CLASSIFICATION OF DAIRY BACTERIA

By H. W. CONN, W. M. ESTEN and
W. A. STOCKING

From the Report of the Storrs (Connecticut) Agricultural
Experiment Station for 1906

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A CLASSIFICATION OF DAIRY BACTERIA.

BY H. W. CONN, W. M. ESTEN AND W. A. STOCKING.

In the Annual Report of this Station for 1899 there was published a preliminary report upon the Classification of Dairy Bacteria as they had been studied in our laboratory. This has been up to the present the only attempt made at a systematic arrangement of these bacteria and it has been used somewhat extensively by bacteriologists. The large amount of work that has been done since that time and the great changes that have occurred in the methods of description and diagnosis of bacteria have made that paper no longer a correct representation of the present knowledge of dairy bacteria. Requests for that paper are still occasionally received, but inasmuch as it is so far from representing the present state of the known facts it is no longer regarded as advisable to distribute the paper in question. For this reason, as well as because of the accumulation of a large amount of further information, it is thought wise to publish at the present time a second paper upon the same subject, bringing the facts up to date. The previous paper has served a purpose. It was recognized when it was published, that it was only a temporary expedient, designed as a stepping stone toward something better. It is hoped that the present may serve a better purpose in the same direction and bring us nearer to a clear, satisfactory classification of dairy bacteria.

Since the publication of the previous paper we have done a large amount of work upon these bacteria. Most of it has been done in the laboratory of Wesleyan University by H. W. Conn and W. M. Esten. A considerable portion has been done in the laboratory at Storrs by W. A. Stocking and this paper is therefore the combined results of the two laboratories. The actual writing of the paper and the arrangement of the scheme of classification has been done by H. W. Conn who is therefore

responsible for the classification and arrangement of groups hereby adopted. It will be seen in the following pages that a large number of new forms have been added to those described in the previous paper and that not a few described previously have been excluded from the present list as insufficiently known. Our work in the last few years has added a large number of new forms to our list. These types which we have studied have been derived from a variety of sources. The larger part of them have been obtained from milk and milk products, either in the vicinity of Middletown or Storrs, and are therefore especially Connecticut forms. We have, however, received many cultures from elsewhere. Dr. Harding of the Geneva Station has kindly sent a large number; Harrison of Guelph, Canada, has sent us some; several have been sent by Marshall from Michigan; a large number were furnished us from the Board of Health Laboratory of New York City and separated from New York City milk which is obtained from a wide territory in the vicinity of that city; some have been sent from Dr. Weigmann of Germany; some from Switzerland by Freudreich; some from Italy by Gorini; and a few isolated forms have come from other sources. The cultures that we have had for examination have thus come from a very wide territory and while by no means complete, they represent fairly the dairy forms in the parts of the civilized world where bacteria are studied. Of the dairy forms described in the following pages the large majority we have ourselves had the opportunity of studying in our laboratory. But in order to make this list of dairy forms as complete as possible we have in the following pages inserted with the types that we have ourselves studied, the descriptions of quite a number that we have not seen but which have been well described by others. This has been done, however, only when the published descriptions are fairly complete and are sufficient to make it possible to determine the relations of the types in question. There have been a large number of bacteria mentioned and partly described in literature in regard to which this is impossible. Many bacteria have been mentioned as occurring in dairy products, and many named, whose description is so meagre that it is absolutely impossible for anyone to recognize them. In the Manual of Swithenbank these names have been collected and the descriptions given.

But most of them are worse than useless at the present time, for no one would be able to identify the bacteria from these meagre descriptions or even to tell whether the bacteria described belong to one or another group of bacteria. In a few cases this is possible, and where we have been able to do so we have associated these forms with some of the groups that we have described more in detail below. But the value of the descriptions in earlier literature varies with the completeness of the description, and the descriptions of a majority of these earlier described bacteria are so meagre that they are *absolutely worthless*. When, therefore, a type of bacterium has been named from characters which will apply to a score or more different types there is nothing to do but neglect it entirely. Not even its name can be retained if the type cannot be recognized. We have therefore, left out of our list large numbers of these types upon the ground that they must hereafter be absolutely omitted from descriptions because of the impossibility of determining what they are. We have tried to include in our list all bacteria that have been sufficiently described to render their recognition fairly sure, but, of course, there may be some omissions.

In our recent work we have tried to include in our technical routine of description all of the characteristics usually adopted for the general description of bacteria. We have, however, not adopted the practice of determining the formation of indol or the reduction of nitrates. The reasons for this omission have been that the data in question have not seemed to us to have much significance in relation of dairy problems with which we have been particularly concerned. Since these data have not been determined in the laboratory routine they have, of course, not been included in the following descriptions. It will be noticed that with some of the forms described by us the description is not complete. This is especially true of some of the earlier descriptions of types which have not been found in abundance and have not reappeared in our later work. Many of our earlier described species, however, though incompletely described at the time, associate themselves so closely with the forms more recently studied that they are merged in groups, which we now recognize and of which we give a more complete description. A few forms, however, are still left with nothing

except the original description made several years ago, a description which may be too incomplete to be of very much value. These are in a few cases retained in the following pages, but only for species that possess very striking characteristics. For instance, *M. l. rubidus*, having the power of producing a brilliant red pigment, is so striking a type that we did not think it wise to omit it even though the description was based upon a single culture obtained several years ago and is more or less incomplete.

It will manifestly require many years before a complete knowledge of dairy bacteria can be obtained. While we have in the following pages described about one hundred and sixty types, we recognize that this is by no means the complete list of even common forms; but to complete the list will require many years of laborious study. When we recognize the extreme variability of bacteria types, especially in physiological characters upon which their classification may be partly based, it becomes evident that to reach an end of the description of dairy forms is almost an impossibility. Our recognized types, as will be shown, vary in all directions and run into each other by more or less complete intermediate links. Every new set of cultures which we obtain from even similar sources show variations in various properties connecting them more or less with other types. This has been forced upon us more and more, as the data we have collected has increased, until we have almost concluded that the task of arranging these forms into species or even groups is hopeless; for even at the very best these groups will show such wide internal variations as to connect them more or less completely with the closely associated forms. Before such groupings can be finally made an immense amount of work and many years' study must be given. But every attempt to formulate our ideas clears our conceptions, and hence it is thought that the present endeavor to arrange the dairy bacteria in a scheme of classification, even though of necessity incomplete and doubtless requiring later changes, will bring us nearer to a proper conception of their relation and help toward a real understanding of problems of bacteriology.

The question of species.—The question of *species* among bacteria is at present an insoluble puzzle. It has become manifest that it is quite impossible to carry over to the classification of

bacteria the conception of word species which zoölogists and botanists have developed in the last century. It has been recognized by the modern zoölogist that the early conception of a species, as something sharp and distinct, is bound to be modified as variations are recognized. If this is true in animals and higher plants, it is more emphatically true of bacteria. Indeed we must practically abandon any thought of using among bacteria the term species with a meaning which has any similarity to that which is used for the rest of the living world. The question as to whether physiological variations are sufficient to characterize a new species is one which we cannot now answer, but this would be involved in an attempt to determine species of bacteria. Into these questions we do not propose to enter at all and shall not make any endeavor to arrange bacteria descriptions in the form of species and genera. The term species, therefore, will not be used at all in the following classification.

As our data has been increasing, it has become more and more evident that the bacteria which we have been studying naturally fall into series of groups. This has been dimly recognized by bacteriologists for some years, the *B. coli* group, for example, being one that for many years has had a somewhat recognized position. As we have collected the dairy bacteria, we have been able to see such groups becoming more and more apparent. In such a group there is usually a somewhat central type, but from this the numerous cultures which may be obtained and compared with each other show variations in all directions. Inasmuch as our characters are chiefly physiological, the variations of physiological properties naturally produce an immense confusion in the attempt to satisfactorily arrange cultures in groups. By these variations our different groups are more or less connected with each other, even when the groups are founded upon clear, sharp, positive distinctions. For example, the power to liquefy gelatin has already been recognized as one of the most fundamental characters, and yet this power of liquefaction is manifestly subject to wide variations. As has been shown several times, organisms which have the power of liquefying gelatin may lose this power more or less completely, and hence it is perfectly clear that some groups which liquefy may perhaps be only physiological modifications of non-liquefying types. Among the cocci described

in the following pages it will be seen that there is a white liquefying and a white non-liquefying group. In other respects these types resemble each other so closely that we are inclined to believe that they must be regarded as representing the same form, but two physiological varieties, one having lost the liquefying power which the other still retains. Similar variability is especially true in regard to pigments. Our experience has convinced us that whereas some pigments, like brilliant reds and deep oranges and greens, are distinctive characteristics of groups, the pale yellow colors and the white pigments are so sure to run into each other by intermediate variations that they are of very little value in distinguishing types. In the following pages it will be seen that a white and a yellow type of coccus has been described and recognized as different groups, but the intermediate forms which we have found convince us that they are really practically the same thing with slight physiological variations. Even in the question of morphology, which is usually regarded as the most manifest criterion for distinguishing types, something of the same appears. The distinction between the short rods forms and the coccus forms is by no means a sharp one. Cultures which have been described in our own laboratory as short rods have been sent to other laboratories and been described as coccus forms. To eliminate the confusion arising in all of these ways is manifestly as yet impossible, but we have endeavored in the arrangement of groups given in the following pages to recognize the sharper distinctions and to state, where possible, the connections of the groups by intermediate forms which have been discovered.

