believes that the feeding power of plants is satisfactorily explained, without the intervention of other acids than carbonic. Since the failure to establish that plants excrete notable amounts of other acids than carbonic, some investigators, as previously stated, have suggested that the differences in feeding power may be due to differences in amount of carbon dioxide excreted. A careful consideration of available data lends little support to this idea. It seems rather that it is the efficiency with which the carbon dioxide is used, and not the differences in amount excreted by different species of plants, that determines whether or not a plant will feed strongly on an insoluble material.

The writer has in preparation a detailed article dealing with the feeding power of plants and the availability of phosphates.

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THE SOCIETY OF AMERICAN BACTERIOLOGISTS[1]

SYSTEMATIC BACTERIOLOGY

Under the supervision of H. A. Harding

A Study of B. subtilis by Means of the Classification Card: H. Joel Conn.

One hundred and thirty cultures of the *B. subtilis* type, isolated from soil, have been studied by means of the classification card adopted by the society. The definition adopted for *B. subtilis* is: a large, peritrichic, spore-producing rod, facultative anærobic in the presence of dextrose, liquefying gelatine, and growing vigorously on ordinary media without chromogenesis, producing a membraneous more or less wrinkled growth on the surface of agar. Two questions have been considered: (1) Do the determinations called for on the card separate these 130 cultures into more than one species? (2) Does the same culture always give identical results upon repetition of the tests?

In answering the first question half of the determinations represented by the "Group Number" on the card were excluded because they are implied by the definition of *B. subtilis*. The determinations taken into account were the fermentation of sugars and glycerin, and the reduction of nitrates. The nitrate reduction determination gives quite clear-cut results and may serve to separate an infrequent nitrate-negative species from an abundant nitrate-positive species. The fermentation tests do not give such definite results. They suggest that the 130 strains do not differ from each other in fermentative powers, but give inconstant results with the present technique.

The second question was answered in the negative as regards the fermentation tests; the nitrate reduction test seemed more constant, but insufficient data is at hand to settle the matter.

These tests indicate that with our present technique different "group numbers" do not always indicate different species. One of the first steps needed in revising the card is to establish the best methods for making the various determinations.

Some Induced Changes in Streptococci: Jean Broadhurst.

Various relatively simple physical and chemical factors (such as changes in temperature and differences in artificial media) differ greatly from such agents as saliva, intestinal extracts, and pure cultures of other bacteria, in their effects upon the physiological activities of selected strains of streptococci. The physiological effects of the former, especially in the various test media containing the sugars and the related substances suggested by Gordon, are mainly of a negative or inhibiting type, and apparently temporary only.

The changes induced by the latter factors (saliva, intestinal extracts, etc.) are, however, markedly different. They are changes in kind not in amount of reaction; they are active and usually include new powers, not merely the inhibition or occasional stimulation of earlier powers or capabilities, and often indicate a complete rearrangement of the fermentative complex. These induced changes have, so far, been practically permanent.

A Study of the Correlation of the Agglutination and the Fermentation Reactions among the Streptococci: I. J. Kligler.

Bacteria have evolved so little along gross structural lines that it is impossible to differentiate members of the same genus on a merely physical basis. We therefore resort to the more delicate criteria of protoplasmic constitution and physiological activity, in which direction remarkable differentiation exists. Tests for the finer structural differences of these organisms are found in their behavior to differential stains, such as the Gram stain, and to the immune substances in-

[1] Abstracts of papers presented at the Philadelphia meeting, December 29, 1914.

duced by them in the animal body. Their physiological activity is measured by the end products of their metabolism. Physiologically, bacteria, generally, have evolved in two main directions—one group possessing marked carbohydrate splitting properties, the other having developed the property of digesting various protein substances. The streptococci belong to the former division, showing but little tendency to proteolysis.

