. H. Bergey, University of Pennsylvania.

Careful search in text-books fails to reveal definite information as to the factors concerned in the Gram method of staining. There is even confusion in different text-

books as to the properties exerted by the ingredients employed in the Gram method.

Investigation shows that the Gram reaction follows the employment of the pararosanilin dyes, especially the violet dyes of this group, such as crystal violet, methyl violet, or mixtures of these known as gentian violet. The influence of the iodin is to form a new compound with the stained protoplasm of certain bacteria, which compound is insoluble or feebly soluble in alcohol. The alcohol serves as the decolorizing agent.

The exact difference in the chemical constitution between species of bacteria that stain positively and those that stain negatively by the Gram method is not definitely known, but the chemical constitution of the bacterial cell influences the behavior of the organism toward the Gram method.

Experiments along these lines are still in progress.

The following new members were elected:

Professor Erastus G. Smith, Beloit College, Beloit, Wis.

Doctor Hideyo Noguchi, assistant, Rockefeller Institute, New York City.

Doctor Francis H. Slack, first assistant bacteriologist, Boston Board of Health Laboratory.

Doctor Eduardo Andrade, bacteriologist, State Board of Health, Jacksonville, Fla.

Doctor Howard T. Ricketts, instructor in pathology, University of Chicago.

Doctor Gustav F. Ruediger, assistant, Memorial Institute for Infectious Diseases, Chicago, Ill.

Professor Wilfred H. Manwaring, Indiana University.

Professor Edwin G. Hastings, University of Wisconsin.

The officers for the ensuing year are:

President—E. F. Smith, Washington, D. C.

Vice-President—F. P. Gorham, Brown University.

Secretary-Treasurer—S. C. Prescott, Massachusetts Institute of Technology.

Council—E. O. Jordan, V. C. Vaughan, Simon Flexner, Joseph McFarland.

Delegate to American Association for the Advancement of Science—W. T. Sedgwick, Massachusetts Institute of Technology.

F. P. GORHAM,
Secretary.

BROWN UNIVERSITY.

THE BOTANICAL SOCIETY OF AMERICA.

THE twelfth annual meeting of the Botanical Society of America was held at New Orleans, in affiliation with the American Association for the Advancement of Science, from the first to the fourth of January, 1906. While the attendance, as in the case of the association, was not large, the meeting was a good one, and the program which follows contained papers of unusual interest.

As officers for the year 1906 were elected, *President,* Professor F. S. Earle, Santiago de las Vegas, Cuba; *Vice-president,* Professor F. E. Clements, Lincoln, Nebr.; *Secretary,* Professor William Trelease, St. Louis, Mo.; *Treasurer,* Dr. Arthur Hollick, Bronx Park, New York City. In addition to these officers and Past-president Harper, of Madison, Wisconsin, the council was completed by the election of Professor B. L. Robinson, of Cambridge, Mass., and of Professor N. L. Britton, of Bronx Park, New York City. Professor E. A. Burt and Dr. D. T. MacDougal were appointed to represent the society on the council of the American Association for the Advancement of Science. Drs. A. F. Blakeslee and G. H. Shull were elected to associate membership.

A vote of thanks was passed for the efficient service of the retiring secretary, Dr. MacDougal.

The treasurer's report showed a balance in the treasury of $3,201.43, of which a grant of $150.00 was made to Dr. C. J. Chamberlain, of the University of Chicago, as an aid in a further field study of *Dioon* and in a morphological study of *Ceratozamia,* and a grant of $100.00 to Professor J.