In our studies, as involved in the following pages, we have endeavored to work upon and describe only such types as are usually found *in nature*. Cultural varieties are not generally included in our list. The work in our own laboratory and elsewhere has shown that by modifications of the culture conditions an endless series of modifications may be produced in the descendants of the same original stock culture. The power of producing pigment may be changed, the power of liquefying gelatin, the shape of the bacteria themselves may be quite modified by different culture methods. It does not fall within our purpose to include such cultural varieties. We are interested, in this paper, not in knowing through what variations

different stock cultures may be forced, by modifying conditions, but rather what are the physiological characters of the types that actually exist under the conditions of nature and which may be found in normal dairy products. Most of the types described in the following pages, therefore, *are actual cultures obtained from dairy materials*, and in no case do they represent modifications of such forms, except such modifications as naturally occur under the normal cultures in the laboratory. A few cultures sent us by others form an exception to this rule.

To our mind the aim of classification of such a group of bacteria at the present time should be as follows:

1. To recognize the *groups* of bacteria in the terms above used, and then to describe these groups in such a way that they may be with tolerable ease recognized by others working upon the same subject.

2. To recognize the kinds of variation possible within the groups. By this is meant the study of as many natural cultures of the members of the group as possible, noting what variations appear in the different cultures, as isolated from milk, and thus determining to what extent the group characters are modified in the different forms of bacteria as isolated from actual milk products.

3. To find, if possible, the natural limits of such groups. By this, of course, is meant to determine the limits within which the variations may occur, and yet the type in question may be legitimately regarded as belonging to the group in which it is placed. If no such limits could be found, naturally the whole question of classification of bacteria would be pretty nearly hopeless. It may be that as information accumulates, it will be found that all of these groups so run into each other as to make it impossible to logically separate them. This is, of course, what would be naturally assumed upon the general theory of evolution of types, but whether such is the case can only be determined after an immense amount of data has been accumulated. At the present time, with the data at hand, it seems to be possible to arrange these forms into fairly distinct groups which, though connected more or less with intermediate types, are nevertheless recognized with as much certainty as the different types of animals and plants may be recognized.

In determining these groups the primary question that arises, of course, is what characters shall be used to separate the groups. While all characteristics should, of course, be taken into account, some are clearly of more importance than others. There is among bacteriologists as yet no consensus of opinion as to what characters are of most importance for this purpose. It is, however, quite generally thought that morphological data are primary and that these should be the first points of distinction between these organisms. We are inclined to believe that this is true, but as intimated above, we have been forced to think that it is necessary to make certain modifications of this statement. The cocci and bacteria certainly run into each other in such a way that it is sometimes simply impossible to determine whether a culture should be called an extremely short bacterium or a coccus type. As already stated, the same culture will be described by one person as one and by another as the other of these two forms. Moreover, the classification of the spherical forms as usually accepted to-day into the streptococcus and micrococcus groups appears to be impracticable in many cases. We have found many forms of bacteria in which it is practically impossible to determine whether we are dealing with a coccus that divides in one plane only or in two planes. Moreover, in one or two cases we have clearly found a spherical form that multiplies for a long time by dividing in one plane, producing a long chain, which would be then naturally called a streptococcus, and then the whole series of spheres divided in the other plane, at once giving chains of pairs. This observation has been made once or twice in our own laboratory and has been confirmed by Winslow. Whether to call such a type a streptococcus or a micrococcus is evidently a problem. We have furthermore found in our study that many forms which are described as streptococci are identical in every character with others described as micrococci except this one point of the method of division. These facts have convinced us that this distinction of the micrococci and streptococci is an uncertain one, and we have not found it possible to follow it out with accuracy. We have therefore used this character as a secondary rather than a primary one in distinguishing types. The question of flagella present upon microorganisms has appeared to us to be of more importance than

some of the other morphological characters. We have in our classification, therefore, sharply separated the motile from the non-motile rods and the peritrichic from the monotrichic bacilli. In a few of our early described species in which flagella were not made out we have found it possible to group them from their other characters with later isolated cultures which are more carefully described.

The question of the liquefaction of gelatine has also been an open one, for the data at our command seem to suggest that here, too, we have a character that is somewhat variable. Some types show this property of liquefaction only after two or three weeks' growth, and we have considerable data to indicate that the power of liquefying may be completely lost; indeed, among the organisms isolated from milk we have occasionally found two that are identical in every respect except in this power of liquefaction, and organisms that have such peculiar character as, for instance, the power of producing a pink fluorescence, as to convince us that we are really dealing with the same organism, but one in which the property of liquefying gelatine is capable of being totally lost.

In the grouping of bacteria in our key we have used prominently the power of fermentation of sugars as a means of diagnosis. This characteristic is one which has become recognized recently as quite significant, and for the purpose of the study of the groups of the bacteria of milk, it is evidently one of exceptional importance. The fermenting power of sugar is closely related to the action of the organisms upon milk, and clearly from the standpoint of dairy bacteriology a grouping of bacteria with this as a basis is one of the most practically useful methods of grouping these organisms. But here, too, it appears to us that variations are common. Some of our groups contain organisms which apparently show considerable difference in their power of fermenting different sugars. It is common to separate in different groups any organisms which would ferment dextrose but not saccharose from one that would ferment the two sugars. As we have compared together the large numbers of cultures from various sources that we have made, we have found such wide variations in this power of fermenting sugar that we have been inclined to believe that here, too, we have a variable factor, and that whereas the general

power of fermenting sugar may be taken as a valuable and important criterion in separating groups, there may be variations among the members of this group in the kind of sugars they can ferment and the readiness with which they can carry on this action. At all events we believe that the variations in the power of fermenting sugar are only of value in separating closely allied varieties from each other, but quite insufficient to characterize different groups.

The production of pigment by bacteria is one of the characters frequently more striking than any other. The question of the value of this characteristic in separating organisms from each other has been much discussed. It has been many times shown that this power of producing pigment is capable of variation. It is certainly true that some organisms that are able to produce pigment under some conditions lose this power after cultivation. *B. rudensis*, for example, which, when first isolated, produced a brilliant red pigment, wholly lost this power in cultivation, and when the organism had reached my laboratory many months from its original source, it was a pure white Bacterium, showing all the other characteristics of the genus, but having lost its pigment-producing power. If the power of producing pigment is thus capable of variation, it indicates, of course, that it is not a criterion to separate types radically from each other. Nevertheless, in the general study of micro-organisms this is one of the characteristics which must be taken into consideration. Even though we recognize that some pigment-producing organisms may, under conditions of long cultivation in the laboratory, be converted into non-pigment-producing types, it is none the less important for us to recognize the pigment-production as a distinct characteristic of the organisms as found in milk productions.

For practical purposes it is a matter of less importance to know that a red pigment may cease to be developed after long cultivation in the laboratory than it is to recognize as a distinct type of dairy organisms a bacillus producing a red pigment. We have therefore used the pigment production as one of the characteristics for separating our groups. From our observations we have been inclined to think that red, orange, brown, and green pigments are commonly distinctively characteristic, that a lemon yellow color is also a character which remains

tolerably constant, but paler yellows are of far less significance. In our classification the color of yellow or yellowish cannot, therefore, be regarded as particularly important, and we are convinced that many of the varieties described as yellow are identical with others described as white. Where we have good reasons for believing this is the case, we have mentioned the fact in the following pages by cross references.

In our opinion the other characteristics commonly used for describing bacteria are less significant. The appearance of the colony in gelatine is subject to wide variations, and while sometimes it is useful, it cannot be relied upon as a rule as of very great importance. The growth on potato varies more or less widely with the nature of the potato, and so decided are these variations that we have ceased to place much confidence upon the characteristics here described. The growth in bouillon has appeared to us to be of less importance than some have thought, even the formation of a scum being somewhat variable in different cultures of the same organism.

The question of nomenclature is, of course, a puzzling one in dealing with a new group of organisms, whose inter-relations are as problematical as that among bacteria. Nevertheless, it is desirable that certain names should be given in our classification. In the following pages, we have adopted the following plan. We have named each of the general groups of organisms which we have recognized as more or less clearly marked types. The names that we have given to these are in all cases where possible names already applied to the chief members of the group. In many cases we cannot find among described bacteria any which agree closely enough to admit of identification, and in regard to many others, while there are among described types some which agree with ours as far as they go, the published descriptions are too incomplete to admit identification. In both of these cases we have given new names. In our names we have in practically all cases used the word *lactis* in the name. We are fully aware of the objections to using three names for a species, but in these cases the manifest advantage of indicating by the name the fact that the organisms are associated with and found in milk products is so great that we regard it as sufficient to overcome the disadvantage. We have used this method in all cases except a few well known organisms where it was manifestly unwise.

Underneath these groups we have tried to recognize the types of varieties which we have found and which are therefore liable to be isolated from any samples of milk, and for the present we have grouped these as varieties A, B, C, D, etc., under the general name of the group. In this way we recognize the general classification of bacteria, and at the same time obtain an impression of the variations that develop within the types themselves.