It appears natural enough to assume that the biologic activities of a cell would correspond with the chemical nature of its protoplasm. Yet such a correlation has not been worked out except in a few isolated cases. Among the streptococci such a correlation, if it exists, would be especially significant in that it would help to differentiate the various members of a genus that has puzzled many investigators.

The agglutination, fermentation and hemolytic properties of sixty strains of streptococci obtained from various pathological conditions, were studied, using four agglutinating sera having a titer of 800–1,000, and six carbohydrates and other fermentable substances as follows:

Disaccharides	Lactose
	Saccharose
Trisaccharide	Raffinose
Alcohol	Mannite
Glucoside	Salicin
Starch	Inulin

Only a limited number of the strains were agglutinated by the sera used. A definite correlation was, however, obtained between the agglutinative and fermentative characters. The serum produced by a strain of one fermentative group (the group that fermented salicin, for instance) agglutinated only cultures of its particular division and failed to agglutinate members of any of the other groups. No such correlation was obtained with the hemolytic property, members of one hemolytic group being agglutinated by the sera produced by strains from other hemolytic groups.

The results obtained indicate that a separation of the streptococci obtained from various pathological conditions into three fermentative types would coincide most closely with their natural relationship.

The groups suggested are:

1. Salicin fermenters only, generally hemolytic —*Str. pyogenes.*

2. Raffinose fermenters, salicin usually fermented, mannite always negative, generally produces a green colony on blood agar—*Str. salivarius.*

3. Mannite fermenters, generally ferment salicin, rarely ferment raffinose, variable in their reaction to blood—*Str. fecalis.*

The Filterability of B. bronchisepticus: with an Argument for a Uniform Method of Filtration: N. S. FERRY.

The purpose of the paper was to place on record a series of filtration experiments with *Bacillus bronchisepticus,* described as the cause of canine distemper by Ferry in 1910, McGowan in 1911 and Torry in 1913.

The experiments were conducted as follows: The organism was grown twenty-four hours both on agar and in bouillon. The bouillon growth was filtered, undiluted, while the agar growth was taken off in bouillon and made into a suspension of about the same density as the bouillon culture. The method of testing the integrity of the filters was that described by Bulloch and Craw in the *Journal of Hygiene* in 1909, which depends upon the measure of the pressure of air as it is allowed to pass through the pores of the candles while immersed in water. The filtration was conducted at room temperature, one hour taken as the length of time for filtration, and three pressures were used; gravity, 15 lbs. (negative) and 225 lbs. (positive).

The experiments proved conclusively that the *Bacillus bronchisepticus* is a filterable microorganism. The work also corroborates the results of previous investigators with regard to the fact that the less pressure used the more easily will some organisms pass through the filters.

Some interesting possibilities were suggested by the outcome of this work. Since 1905, when Carre claimed he had produced typical symptoms of distemper in susceptible dogs from the filtered discharges of diseased dogs, the majority of writers have classified the etiology of canine distemper as a filterable, invisible or ultramicroscopic virus, and it is so described in many textbooks. The work of Ferry, McGowan and Torry with the *Bacillus bronchisepticus* tends to refute the statements of Carre.

The results of the present filtration experiments puts an entirely new light on the subject. If the *Bacillus bronchisepticus* is the cause of canine distemper, then the experiments corroborate the work of Carre. If the work of Carre was correct, and if the causative agent of canine distemper is a filterable virus, then the experiments point very conclusively to *Bacillus bronchisepticus* as the etiological factor and confirms the findings of the three previously mentioned investigators.

The author suggests that steps be undertaken for making out some uniform method of conducting filtration experiments of testing the efficiency of the candle and expressing or recording the results.

Influence of the Concentration of the Nutrient Substrate upon Microorganisms: Zae Northrup.

1. *Determination of the Influence of the Concentration of the Gelatin in Nutrient Gelatin, upon Liquefying and Non-liquefying Organisms.*—Gelatin media, having the same amount of other nutrient substances than gelatin, per unit volume were prepared, using 15 per cent., 25 per cent., 35 per cent., 50 per cent. and 75 per cent. gelatin.