Method of Description.—In describing a long list of species of bacteria such as are included in the following pages, there are two different purposes to be considered, each of which would involve a different method of procedure. First, there is the question of *diagnosis*, which would call for such an arrangement of important characters as will enable other bacteriologists to identify forms which they may have in hand with those already described. There is, second, the need of such a complete description of the organisms in question, with *all* its other characters as will finally and definitely describe them for a permanent record. In accordance with which of these two purposes we have in mind the method of description would be varied.

In our own opinion at the present time the first of these two points is the more important. Until we know more about the general relations of bacteria, it is more important to have such a grouping of the types as will enable others to identify the cultures which they may have under observation with those already described. The more minute detailed description of species will undoubtedly of necessity come later. But when we recognize that such considerable variations of the same type are possible, and certainly do occur under cultural conditions, it becomes evident that the minute description of the physiological characters of the different cultures becomes of comparatively little value. If the types are not constant in all of these points, it is certainly of no great value, at least in the present state of bacteriological science, to describe the particular characteristics of a culture at any particular time, especially if it is true that when cultivated in the laboratory for six months, these characters may largely change. Hence we have concluded that with the present state of bacteriological science the more valuable form of classification will be one which will

select the salient features, and so far as possible those that are the least subject to variation, and so tabulate them as to make it feasible and convenient for other students to recognize the types in question and to identify them with any other form they may be studying. We have, therefore, in the following pages placed emphasis wholly upon the question of *brief diagnostic characters* and in arranging them in such a form that they can be easily utilized, rather than in a detailed description of species.

We have also learned from experience that unless the characteristics of species can be clearly and distinctly *tabulated*, it is almost a hopeless task for anyone to identify a new culture with one previously described. The methods of description that have been in vogue in the first decade of the study of bacteria have been to describe in somewhat verbose detail all sorts of minute characteristics, which later discovery has shown to be of little or no significance. These have been commonly arranged in no order and have never been tabulated. The result has been that it is almost impossible to identify any previously described type of bacteria. Consequently the bacteriological literature has seen a constant succession of new types described, and when these descriptions are compared with each other it becomes more and more evident that the same general type of bacterium has been described over and over again by different bacteriologists and given name after name. Undoubtedly some of our most common dairy organisms have been given a dozen or fifteen names, due to this unfortunate method of describing species by long details. We have endeavored, so far as possible, to correct this error in our classification, by excluding all unimportant data and keeping only what seemed to be the distinctive and more or less constant characters, and then to arrange these in such tabular form by methods already known so as to make it possible to use them conveniently.

We have arranged our classification as follows: We have first inserted a detailed description of the different bacteria which we recognize under different names. Instead of attempting however, to describe these to any great length, as would be necessary if all of the characters were filled out which are demanded by the description blanks of the American Society of

Bacteriologists, we have endeavored to insert only the salient characters. It has appeared to us that the numerous details only confuse one in an attempt to identify and classify bacteria. When it is so evident that these physiological characters, which are largely the basis of these long descriptions, are capable of such great modification by culture, when it is evident that the cultures of bacteria which one obtains are likely to come from a great variety of sources and therefore to have been subject to a large variety of different conditions, it is evident that we may expect an almost endless variation in the physiological characters of the different cultures. If we do not identify two cultures of bacteria as the same until we find that they agree *exactly in all* of the long series of characters given in the society blanks, we shall practically never identify any two types of bacteria as the same. It has seemed to us that this is an absurdity and that the recognition of the relations of bacteria will be advanced by omitting insignificant details, concentrating our attention upon the more important characters. For this reason we have in these descriptions omitted much of detail and have only given such points of description as have seemed to be most salient.

Following the detailed descriptions we have given analytical keys and tables covering all the types. We recognize the fact that the bacteria of milk may be grouped in three general groups, the *Coccaceae*, the *Bacteriaceae*, and *Bacillariaceae*. These groups are morphological ones, and while, as has already been pointed out, we are convinced that to a certain extent they run into each other and cannot be sharply distinguished, they do represent fairly well marked groups. Under each of these heads we have given an analytical key, in which the important characters are used for purposes of diagnosis. By means of this key it is possible to trace readily to its group or to its allies any type of bacterium of which we have a tolerably complete description. These analytical keys are given for each of the great groups of bacteria. Following these analytical keys we have given finally a tabulated key of all of the important characters of the different species. In the preparation of this key we have made use of the plan adopted by the American Society of Bacteriologists, selecting the important characters which can be indicated by positive

and negative signs. This plan, advanced some years ago by Fuller, has proved useful, and inasmuch as it has been provisionally adopted by the American Society, we have made use of it in the tables of our key. In our own opinion the plan advanced by Gage and Phelps in using numbers that have certain arbitrary meanings is far more satisfactory and far more usable than this plan which the American Society has adopted; but it is better to have uniformity in the matter even at the expense of some loss, and we have therefore adopted the plan of the American Society. All of our species are arranged in tables in this way, the tables being so grouped that organisms which are most closely related to each other come in proximity. With this plan it is only necessary in order to identify the relations of a new type of bacterium, to fill out on one of the cards prepared by the American Society, the blanks left for the insertion of the characters, and then to place this blank upon the table in the following pages and running it up and down the page until there is found one organism with which the plus and minus signs practically agree. When this is found it is sure that the allies of the species under consideration have been identified, though it may not agree in all details. In this table we have also used the group numbers as directed by the American Society of Bacteriologists, this group number being another important aid in identifying a culture and placing it among its allies.

In a number of cases we have found that what we believed to be the same organism comes under two different heads in our classification; for instance, a culture has been described as a Bacterium and another one as a Coccus, and yet when carefully studied out in all other respects they are found to be the same. Under these circumstances we have been uncertain as to where it should be classified and we have therefore placed it under both divisions of Coccaceae and Bacteriaceae. The same thing has occurred occasionally with organisms that liquefy or do not liquefy gelatine. In all such cases cross references are inserted to indicate these probable relationships.

It should be finally stated that the forms which we recognize in the following pages which we name must be regarded as groups and not species. This is not at all material, inasmuch as we have no conception whether the term species has any

meaning whatsoever among bacteria. Our names, therefore, refer to groups under which there are in some cases many sub-varieties, in other cases few and in some cases no sub-varieties. From the standpoint of practical dairying the sub-varieties are sometimes of more significance than the general type. For example, under the head of *Bacterium lactis acidii* there are certainly a number of varieties differing in respects which are of extreme importance to the dairyman. They differ in the amount of acid they produce and in their power of curdling milk. So, too, under the head of *Bacterium lactis aerogenes* we have clearly a group of organisms differing in many important characters. Among these characters there is wide difference in the extent of the gas production; some cultures producing only a small amount of gas, others producing gas in prodigious quantities. While these variations in the amount of gas cannot, in our opinion, be regarded as points by which the group should be separated from each other in a scheme of classification, they are of the highest importance to the dairyman. The variety which produces a small amount of gas would be consistent with the best dairy products, while the presence of the other variety would totally ruin a lot of butter and more surely ruin a lot of cheese. These sub-varieties, in short, are of extreme importance to dairymen, and the careful study of these varieties is a problem which should be considered carefully in the future; but in a general classification of bacteria it is, in our opinion, at present quite impossible to recognize all of these types by name, and our present plan is, therefore, only to group them as varieties under the general group name.

METHOD OF STUDY AND DESCRIPTION.

A few words are needed as to the method of obtaining the data tabulated in our descriptions. The *isolation* of the bacteria is usually accomplished by the use of litmus gelatine. The preparation of this litmus gelatine has been described in a former publication. (Bacteria in Milk and its Products, P. Blakistons Sons.) This has been used because our experience has shown that it gives a better differentiation of colonies than other solid media. After isolation the cultures are *purified* by the ordinary

method of replating, and, after purification, they are inoculated, upon *agar streaks*. After about two days' growth on agar they are inoculated into the various other culture media.

The *morphology* of the various organisms was determined from fresh agar streak cultures although, to determine the formation of spores, it was sometimes necessary to use older cultures. To determine the *motility* we have usually used an ordinary bouillon culture of 12-24 hours' growth. We have found that a careful study of a hanging drop of such a bouillon culture is most satisfactory for this purpose. To determine the presence of *flagella* we proceeded as follows: from an actively growing bouillon culture a drop was removed with a platinum loop and spread thinly over the surface of solidified agar. This was then incubated at 37° for 12 hours. A small quantity was then removed, diluted in three successive drops of sterilize water and stained by the well known Loeffler method.

The culture media we have used have been those ordinarily employed. Our bouillon, gelatine and agar have been made with Liebig's beef extract instead of chopped beef, because of greater convenience and uniformity. The *fermentation tube test* was made with dextrose, lactose and saccharose, 1 per cent. of these sugars being added to ordinary bouillon. In all the cultures tested by us in the last five years we have used all three sugars. The *milk* which we have used has in all cases been skimmed milk, sterilized by boiling for 10-15 minutes on three successive days. *Potato cultures* have been made by cutting plugs from large clean potatoes, slicing them once obliquely, and then soaking them in running water over night. After this they were placed in tubes and sterilized in an autoclave.

In general the terms which we have used in our descriptions have been those suggested by Chester and adopted by The American Society of Bacteriologists. In stating whether or not an organism produces *acidity* we refer to the production of acidity in dextrose bouillon. In some cases, as will be seen, organisms produce acidity in lactose but not in dextrose; but these are rare. The detection of acidity has been made by means of carefully prepared litmus paper. This paper we have prepared ourselves from neutral litmus. The test for acidity has been made in about two days, and also later. This method has been adopted, both for the fermentation tubes and for milk. In determining

the *action upon milk* we have always used both the temperature of 20° and 37°. In our descriptions in the following pages we have not referred to the action of these two temperatures, except in cases where it was different at the two temperatures. It will be understood, then, that the actions given as taking place in milk occur at both temperatures unless otherwise specified, although usually, of course, more rapidly at the higher temperature. The *digestion* of milk has been determined by eye only.

We have hitherto done no work upon strictly *anaerobic* bacteria. Most of the forms which we have described have been aerobes, although some grow more readily if they do not have too abundant a supply of oxygen. The question of their relation to oxygen we have determined from their surface growth on various media, their growth in the closed arms of the fermentation tubes, and also from their growth under a mica plate which we have always made and frequently found useful, although we have not tabulated it in the following pages.

DETAILED DESCRIPTION OF TYPES.

A. NON-LIQUEFYING COCCI.—STREPTOCOCCI AND MICROCOCCI.

I. Types that do not produce acid.

M. lactis rosaceus n. s. *A pink Micrococcus*. This organism, originally found in 1903, has been found twice since, in milk, in this vicinity. All cultures agree in essential particulars.

Morphology.—Size, .8 μ in diameter. Stains by the Gram method.

Gelatine colony.—Surface colony reaches the size of 1 mm., with a nucleus and a light outer zone. The color is pink. On *litmus gelatine* it produces a bluish colony which is not acid.

Gelatine stab.—A needle growth and a spreading pink surface.

Agar streak.—A luxuriant pink growth.

Fermentation tubes.—No acid nor gas is produced in any sugar bouillon, and no growth in closed arm.

Bouillon.—A sediment and a slight turbidity, but no pellicle.

Milk.—Rendered slightly acid and shows a slight pinkish sediment. It becomes somewhat slimy.

Potato.—A very luxuriant, thick, moist growth of a pink color.

Grows at both 20° and 37°. Aerobic.

M. lactis citreus B n. s. *A yellow, non-acid Coccus*. (Perhaps=*M. citrinus Migula*). The similarity of this culture to our yellow liquefying coccus leads us to think that they are perhaps the same and hence the use of the same name. (See *M. lactis citreus* A, p. 118.) This organism has been found but once in milk in Middletown. Its characters are as follows:

Morphology.—Size, 8 μ . No chains produced. Stains by the Gram method.

Gelatine colony.—A distinctly round, thin, yellow surface colony, which becomes about 1 mm. in size. Not characteristic.

Gelatine stab.—A good needle growth and a moderately luxuriant surface growth, of a yellow color.

Agar streak.—A luxuriant, yellow growth.

Fermentation tubes.—There is no production of acid or gas in any sugar bouillon, and no growth in closed arm.

Bouillon.—An abundant sediment and turbidity, and a thin pellicle on the surface.

Milk.—At both 20° and 37° the milk is rendered acid, but it is not curdled or otherwise affected. The acidity is very slight.

Potato.—An abundant, canary-yellow growth, and the potato is discolored.

Grows well at both 20° and 37° Aerobic.

M. lactis flavus n. s. An orange, non-acid *Micrococcus*.

Morphology.—A micrococcus. Size, .5μ-.8μ. Stains by the Gram method.

Gelatine colony.—A round, smooth, thick, homogeneous, orange-colored surface colony.

Gelatine stab.—A good needle and surface growth, with an orange color.

Agar streak.—Luxuriant, moist, smooth, of an orange to a red-brown color.

Fermentation tubes.—No acidity or gas in any sugar bouillon. Dextrose and lactose may be rendered alkaline. No growth in the closed arm.

Bouillon.—A sediment, a turbidity, and a pellicle; or the turbidity and pellicle may be wanting, the latter characteristic belonging to a second culture isolated at a different time.

Milk.—Is rendered acid both at 20° and 37°, but no other change is produced, except a slight yellow color in some cases.

Potato.—Moderate to luxuriant, moist, smooth, red-brown to orange.

Grows at 20° and 37°. Aerobic.

We have found this organism several times. The different cultures differ, however, in small points. The chief lines of difference were the following: In color it ranges from orange to bright yellow. In two cultures the gelatine colony was white rather than yellow, and in these same two cultures milk was not rendered acid. One culture curled milk, acid. One culture grew in the closed arm of the fermentation tube, while the others did not. We are not inclined to think these differences sufficient to separate them as varieties.

Apparently this is identical with *Micrococcus D.* of Barthel, and perhaps with *M. aurantiacus* Cohn.

M. lactis viscosus C n. s. A slimy milk *Micrococcus*. This culture was sent us by Harrison and was isolated by him from Pasteurized milk. We have not found it ourselves. Its failure to make milk acid and its dark colored growth on potato seem to distinguish it from any other slimy milk micrococci. Hence we call it variety B. (See p. 114). The characters are as follows:

Morphology.—A micrococcus. Size, .8μ-.9μ. Gram stain positive.

Gelatine colony.—A thick, round, smooth, white colony. In old cultures the gelatine is turned green. On *litmus gelatine* the colony is coarse, granular and nucleated. The surface growth is rather transparent. Below the surface it is opaque and brownish.

Gelatine stab.—A vigorous needle and surface growth.

Agar streak.—Moderately thin, spreading, white.

Fermentation tubes.—Neither of the sugar bouillons is rendered acid, nor is there any gas produced, nor growth in the closed arm.

Bouillon.—A sediment and turbidity, but no pellicle.

Milk.—No change except the production of a decided sliminess.

Potato.—A luxuriant, thick growth of a slaty-gray color, turning to blue or black, and old cultures to an olive-green. The potato itself is discolored.

It grows well at both 20° and 37° Aerobic.

The organisms that produce slimy milk are numerous. They do not by any means form a class by themselves, for this property of rendering milk slimy seems to be found scattered widely among bacteria. Apparently the type that most commonly produces trouble in dairies is a Bacterium. The following organisms have been described as causing sliminess, some of which are included in our list and others omitted as insufficiently described: *M. viscosus* Bechamp, *Actinobacter du lait visqueux* Duclaux, *Actinobacter polymorphus* Duclaux, *B. mesentericus vulgatus* Flügge, *M. mucilaginosus* Migula Migula, *B. lactis pituitosi* Loeffler, *Strep. Hollandicus* Weigmann, *Bact. lactis longi* Troili-Petterson, *B. Guillebeau c.* Freudenreich, *Bact. Hessii* Guillebeau, *M. Freudenreichii* Guillebeau, *Carpho. coccus pituitoparus* Hohl, *Coccus lactis viscosi* Grüber, *B. lacto rubifaciens* Grüber, *Bact. lactis acidi* Leichmann, *Bact. aerogenes* Escherich, *B. Harrisonii*, *M. viscosus* A, and some others. This list includes almost every type of bacteria, so that the characteristic of slimy milk cannot be taken as forming a group mark.

M. lactis arborescens.—This was described in our former report and has been found twice since.

Morphology.—A micrococcus. Size, .7 μ in diameter.

Gelatine colony.—A myceloid colony about 1 mm. in diameter. Sometimes it is smooth.

Gelatine stab.—An arborescent needle growth and a surface growth.

Agar streak.—A luxuriant, white, smooth moist growth.

Fermentation tubes.—Not determined, but doubtless neither acidity nor gas.

Bouillon.—A turbidity, sediment and a tenacious scum.

Milk.—No action or a very slight alkalinity.

Potato.—A spreading, brownish growth, not very luxuriant.

Grows at 20° and 37°. Aerobic.

Galactococcus versicolor Lux. A white non-acid coccus. One of the most common types of milk organisms proves to be a small white coccus that does not produce acid and without any very marked characters. There are a large number of these which, although showing some variations, are so much alike that we regard them as forming a single type. They are extremely common in milk and are certainly found in the udder very commonly. We have adopted the name first given by Lux as fairly distinctive, although we think it would be better to use the name *Streptococcus versicolor*. We can recognize two types although no sharp line can be drawn between them.

Morphology.—A streptococcus. Size, .5 μ –1.5 μ . Never more than three or four joined together. It commonly stains by the Gram method.

Gelatine colony.—Spreads slightly over the surface, white, yellowish or brownish, lobed or round or rather thin. The colony is not characteristic.

Gelatine stab.—An abundant needle and a surface growth.

Agar streak.—White to yellowish, moist, not luxuriant.

Fermentation tubes.—No acidity in any sugar bouillon; there is no gas or closed arm growth.

Bouillon.—A sediment and turbidity but no pellicle.

Milk.—Usually no action, although a few cultures show a slight acidity.

Potato.—Very scanty, grey-white, or sometimes no growth.

Grows at both 20° and 37°. Aerobic.

Variations from the above are shown in color, from a white to a yellowish; in the formulation of a pellicle in one culture; and in the failure to stain by the Gram method.

Variety A.—We recognize as a distinct variety one form that grows luxuriantly on potato and has a less tendency to form a yellowish pigment. This type has also a slight tendency to produce an acidity in milk, in this respect agreeing with *M. candius* of Barthel, which is probably the same. Variety A is equally common with the type described.

One culture which would naturally belong here shows relations to *M. lactis albus* in that it digests milk, but does not liquefy gelatine. Its characters are as follows:

Variety B. Digesting milk without liquefying gelatine.