Difficulties were met in the preparation of the highest percentage of gelatin on account of the thick sticky nature of the mass, but an excess of water was added to make the mixture homogeneous, this water being then driven off by evaporation on a water bath.

Pure cultures of both liquefying and non-liquefying organisms were plated on the different concentrations of gelatin.

On account of the extreme viscidity of the 75 per cent. gelatin it could not be plated in the usual manner; a thin film of the gelatin was spread over a sterile glass slide in a sterile petri dish and inoculated by spreading a small drop of a 24-hour culture of the organism on the surface of the gelatin.

The number, size and appearance of colonies were to be noted on the media of the respective concentrations.

In counting, the low power of the compound microscope ocular No. 1 and objective No. 7 was found to give counts 3–4 times as high as the ordinary counting lens.

The numbers of organisms developing on the plates are influenced to some but not to any marked extent, if the mechanical difficulties of inoculating the gelatin and pouring the plates are taken into consideration. The decrease or variations noted may be due only to experimental error.

The size of the colonies was found to be inversely proportional to the concentration of the gelatin. This was especially marked in the case of the organisms which are the most active in liquefying gelatin.

The type and appearance of the colonies were also found to be worthy of note. The subsurface colonies of both liquefying and nonliquefying organisms appeared like very fine gas bubbles distributed throughout the medium. The active liquefying organisms began to show a rectangular instead of a concave depression in surface colonies on 35 per cent. and 50 per cent. gelatin, while with the slow liquefier a new type of growth, a stalagmite-like or apiculate growth, appeared on the 50 per cent. gelatin. This type of growth was noted in the 25 per cent. gelatin of colonies of the non-liquefying organisms.

B. typhosus was the only organism among the eight types used in the experiment which refused to grow on the 50 per cent. gelatin. However another trial might prove successful.

The different phenomena observed in the course of this experiment will most probably call upon the sciences of physics and of physical chemistry for their interpretation.

Several questions have been called forth by the results of this experiment and most of them remain as yet unanswered.

What part does the medium or substratum and what part does the organism play in the formation of the so-called characteristic growths which are obtained in solid media? What force or forces cause the variation in types of liquefaction produced by various proteolytic enzyme-forming organisms? Does the inherent nature of the organism or its secretions play the greater part or are physical or physico-chemical forces the greater factor?

Why is the size and the structure of the colony so markedly influenced by the media of increasing concentration? It is not due to osmotic pressure, as gelatin is a colloid and consequently will exert no osmotic pressure.

Is it due to the lack of water or is it due to some physical property of the gelatin, as surface tension, which is more evident in greater concentrations?

What force causes the colony in a nutrient gelatin of high concentration to show a rectangular depression when in ordinary nutrient gelatin the depression is concave?

In the liquefaction of ordinary nutrient gelatin what part does the force of gravity play?

An interesting occurrence was noted in the "plates" made with the 75 per cent. gelatin. Upon examining these plates, several days (exact period of time not noted) after they were made, the glass slides were found in very fine pieces as if crushed by a powerful force. This occurred in every case. The crushing of the slide was evidently due to the contraction of the highly concentrated gelatin upon cooling and solidification. Just how much energy it will take to crush slides by mechanical force is yet to be determined.

The determination of the various physical and physico-chemical forces will serve to give some idea of the factors which microorganisms have to overcome in growing in gelatin and similar media of high concentration.

This experiment was worked out by Mr. O. M. Gruzit, a senior student.

Induced Variations in Chromogenesis: M. R. SMIRNOW.

Of the various biological characters of bacteria, one of the most interesting yet least important is that of pigment production. Though considerable efforts have been expended in the study of this function, little of real value is as yet available. It appears that this property is especially prominent amongst the saprophitic organisms, and depends to a greater or less extent, on certain conditions of environment which vary with different bacteria, and is, as a rule, more or less constant for the same organisms.