Morphology.—A micrococcus. Size, .6 μ . The Gram stain is negative.

Gelatine colony.—Very small, yellowish, surface colonies, smooth, convex, entire, moist.

Gelatine stab.—A needle growth and a convex surface.

Agar streak.—Filiform, raised, smooth, transparent, moist, luxuriant. In some cases wrinkled.

Fermentation tubes.—No acidity or gas in any bouillon. Usually no closed arm growth, but one culture showed growth in closed arm.

Bouillon.—A sediment and turbidity; a pellicle sometimes forms and sometimes not.

Milk.—Becomes alkaline and digests. This digestion becomes complete in three weeks, and is unusual since the organism does not liquefy gelatine.

Potato.—Luxuriant, filiform, capitate, cream color. Potato not discolored.

Grows at 20° and 37°. Aerobic.

The description of this Coccus appeared to us to agree closely with that of *Streptococcus pyogenes*. To see how closely they agree, we obtained a culture of the latter from the bacteriological laboratory at Yale Medical School, and compared it with our cultures side by side. In all cultural characters we found that the two agreed so closely as to be indistinguishable. Whether this indicates that they are identical we would not at present determine, but the identity in cultural characters of this common pathogenic Coccus and this most common dairy organism is at least suggestive. In fresh milk this type is probably the most common of all dairy bacteria.

II. Types that produce acid in dextrose or other sugars.

S. lactis fulvus n. s. *A brownish-red Streptococcus*. This was obtained once directly from the udder and once from city milk. Another culture was obtained from stable dust which agree in all points except those indicated in brackets.

Morphology.—A streptococcus. Size, $.7\mu$. Gram stain is positive.

Gelatine colony.—Small and dense, 5 mm. in diameter, rather thick, round, white. The colony in litmus gelatine is acid. [Brownish-pink to orange.]

Gelatine stab.—A needle growth and a thin surface growth.

Agar streak.—Luxuriant, thick, moist, translucent, reddish-brown [orange-yellow].

Fermentation tubes.—All three sugar bouillons rendered acid; there is no gas or closed arm growth.

Bouillon.—A sediment, turbidity, but no pellicle. [No turbidity.]

Milk.—Is acid and curdles.

Potato.—Luxuriant, brown.

Grows at 20° and 37° Aerobic.

One culture obtained from New York milk shows some rather striking characters, and we recognize it as a separate variety as follows:

Variety A.—This agrees with the above except in the following: Size, 1.2μ – 1.4μ . Gram stain negative. Gelatine colony ringed and irregular, showing acid in litmus gelatine. Bouillon with a pellicle. Milk, curdled acid at 37° only. Potato has luxuriant growth and is discolored.

M. lactis aureus n. s. Many different cultures of *yellow, acid Cocci* have been found in this vicinity, from Canada and New York. They are found in milk, in cheese, in butter, and in stable dust. Although they do not liquefy gelatine, we are confident that they are to be looked upon as non-liquefying forms of *Staph. pyogenes aureus*. (See p. 121.) The general description is as follows:

Morphology.—A micrococcus. Size, $.5\mu$ – 1.2μ . Gram stain positive.

Gelatine colony.—Round, moderately thick, smooth, translucent colonies, of a lemon-yellow color. *Litmus gelatine* is acid.

Gelatine stab.—A needle growth and a yellow surface growth.

Agar streak.—Luxuriant, moderately thick, smooth, translucent, yellow.

Fermentation tubes.—All sugar bouillons are rendered acid, but there is no gas. Occasionally there is growth in the closed arm.

Bouillon.—A sediment and turbidity, and rarely pellicle.

Milk.—Rendered acid and usually does not curdle. Some cultures, however, curdle milk after several days at 37° . Milk may be rendered yellowish.

Potato.—Usually a moderately thick, yellow growth, with the potato discolored.

Grows better at 37° than at 20° . Facultative anaerobic.

Among the many cultures which we have studied, variations are found in the following points: Size, from $.5\mu$ – 1.2μ . Litmus colony is sometimes acid and sometimes not. Color varies from lemon-yellow to pale-yellow. In a few cases there has been closed arm growths in the dextrose, and sometimes in lactose. In two cultures a pellicle formed on bouillon. Milk always acid but

only about half of our cultures curdle it. Potato varies from scanty or no growth to luxuriant. We do not regard these variations as sufficient to warrant us in distinguishing them under different names.

S. lactis aureus n. s. *An orange red Streptococcus.* This organism is apparently sufficiently different from the last to be separately named. It was found in a Camembert cheese sent from France. Several varieties occurred all together in the same cheese and they are, doubtless, physiological varieties of the same organism.

Morphology.—A streptococcus. Size, 1μ – 1.2μ . Gram stain positive.

Gelatine colony.—A round, thick, rough, opaque, surface colony, of a creamy-white color. On litmus gelatine it is dense and not acid, with an irregular edge. One culture was thin.

Gelatine stab.—A needle growth and a raised surface growth; orange.

Agar streak.—A luxuriant, rough, orange-yellowish to greenish-yellowish color; sometimes dull and wrinkled.

Fermentation tubes.—All sugar solutions are rendered acid, but there is no closed arm growth nor gas.

Bouillon.—A sediment and a turbidity are produced, and, after several days, a pellicle.

Milk.—After some days rendered acid and curdled. Some of the cultures did not produce acid and did not curdle; a slightly sour odor.

Potato.—A thick, rough, opaque, yellow, luxuriant growth; potato may be discolored.

Grows at 20° and 37° . Aerobic.

We have found several white coccus forms that produce *slimy milk*. The differences between them are considerable, although it may be that they are all variations of the same type. At present we recognize three.

S. lactis viscosus n. s.

Morphology.—A streptococcus. Size, $.8\mu$ – $.9\mu$. The Gram stain is negative.

Gelatine colony.—A shiny, pale-yellow, round or lobate colony, 1 to 2 mm. in diameter. It is commonly viscous.

Gelatine stab.—A needle growth and a surface growth, producing a nail culture.

Agar streak.—An irregular lobate surface growth, quite luxuriant, viscous.

Fermentation tubes.—All three sugar bouillons become acid and there is growth in the closed arm, but no gas is produced.

Bouillon.—A sediment and a turbidity, and also a pellicle.

Milk.—Rendered acid and curdled after three or four days. The milk becomes very slimy.

Potato.—A luxuriant, dull, paste-like growth, of a gray to a yellowish color.

Grows at both 20° and 37° . Facultative anaerobic.

This description is from the slimy milk organism of group V. as given by Harrison. We have found an organism at Storrs which is apparently the same, and which we had previously named *M. lactis viscosus*. We will call this a distinct variety, and it differs from the above described in the following points:

Variety A.—The agar growth is scanty and not viscous. No pellicle is formed on bouillon. Growth on potato is luxuriant and the potato is discolored.

To this same group apparently belongs *M. Freudenreichii*, as well as can be determined by the incomplete descriptions given.

Reference must also be made to *M. lactis viscosus* B., differing from either of those described, so as to be located elsewhere in our scheme. (See p. 109.)

The white cocci which produce *acid and no slime* are very numerous and their separation into groups is uncertain. We have tried to divide them into several types but the divisions between them are not satisfactory. Each of the groups shows variations, and it may be better to unite them altogether. For the present, however, we recognize the following divisions, based primarily upon their action on sugar and the abundance of their surface growth.

S. lacticus Kruse. This includes the smaller acid cocci with a scanty surface growth. This type is very closely related to, if not identical with, *Bact. lactis acidi*. The organism that commonly causes the souring of milk shows some variation in its morphological structure. It is sometimes clearly a short coccus form, while in other cases it is decidedly more elongated. In cultures, too, similar variations have been found. It commonly appears as a short rod, clearly longer than broad, and as such has been usually described as a Bacterium and named *Bact. lactis acidi* by Leichmann. By this name it is commonly referred to in literature. But cultures of *Bact. lactis acidi* when grown in lactose bouillon frequently, at least, appear as streptococci, and Kruse (Cent. f. Bac. u. Par. I., XXXIV., p. 737, 1903), and Heinemann (Cent. f. Bac. u. Par. II., XVI., p. 538, 1906), have recently insisted that there is no such thing as *Bact. lactis acidi*, all of the organisms which have been so named being really cocci, which should be called *S. lacticus*. Upon this point we will at present express no opinion beyond the statement that the organisms as found in souring milk are sometimes streptococci and sometimes clearly longer than broad, and hence would be naturally classed as Bacterium. We regard it better at present, therefore, to retain the name *Bact. lactis acidi* for the milk type as it commonly appears, and to recognize the streptococcus form independently under the name given by Kraus. It has the following characters:

Morphology.—A streptococcus with commonly short, but sometimes long, chains. Size, .5 μ –1 μ . Gram stain positive.

Gelatine colony.—Extremely minute, white colonies, occasionally slightly yellowish, rough and dense. In *litmus gelatine* the colonies are always acid. The surface growth is always slight and usually absent entirely, and it grows better under a mica plate. Sometimes the colonies in litmus gelatine show minute spines on their edge.

Gelatine stab.—A moderate needle growth but no surface.

Agar streak.—The growth is hardly visible, but when it occurs is the faintest transparent film.

Fermentation tubes.—All three types of sugar are rendered acid and there is commonly a growth in the closed arm. No gas is produced.

Bouillon.—Growth is extremely slight and sometimes invisible. A slight sediment and turbidity may be produced.

Milk.—Rendered acid and curdled promptly, producing a typical, smooth, acid curd.

Potato.—The growth is usually invisible, but sometimes a scanty surface film may be seen.

This streptococcus is very common and in some specimens of soured milk comprises ninety-nine per cent. of all the Bacteria present. It has been found frequently in the milk in Middletown, in Storrs, in New York City, and elsewhere. Where it occurs it is commonly the cause of the souring of milk, but it is not so common as the elongated form which we have classified as *Bact. lactis acidi*.

The white cocci that produce *acid but no slime* are very numerous and their separation into groups is uncertain. We have divided them into six groups, but the distinctions between them are not satisfactory. Each of the groups has slight variations and it may be that some of them would be better united together. For the present, however, we recognize the following, dividing them first according to their action on various sugars, and secondly by the abundance of surface growth produced on media.

S. lacticus I. The essential character of this type is the production of acidity in dextrose but not in other sugars.

Morphology.—A streptococcus in pairs or short chains. Size, $.6\mu$ – 1.2μ . The Gram stain is positive.

Gelatine colony.—This is not characteristic. It is round, white to yellowish and spreads over the surface, forming a colony 1 mm. in diameter.

Gelatine stab.—A needle growth and a surface growth.

Agar streak.—Moderately abundant, white.

Fermentation tubes.—Dextrose bouillon is rendered acid, but no gas is produced and no acidity in other bouillons.

Bouillon.—A sediment and turbidity and sometimes a pellicle.

Milk.—Becomes acid, but is not curdled, and no other change is noticed.

Potato.—Growth very slight or wanting.

Four cultures of this group have been studied—two derived from milk fresh from the udder, one from the dust of the stable, and one from cheese. They differ slightly, as follows:

Variety A.—This culture, from the udder, produces no turbidity, but a slight pellicle in bouillon.

Variety B.—From the udder. Produces turbidity and no pellicle in bouillon and does not make milk acid.

Variety C.—From stable dust. Has a negative Gram stain, a turbidity and a pellicle in bouillon, and milk is not acid.

Variety D.—From cheese. Shows a turbidity and a pellicle in bouillon; milk is acid, and there is a luxuriant potato growth.

S. lacticus II.—This produces acid in lactose and saccharose but not in dextrose. Its other points of difference are as follows: Gram stain is negative; the gelatine colony is very small and transparent; no pellicle is ever formed on bouillon; there is no action on milk and there is an abundant potato growth.

S. lacticus III..—This shows a pellicle on bouillon; the milk is usually acid and occasionally curdled, but in some cases shows no trace of acid to litmus paper. The potato growth is usually scanty or wanting, though sometimes more luxuriant.

M. lactis acidi..—This name includes the smaller acid-producing cocci that have a luxuriant surface growth on various media. There are very many of them. Probably *M. candicans* of Flügge belongs here. Their general characters are as follows:

Morphology..—A micrococcus. Size, $.5\mu$ – 1.2μ . Gram stain is positive.

Gelatine colony..—Not characteristic. The colonies are round, thin, smooth, rather opaque and white in color. On *litmus gelatine* no acid is produced.

Gelatine stab..—Needle growth and surface growth.

Agar streak..—A moderate, white, smooth growth, which is sometimes rough.

Fermentation tubes..—All fermentation tubes show an acid production, but no gas and no growth in closed arm.

Bouillon..—A sediment and turbidity, but no pellicle.

Milk..—Sometimes acid and sometimes not acid. Usually not curdled, though some cultures curdle the milk by acid production. Occasionally a slightly sour odor.

Potato..—The growth is usually scanty and sometimes absent. It is whitish; not characteristic.

Grows at 20° and 37° . Aerobic.

This is the most common of the milk cocci. It is found almost constantly in common fresh milk, both here and elsewhere, where we have had an opportunity of studying it. It is clearly very similar to *S. lactis acidi III.*, the primary difference being in the amount of surface growth. We have studied very many different cultures of this type and naturally found many variations. The most noticeable points of variation are as follows:

Size, from $.5\mu$ – 1.2μ . Gelatine stab in two cases showed no surface growth; colony on gelatine varies much in thickness and is sometimes slightly yellowish; the litmus gelatine colony is occasionally acid; the growth on agar varies much in abundance, but is always more than in the lactic type.

M. lactis gigas n. s.

Morphology..—A very large coccus. Size, 1.5μ . Gram stain is positive. No chains.

Gelatine colony..—A round, thick, smooth, homogeneous, entire translucent, cream-white colony.

Gelatine stab..—A needle growth is produced but no surface.

Agar streak..—Growth is scanty, beaded, translucent and white.

Fermentation tubes..—All three sugar bouillons are rendered acid but there is no growth in the closed arm and no gas.

Bouillon..—A sediment but no turbidity and no pellicle.

Milk..—Becomes very slightly acid, sufficiently to curdle the milk in about four weeks.

Potato..—No growth.

Grows at 20° and 37° . Aerobic or facultative anaerobic.

This coccus is characterized by its very large size and has been found several times by us in Middletown and Storrs. It was first isolated in 1896.

B. LIQUEFYING COCCI.—STREPTOCOCCI AND MICROCOCCI.

I. No acidity produced in dextrose or other sugars.

M. lactis erythrogenes Grotenfelt n. s. *A pink, fluorescent coccus.* Several specimens of a micrococcus giving a pink fluorescence have been found. We have given them the above name, although in our cultures they do not render milk red. As studied in our laboratory their characters are as follows:

Morphology.—Size, .8 μ . No chains.

Gelatine colony.—A smooth, flat colony, .5 mm. in diameter in four days, which later sinks into a shallow pit.

Gelatine stab.—A white needle growth, with a slow liquefaction and a dense scum, stratiform.

Agar streak.—A luxuriant, white to yellowish growth, with a pink fluorescence.

Fermentation tubes. No acidity and no gas.

Bouillon.—A sediment and a turbidity formed, but no pellicle.

Milk.—Rendered slightly acid and digested into a semi-transparent yellowish liquid; not curdled.

Potato.—A luxuriant, yellow, moist growth.

Grows at both 20° and 37°. Aerobic.

This organism is clearly allied to *B. lactis erythrogenes* if it is not identical with it.

M. lactis rubidus n. s. *A red coccus.* This organism has not been found since its previous description. It resembles *M. cinnabarius* of Flügge, differing from it in the points shown in brackets. The following is the description of our organism as given in our previous publication:

Morphology.—Size, 1 μ . No chains.

Gelatine colony.—A rapidly liquefying, red colony. Some of the colonies on the same plate are not red. [Dull red color.]

Gelatine stab.—Infundibuliform growth, which later liquefies completely, forming a red liquid. [Liquefaction only partial.]

Agar streak.—A thick, moist, blood-red growth. [Yellowish brick-red.]

Bouillon.—A sediment and turbidity, but no pellicle; the sediment is pinkish.

Milk.—No change in the action. Old cultures show a digestion with a pinkish surface.

Potato.—A very luxuriant, blood-red growth [yellow to red] spreading over the potato.

Grows at both 20° and 37°. Produces no pigment at the latter temperature. Aerobic.

While this organism is quite similar to Flügge, his is too insufficiently described to be sure of the identity.

M. lactis citronus n. s. *An orange-colored, liquefying Micrococcus.* This was found in the red slime on Camembert cheese.

Morphology.—Size, .8 μ –.9 μ . Gram stain irregular; no chains.

Gelatine colony.—A yellowish, slowly-liquefying colony, with a clear liquid. *Litmus gelatine* is reddish-brown in color.

Gelatine stab.—Liquefaction begins in four days and is never complete. Stratiform.

Agar streak.—A spreading, thick, smooth growth of an orange color, somewhat viscous.

Fermentation tubes.—No acidity, no gas, no closed arm growth in any sugar bouillon.

Bouillon.—A sediment, turbidity and a pellicle.

• *Milk*.—No action on milk.

Potato.—Luxuriant, thick, smooth, orange-brown.

Grows at 37° Aerobic.

S. lactis citreus I. n. s. A lemon-yellow, liquefying *Streptococcus*.

Morphology.—A streptococcus. Size, .6μ–1μ. Gram stain positive.

Gelatine colony.—A small colony, with clear liquid and slow liquefaction. Not characteristic.

Gelatine stab.—A slow liquefier with stratiform liquefaction.

Agar streak.—A luxuriant, thin, lemon-yellow growth.

Fermentation tubes.—No acidity, no gas, no growth in closed arm in any sugar bouillon.

Bouillon.—A turbidity, a sediment, and a pellicle.

Milk.—Slightly alkaline, or no change in reaction, and a slight digestion.

Potato.—Luxuriant, moderately thick, lemon-yellow.

Grows best at 20°; less luxuriantly at 37° Aerobic.

The culture from which this was taken was from the centre of a cheese.

Variety A.—A second culture from the same cheese near the surface, differed in producing an acidity in dextrose and saccharose, and no pellicle nor digestion of milk.

Variety B.—From Storrs; found in milk. Did not liquefy the gelatine plate, but produced a thin, flat colony, 2.5 mm. in diameter.