With the exception of *Spirillum rubrum* and possibly a few others, the chromogenic bacteria require an abundance of free oxygen, giving no pigment under anærobic conditions of growth. Temperature also seems to determine pigment production of some bacteria, thus the *B. prodigiosus* will give no pigment at 37° C.

Perhaps the most important influencing agent on the function of chromogenesis is the medium on which the organism is grown. With other factors of environment constant, chromogenesis will vary with the medium employed. Gessard, for instance, has shown that the *B. pyocyaneus* will produce only a blue color, of a most beautiful shade, in a two-per-cent. solution of peptone, which may be increased in intensity by the addition of five-per-cent. of glycerin. When grown on egg-white or other albumen or on weak glucose media it would produce a fluorescent green. This same organism when grown on a five- or six-per-cent. glucose medium or on immune serum would give no pigment. He believes that phosphates are required for the production of the fluorescin.

Substances that enhance the value of culture media, in a general way increase also the pigment production. Other substances, as acids or alkalies, may diminish or even inhibit its production. Some organisms may give different colors on media of different reaction. Thus the *B. prodigiosus* gives a distinct yellow color on alkaline, and a violet-red on acid media.

In what manner the pigment is produced is not yet known. It is regarded that the property of pigment production keeps pace with other biological characters, as enzyme formation. This, the writer does not feel to be correct, inasmuch, as will later be shown, he has succeeded in increasing the chromogenic properties of some bacteria with a coincident decrease of enzyme formation. Some of the higher forms of organisms give rise to pigment as a function closely related with their nutrition and may possibly be regarded as products of metabolism. In these cases the pigment is obtained from the medium and is stored up in the bodies of the cells, as in the case of sulphur bacteria. Or, it may be produced on certain media containing iron, as evidenced in the so-called iron bacteria, through the products of metabolism and the production of sulphide or iron.

Chromogenesis may be increased not only by growing the bacteria on more favorable media and environment, but also by the process of selection, transplanting each time from portions of the culture or from a colony that shows the most pronounced pigment.

Experimentally induced variations in the chromogenic properties of the *Staphylococcus pyogenes aureus* may be brought about by exposure to phenol or by growth in phenol, glucose, sodium sulphate or sodium chloride broth. Nine different strains of the *Staphylococcus* were used in the work here reported. Five of these were old stock cultures giving little or no color; the remainder were a few months old and showed a fair amount of pigment at the beginning of the experiments.

The organisms were grown in the above media for from six to ten weeks, being transplanted every three or four days during the entire time. They were then grown on potato and blood serum media for from 24 to 120 hours, and the effect on chromogenesis noted.

The increase of chromogenesis is brought about more readily by growing the organisms in phenol broth than by exposing them to 75 per cent. phenol solution and transferring on to agar. Of the nine strains used phenol markedly increased the chromogenic properties in six, Nos. 1, 2, 5, 6, 7, 8; slightly increased it in Nos. 4 and 9 and left No. 3 practically unchanged or even slightly diminished. Growth in dextrose, sodium chloride and sodium sulphate broth invariably decreased or left unchanged the quantity of pigment produced. Often almost a pure white growth of the various cocci, subjected to the growth in NaCl and Na_2SO_4 broth, would be seen when transferred to potato or blood serum.

An old stock culture of the *B. prodigiosus* was also used. This organism gave the slightest trace

of color at 20° C. at the beginning of the experiment. The organism was subjected to phenol only, beginning with a few minutes' exposure to a 0.75 per cent. solution and increasing as it became more resistant up to fifty or sixty minutes. Cultures were made on agar and grown at 20° C.