Variety C.—From milk in Middletown. Produced a slowly-liquefying, granular colony, 2–3 mm. in diameter; a wrinkled growth on agar; a pellicle upon bouillon, and a digestion of milk.

Variety D.—Showed no potato growth, but an acidity in milk, although the milk digested.

Variety E.—From cheese. Showed no potato growth and a very slow liquefaction, with no milk digestion. We have no hesitation in regarding them all as belonging to the same type.

S. lactis Rogeri n. s. A lemon-yellow, liquefying *Streptococcus*.

Morphology.—Size, .7μ–1μ. Gram stain irregular; chains produced.

Gelatine colony.—A slow liquefaction, with a thin, transparent, granular colony, at first white. *Litmus gelatine* is intensely alkaline, with a spindle-shaped colony.

Gelatine stab.—A needle growth and a stratiform liquefaction beginning in one day.

Agar streak.—A luxuriant, thick, lemon-yellow to brown growth.

Fermentation tubes.—No acid, no gas, no growth in closed arm in any sugar bouillon.

Bouillon.—A sediment and turbidity.

Milk.—Rendered alkaline. Sometimes there is a curdling and sometimes not, but the milk always digests.

Potato.—Luxuriant, yellow to lemon-yellow, with a discolored potato.

Grows best at 20°; slightly at 37°. Aerobic.

This was very abundant in a Camembert cheese sent us from France. It differs only slightly from the last described type, being intensely alkaline on litmus gelatine, and having a very luxuriant potato growth. The two are closely allied and are perhaps the same.

M. lactis minutissimus. *A minute, liquefying Coccus*. Has been found but once, from milk in Middletown.

Morphology.—Size, .2 μ –.3 μ . Gram stain negative.

Gelatine colony.—Round, thin, smooth, in a clear, liquefying pit. Liquefaction slow.

Gelatine stab.—An infundibuliform liquefaction, with a granular layer on the surface, or sometimes only a deep, dry pit.

Agar streak.—Scanty, thin, smooth, yellow to lemon yellow.

Fermentation tubes.—Lactose is acid; the other sugars are not acid, and no gas nor closed arm growth is produced.

Bouillon.—A sediment and turbidity, but no pellicle.

Milk.—No change in the reaction, but the milk is promptly curdled and then digested, with a prominent odor.

Potato.—A white, dry, wrinkled, luxuriant growth.

Grows best at 20°; slightly at 37°. Aerobic.

M. lactis aureus A n. s. *A yellowish, liquefying Micrococcus*. This organism is very much like *M. lactis varians*, and may be the same. (See p. 121.) The only essential difference is the lack of acid production, and on this ground we separate them, although we regard them as allies, or identical. This type is comparatively rare, while the *variens* type is very abundant. Certain variations from the described type are found in other cultures, and are indicated in brackets.

Morphology.—Size, .8 μ –1 μ . Gram stain positive.

Gelatine colony.—A V-shaped colony is formed in a pit surrounded by a halo. It is irregular, globate or entire; later it liquefies.

Gelatine stab.—A stratiform liquefaction, with a yellow sediment. Liquefaction begins on the second day and is complete in fourteen days.

Agar streak.—A luxuriant, brownish-yellow growth. [White with yellowish tinge.]

Fermentation tubes.—No acidity nor gas is produced.

Bouillon.—A pellicle and turbidity, with no sediment. [Sediment and no pellicle.]

Milk.—Alkaline and curdled; the milk subsequently digested into a clear liquid, with a half inch of sediment. [No digestion.]

Potato.—A luxuriant, brownish-yellow growth.

Grows at both 20° and 37°. Aerobic.

Two types of yellow cocci, unnamed, described by Freudenreich, belong here.

The white liquefying cocci are very numerous and we have studied many separate cultures of them. Whether they should all be grouped together is uncertain. We have endeavored to arrange them in sub-groups, as shown below, but are convinced that these represent only physiological varieties of the same type. We are also of the opinion that this group is simply a liquefying form of the white coccus, *Gal. versicolor*. (See p. 110.)

M. lactis albus n. s.

Morphology.—Size, $.7\mu$ – 1μ . Gram stain is positive; no chains.

Gelatine colony.—Round, moderately luxuriant, thick, smooth colonies, which may liquefy or in some cultures do not liquefy. In *litmus gelatine* the colony is dense and alkaline.

Gelatine stab.—A very slow liquefier, that commonly forms a dry pit with a white growth on its sides.

Agar streak.—Moderately luxuriant, opaque, whitish.

Fermentation tubes.—No acidity, gas, or closed arm growth in any sugar bouillon.

Bouillon.—A very slight growth, showing a slight turbidity and a sediment but no pellicle.

Milk.—Rendered alkaline and digested without curdling. Later the milk becomes pinkish and slimy.

Potato.—Luxuriant; thick white.

Grows at 20° and 37° . Aerobic.

Variety A.—Shows a viscosity on agar and sliminess in milk.

Variety B.—Shows no viscosity; a wrinkled growth on agar; a pellicle on bouillon and a discolored potato. Milk is curdled at 37° .

Variety C.—Has a moruloid colony that slowly liquefies, produces a pellicle on bouillon, and no milk digestion.

Variety D.—Liquefies more rapidly and curdles milk.

Variety E.—A very large coccus, 2μ in diameter.

Variety F.—A very slow liquefier, curdling and digesting milk; the milk after some weeks turning to a dark mahogany color. Potato growth is dry and velvety.

To this same group belong doubtless the small coccus *b.* of Freudenreich.

II. Acid in dextrose or other sugars.

M. lactis fluorescens n. s. Only one *fluorescens coccus* has been found, in stable dust.

Morphology.—Size, $.5\mu$ – $.6\mu$. Gram stain negative.

Gelatine colony.—A round, moderately thick, smooth, entire colony, with a greenish liquefaction. *Litmus gelatine* shows liquefying pit that is not acid.

Gelatine stab.—A stratiform liquefaction.

Agar streak.—A luxuriant, narrow, rather thick, smooth, white growth, with a green fluorescence.

Fermentation tubes.—Dextrose is rendered acid, the other sugars alkaline; no gas is produced. Growth appears in closed arm.

Bouillon.—A sediment, turbidity, and pellicle.

Milk.—Rendered slightly acid, curdled, and later completely digested into a greenish-yellow liquid.

Potato.—Scanty, thin, smooth, white.

Grows at both 20° and 37°. Facultative anaerobic.

M. lactis varians n. s. *Yellow, liquefying, acid-producing Cocci*. This is perhaps the most common and widely diffused type of coccus found in milk. We have found it in milk from many localities, and it is very abundant. It is frequently present in milk directly from the udder. It shows a wide range of variations affecting nearly every character. We have studied some scores of independent cultures from different sources, and find that the different types grade into each other by such slight differences that we have no hesitation in putting them all together as one type. This organism liquefies gelatine, but in some cases so slowly as to form only a dry pit. From this it is only a step to a non-liquefying form. We, therefore, are inclined to believe that this type is identical with, or at least closely allied to, *M. lactis aureus* (p. 112). The characters as we have studied them agree essentially with those of *Staph. pyogenes aureus*. Cultures of the latter organism, sent us by Retger, have been compared side by side with our cultures and no essential differences are seen. We are inclined to think, therefore, that our type is the common *Staph. pyogenes aureus*. In the description that follows the limits of variations will be given under each head and no attempt will be made to distinguish distinct varieties.

Morphology.—Size, .4 μ –1.4 μ . Gram stain positive.

Gelatine colony.—Deep colonies are opaque; surface colonies form white or yellowish beads or a slow liquefying colony with a clear liquid and a granular, mottled, irregular growth, distributed in the liquid. It is usually slightly acid in litmus gelatine, sometimes decidedly so, forming a bright red liquid colony.

Gelatine stab.—Liquefaction is usually slow but sometimes rapid. It commonly begins in from two to four days, but is never complete. In some cultures liquefaction is so slow that only a dry pit is formed, with a broken, yellow growth on its side. It is usually napiform.

Agar streak.—A luxuriant growth, frequently tending to be rough, though never wrinkled. It does not spread profusely. Its color is typically pale orange-yellow, but varies from this to nearly orange and to practically white. In the latter case this is quite indistinguishable from *M. lactis albidus* (p. 123). The agar growth may be dry or moist.

Fermentation tubes.—All three sugars are rendered acid and growth appears in the closed arm. No gas is ever formed. Some cultures do not grow in the closed arm and some have failed to render lactose and saccharose acid.

Bouillon.—A flocculent sediment is produced and a slight turbidity. Two cultures showed a granular pellicle.

Milk.—Is always rendered acid and commonly curdled and digested with a yellow sediment. In the non-liquefying cultures the curdling may not appear nor any noticeable digestion.

Potato.—Is usually luxuriant, though sometimes scanty, of a pale orange-yellow color, showing the same variations as mentioned in agar streak. It is frequently dry.

Grows better at 37° than at 20°. Facultative anerobic.

These variations clearly run into *M. lactis aureus* on one hand and *M. lactis albidus* on the other, so that a sharp separation is impossible. This corresponds to *Mic. E.* of Barthel and is probably everywhere distributed.

This organism is not only common in this country but it appears to be common in Europe as well, several described forms doubtless belonging to the type, for example, *Stall-luftbacterium I.* of Koning and others. Koning's *Stall-luftbacterium II.*, which he regards as different, shows such slight variations from this that it also comes within the limits that may properly be regarded as covered by our *M. lactis varians*.