A striking increase in color production on all media resulted, the color becoming deeper and deeper until the maximum was reached at the thirteenth exposure. Up to the nineteenth exposure the color of each succeeding growth became most pronounced in 48 hours. From thereon, with increasing time exposures, the color production was slower, the color reaching its maximum in three or four days. Different shades of red were produced on different media. On agar, the color was deep brick red; on blood serum it had more of a scarlet hue; while on potato the color was somewhat variable and not as marked as on the other media. It was, however, on glycerin agar and glycerin potato that the most striking results were observed. The original strain gave no color on glycerin agar, and only a pale, delicate reddish color on glycerin potato. Transplants made from the phenol exposed organisms gave a brilliant cherry-red color on glycerin potato spreading to surround the entire surface of the medium. On glycerin agar, a dull cherry-red color was obtained.

In summing up what has been said concerning chromogenesis, it becomes evident that this faculty is more or less closely associated with the metabolic activities of bacteria, nutritive or otherwise. It varies with the strain and is more or less dependent on oxygen, temperature, and the medium used. An organism may produce more than one color at once and the same time or it may produce different colors, depending upon environment and the medium used, particularly the latter. Finally, chromogenesis may be varied through the agency of chemicals, as seen by the work here outlined, phenol generally increasing, and glucose, sodium chloride and sodium sulphate diminishing this function.

Induced Variations in the Cultural Characters of B. coli: M. R. Smirnow.

The same technique that was used in the experiments on chromogenesis was made use of here. In all, 21 different strains of the various bacilli of the colon-typhoid group were used, but this report is confined only to the *B. coli,* of which seven different strains were experimented on. All of these strains were obtained from the Museum of Natural History of New York through the kindness of Dr. C.-E. A. Winslow, and were the stock Nos. 19, 44, 45, 46, 52, 57 and 95. The transplanting was carried out every three or four days over periods varying from one to three months, thus allowing from ten to thirty or more transfers. The results obtained in each set of experiments were rather constant, though not altogether so, inasmuch as some of the strains reacted quicker or different in the degree of the action at one time than another.

Control cultures were carried on in plain broth throughout the experiment. It might be stated at once that there were very slight variations between the original stocks and these control cultures, but no more than would be expected as normal variations. These were seen as slightly increased or decreased amounts of gas or acid formation, in time of coagulation, or slight changes in the growth on potato. At no time, however, were the biological characters markedly changed nor enzyme production completely inhibited simply by continual passage through broth.

Growth on Potato.—Dextrose seemed to have a special effect upon the character of growth of *B. coli* on this medium. Five of the seven strains showed at best only a slight yellow or a very light brownish growth on ordinary potato, with practically no discoloration of the medium. Very frequently, indeed, the dextrose-affected organisms would give the typical "invisible" growth seen with the *B. typhosus.* Both the original stock and the control showed the characteristic colon growth on this medium. This change was noted so many times that the explanation based on differences in the composition of the potato could be excluded. Three of these five strains also showed this change after exposure to phenol. One strain of the *B. coli,* not changed in this respect with either dextrose or phenol, showed this same variation after growing in either sodium chloride or sodium sulphate broth.

Action in Milk.—Both phenol and dextrose diminished the acid production and inhibited the formation of lab enzyme in three of the seven strains of the *B. coli,* either entirely or for a period of two weeks at least. These results were not seen with the use of the strong saline or sodium sulphate broth.

Fermentation of Sugars.—The results obtained with these substances on *B. coli* with reference to variations in sugar fermentations can be best seen in the accompanying charts. The most striking changes here also were seen in those organisms

exposed to dextrose and phenol. The former completely inhibited both acid and gas formation and all the sugars tested in three different strains. In two other strains dextrose varied the fermentation of the sugars as to amount of acid and gas formation, some of which were totally inhibited. Phenol inhibited these fermentations in all of the sugars in only one case, and in four other strains it at times diminished this reaction to the point of inhibition. Sodium chloride and sodium sulphate had less effect than did phenol, giving usually slight variations in amount of acid or gas produced with an occasional inhibition.

Inhibition of all the sugar fermentations in any one experiment was almost always accompanied by inhibition in the usual changes in milk, the characteristic growth on potato, and the formation of indol.

Variations in Indol Production.—The production of indol is regarded by many bacteriologists to be as important a biological characteristic of *B. coli* as its fermentations of the sugars, and is even thought to be of greater importance in its differentiation. This quality, however, under normal conditions, varies considerably in its quantity and time of appearance with most strains, and at times requires more delicate tests than the usual Salakowsky method for its determination.

In the experiments here reported it appears that of the variations induced in *B. coli* that of indol production is the first to take place, often disappearing in the third or fourth culture in dextrose broth. This does not hold however when the bacteria grow in the other media, as evidenced below.

Each strain of *B. coli* was grown in plain broth as control, in dextrose, phenol, sodium chloride and sodium sulphate broth and on potato. Thirty-five sub-cultures were made in all. Indol was tested for after the 10th, 15th, 25th and 35th transfers. The tests for indol were made by inoculating one loop of culture from the respective media to which each strain was subjected into standard peptone solutions, grown for seven days at 37° C. and then tested by the Salakowsky method. All the tests were done at the same time, using the same batch of peptone solution throughout the experiment.

All the controls, grown in plain broth gave good indol tests even after the 35th sub-culture. Those grown in dextrose broth gave none at the 10th subculture nor thereafter. In phenol broth the property of indol production seemed to be somewhat increased, judging from the intensity of the reaction. Sodium chloride and sodium sulphate and prolonged cultivation on potato practically exerted no influence, or if any, showed but a slight inhibitory effect.

Experiments were then carried out to see how soon the property of indol production is interfered with by growth in three per cent. dextrose broth, and it was found that *B. coli* lost this property usually on the third and at times on the second transfer over a period of from seven to ten days. In one experiment sub-cultures were made every 24 hours, with a total disappearance of the indol tests in 48 or 72 hours in all the strains.

In order to exclude the possibility of interference in the indol test by the presence of three per cent. dextrose, several cultures in plain broth, also peptone, were made and grown at 37° for seven days. Dextrose was added to each of the cultures and then tested for indol. Positive tests were obtained in all, hence excluding any possibility of such interference by the presence of the carbohydrate.

Experiments were then carried out to determine the permanency of this change. The cultures in dextrose broth after the 35th transfer were taken and grown in plain broth, transplanting every day and tested on the seventh day of incubation. Four of the strains of *B. coli*, Nos. 44, 45, 46 and 52, gave slight indol reactions on the third transfer, No. 46 gave a good positive on the fifth transfer, but the others took five to ten more transfers before they could be called "+" or "++" positive. Nos. 57 and 95 took six transfers before a trace of indol appeared. No. 19, a very feeble indol producer in the control, remained negative up to the fifteenth transfer, at which time the experiment was discontinued.

In summing up then, it can be said that dextrose and phenol, particularly the former, cause partial inhibition or total disappearance of acid and enzyme formation in some strains of *B. coli*. These changes, together with the suspension of the production of indol and the characteristic colon growth on potato, make the *B. coli* approach if not entirely appear like the *B. typhosus* type organism. These changes have been noted time and again, but in varying degrees, in those strains that are susceptible to variations, but for some unexplained reason can not be regarded as altogether constant. Indol formation would invariably return when these altered bacteria were transplanted into plain broth at frequent intervals. Lab enzyme would also return in most of the altered strains, but not invariably so. The same can be said of the fermentative properties, but even to a less extent. Very often, however, these characteristics appear to be entirely done away with, the change

being permanent as far as could be made evident by sub-culturing into plain broth. In these cases observations were made up to two months after the last exposure to the influencing substance, making frequent transfers. There seemed to be no definite rule of reversion, no constant results and no relation between the reappearance of one enzyme and another. The reappearance of the fermenting enzymes in one sugar was not necessarily accompanied by those in other sugars. At times the fermentation of one sugar might have returned to nearly normal, while others might show little or no presence of gas with the same strain of *B. coli*.

Halophytic and Lime Precipitating Bacteria: K. F. Kellerman and N. R. Smith.

Of approximately 70 cultures isolated from the Great Salt Lake and from sea water from Florida and the Bahamas three types of organisms were secured. *Pseudomonas calcis*,[2] a new spirillum and a new bacterium were isolated from the sea water. Closely similar varieties of species of *Spirillum* and *Pseudomonas* were found in water from the Great Salt Lake. Both in sea water and in the Great Salt Lake these bacteria are associated with the precipitation of calcium carbonate.

Relation of Crop to Bacterial Transformation of Nitrogen in the Soil: K. F. Kellerman and R. C. Wright.

Progress report upon continuation of work reported[3] previously.

The Influence of Hydrogen-ion Concentrations upon the Physiological Activities of Bacillus coli: Wm. Mansfield Clark.

Attention is called to the importance of hydrogen-ion concentration for the physiology of cells and to to its importance for the solution of various problems of bacteriological chemistry. The experiments of Michaelis and Marcora upon the limiting hydrogen-ion concentration for *B. coli* have been elaborated and it is shown that although minor differences exist there is a limiting concentration at or above which all activity ceases. The same results were obtained with various cultures of the true colon bacillus. At the limiting hydrogen-ion concentration proteolysis is inhibited. With increase in temperature the effect of hydrogen-ion concentration increases. The relation of this fact to the so-called thermal death point is pointed out.

An example is given showing the usefulness of the hydrogen-electrode in bacteriological research. By a study of the reaction of the medium at the close of the fermentation it was shown that by the use of *p*-nitro phenol a separation of the colon arogenes family could be accomplished. The groups so separated were rigidly correlated with the gas ratio.

Bacteria of the Colon Type Occurring on Grains: L. A. Rogers, William Mansfield Clark and Alice C. Evans.

In an earlier paper it was shown that the colon bacteria of bovine feces belong to a very sharply defined type which was characterized by the production of a relatively small amount of gas composed of hydrogen and carbon dioxide in almost exactly equal parts. A study of the gas production by 166 colon-like cultures from grains as determined under carefully controlled conditions showed that these cultures could be divided into three physiological groups. These were (1) cultures giving a low volume composed of carbon dioxide only; (2) those giving a low volume and a carbon dioxide-hydrogen ratio of 1.06 and (3) those giving a high volume and a ratio varying from 1.90 to 2.90. The cultures producing a carbon dioxide only were also distinguished by the rapid liquefaction of gelatin. The low-ratio cultures, although agreeing with the fecal type in the gas production, were distinguished by the production of a yellow pigment. The 151 high-ratio cultures were divided into four types. Ninety of the 151 liquefied gelatin slowly, gave a carbon dioxide-hydrogen ratio of 2.50 to 2.80, produced a light cadmium pigment, failed to form indol from tryptophane, fermented saccharose and glycerine, and failed to ferment starch, inulin and adonite. Forty cultures failed to liquefy gelatin, gave a gas ratio of 2.20 to 2.50, and produced a light cream-colored pigment, did not produce indol from tryptophane, fermented saccharose, lactose and raffinose, but almost always failed to ferment the other test substances.

Two other groups, differing in their gas ratio and fermentation reaction were made but they included a relatively small number of cultures.

A. Parker Hitchens,
Secretary

(*To be continued*)

[2] Kellerman, Karl F., and Smith, N. R., "Bacterial Precipitation of Calcium Carbonate," *Jour. Washington Academy of Sciences*, Vol. IV., No. 14, August 19, 1914, pp. 400–02.

[3] Kellerman, K. F., and Wright, R. Claude, "Mutual Influence of Certain Crops in Relation to Nitrogen," *Journal American Society of Agronomy*, Vol. 6, 1914, pp. 204–10.