M. lactis varians A.—Under this head we recognize two cultures derived from milk fresh from the udder, agreeing with the type in all points except the following:

Acid is produced in dextrose only.

Milk is not rendered acid, although it may be curdled and digested.

We do not think this is sufficiently different to constitute a new type, but tabulate it here separately.

M. lactis giganteus n. s. *An extremely large, liquefying Coccus.*

Morphology.—Size, 1.4μ – 3.5μ . A micrococcus which accepts the Gram stain. Its peculiarity is the very large size which the coccus sometimes reaches— 3.5μ in diameter.

Gelatine colony.—A clear, liquefying pit, which is slightly cloudy and white. *Litmus gelatine* is not acid.

Gelatine stab.—Begins to liquefy in one day, infundibuliform.

Agar streak.—A moderately luxuriant, smooth growth, of an orange color.

Fermentation tubes.—All sugar bouillons are made acid, but there is no gas nor closed arm growth.

Bouillon.—A sediment, but no turbidity nor pellicle.

Milk.—Rendered acid and digested into a yellow liquid at both 20° and 37° .

Potato.—A scanty, beady, brownish growth.

Grows at both 20° and 37° . Aerobic.

This agrees with the last in its physiological properties, but the extraordinary size of the cells is so peculiar that we have given it independent rank.

M. lactis rugosus n. s. *A salmon-yellow Coccus.* Perhaps *M. acidi lactici* of Krüger.

Morphology.—Size, 1μ – 1.2μ . Gram stain irregular; micrococcus.

Gelatine colony.—A liquefying pit, with a clear centre and a ring of granular matter; white.

Gelatine stab.—A slow liquefier, crateriform or stratiform.

Agar streak.—A salmon-yellow growth, luxuriant and viscous, wrinkled, with a dull surface and a salmon-yellow color.

Bouillon.—A sediment, turbidity, and a ring-like pellicle.

Milk.—Rendered acid and curdled, with an orange color and a sour odor; no digestion.

Grows well at 20° and 37° . Aerobic.

We have found this only once. Krüger apparently found the same in milk and butter. His organism differed from ours in digesting milk. The salmon color gives it independent rank.

The white, liquefying, acid-producing cocci form another series of extremely common Bacteria, with a long list of variations. As already mentioned, they pass, by imperceptible grades, into the yellow acid cocci, and should, perhaps, be united with them. These white cocci appear identical in cultural characters with *Staph. pyogenes albus*, a culture of which, when compared side by side, showed no essential differences. *Staph. mastitis albus* of Guillebau seems also to be essentially the same, as well as four different varieties of white cocci described by Freudenreich. (*Milchztg.* 1905, pp. 628 and 643.) These white cocci are extremely common in milk, in the udder, in the dust of the stable, and have been found in many samples of milk here and elsewhere. Among the many variations some are quite striking, and we have, therefore, endeavored to separate the various strains studied in groups, as follows:

M. lactis albidus n. s.

Morphology.—Micrococci. Size, $.6\mu$ – 1.2μ . Stains by Gram method.

Gelatine colony.—An opaque colony, usually white, and soon liquefying. It is not characteristic. On *litmus gelatine* it is sometimes acid and sometimes not acid.

Gelatine stab.—Liquefies in from one to three days, infundibuliform. Sometimes a dry pit is formed, which may liquefy after several days.

Agar streak.—A moderately luxuriant, smooth, white growth; not very thick.

Fermentation tubes.—All sugars are rendered acid but no gas is formed, and there is usually no closed arm growth. (One culture did not produce acid or grow in bouillon.)

Bouillon.—A sediment and turbidity, but no pellicle.

Milk.—Usually rendered acid, and may or may not curdle. Curdling is more common, however, than not curdling. The milk is digested, except in those cultures that do not liquefy gelatine. One culture, however, liquefies gelatine, but does not curdle milk.

Potato.—A moderate growth, white to yellow; not characteristic.

Grows at 20° and 37°. Facultative anaerobic.

To this type belongs the white cocci of Guillebau and Freudenreich.

Variety A.—This differs from type Variety A chiefly in producing a snow-white growth on agar. Two cultures were studied, one of which produced a snow-white colony in gelatine, and the other lacked snow-white color. One of the two was snow-white on potato, while the other produced a thin, scanty growth. In all other respects they resemble the type.

Variety B.—This is separated from the others by its more anaerobic character, as shown by growth in the closed arms of the fermentation tubes. The colonies on litmus gelatine are acid; the agar growth is scanty, as is also the growth on potato. This was found on Cheddar cheese and Camembert cheese.

Variety C.—Distinguished from the others chiefly by not making milk acid and by not curdling. One culture of this variety renders the milk alkaline,

curdles and digests it. The acidity in sugar bouillon is less pronounced than in the other varieties, and in lactose there is usually no acidity. The litmus gelatine colonies are usually acid, though not always. In other respects they agree with the type.

M. Freudinreichii of Guillebeau appears to belong here, differing from those mentioned above in rendering milk *slimy*.

THE GENUS SARCINA.

We have not found the genus *sarcina* very commonly represented in milk products. The cultures which we have found may all be reduced to four types; for, while the different specimens show some variations, they are not very great, and not so great but what they may be properly included under one of the four types.

Sar. lactis albus n. s. *A white or yellow non-liquefying Sarcina.*

Morphology.—Size; .7 μ . The Gram stain is positive and there is no motility.

Gelatine colony.—Round, convex, smooth, homogeneous, entire, yellowish or white.

Gelatine stab.—A needle growth and a convex surface growth.

Agar streak.—Beaded, raised, smooth, translucent, cream-white, moist, not luxuriant.

Fermentation tubes.—Acidity and closed arm growth in all sugar bouillons, but no gas. Sometimes lactose shows no closed arm growth.

Bouillon.—A sediment and a slight turbidity, but no pellicle.

Milk.—Becomes sufficiently acid to curdle on boiling. No other change.

Potato.—A very slight cream-white growth.

Grows at 20° but scarcely at all at 37° Facultative anaerobic.

Sar. lactis lutea n. s. *A yellow, liquefying Sarcina.* Resembles *Sar. lutea* of Flügge.

Morphology.—Size, .7 μ –1 μ . Gram stain positive. Not motile.

Gelatine colony.—A slow liquefying pit, with a nucleus surrounded by granular area; yellowish. Litmus gelatine not acid.

Gelatine stab.—Begins to liquefy in about three weeks, crateriform, and never complete.

Agar streak.—Filiform, raised, smooth, opaque, lemon-yellow, luxuriant.

Fermentation tubes.—No acidity, no gas, no closed arm growth in any sugar bouillon.

Bouillon.—A sediment, but no turbidity nor pellicle.

Milk.—Becomes alkaline and slowly digests, with a yellow color, but does not curdle.

Potato.—Beaded, raised, opaque, lemon-yellow, luxuriant.

Grows at both 20° and 37° Aerobic.

Among the variations found in our several cultures the following may be mentioned: Size, from .7 μ –2 μ ; color, lemon-yellow to whitish; the reaction on milk is frequently not changed; the growth on potato ranges from luxuriant to no growth.

Sar. lactis aurantiaca n. s. An orange, liquefying *Sarcina*. This may be the same as the last, but its color is quite different and it shows some other differences. Probably *Sar. aurantiaca* of Flügge.

Morphology.—Size, 1μ . Gram stain positive, non-motile.

Gelatine colony.—A liquefying pit, forms with an orange pigment. Litmus gelatine is not acid.

Gelatine stab.—A slow liquefaction, stratiform.

Agar streak.—Filiform, raised, smooth, moist, orange, luxuriant, slightly viscous.

Fermentation tubes.—No acidity, no gas, no closed arm growth in any sugar bouillon.

Bouillon.—A sediment, a membranous pellicle and a slight flocculent sediment.

Milk. No change in reaction, or a slight alkalinity; the milk is curdled and digested. Digestion is nearly complete, with an orange sediment.

Potato.—Spreading, capitate, contoured, orange color, luxuriant; potato discolored.

Grows at both 20° and 37° Aerobic.

Sar. lactis acidi n. s. An acid, yellow *Sarcina*.

Morphology.—Size, $.8\mu$ – 1μ . Gram stain positive; not motile.

Gelatine colony.—Round, raised, smooth, homogeneous, opaque, brownish, slowly liquefying.

Gelatine stab.—Liquefaction very slow and sometimes only a dry pit is formed, with a yellow bacterial growth.

Agar streak.—Filiform, raised, smooth, cream-white, moist, not very luxuriant. Color is sometimes yellow.

Fermentation tubes.—Acidity produced in all three sugar bouillons (it may be lacking in saccharose), but there is no gas nor closed arm growth.

Bouillon.—A sediment and a slight turbidity, but no pellicle. (Turbidity sometimes absent.)

Milk.—Becomes acid, but does not curdle or digest.

Potato.—Does not grow well, but there is a slight cream-colored growth.

Grows at both 20° and 37° , though not very abundantly at 37° Aerobic.

THE GENUS BACTERIUM, NON-LIQUEFYING.

I. No acid in dextrose or other sugars.

Bact. lactis salmonis n. s. A salmon-colored *Bacterium*.

Morphology.—Size, $.6\mu$ x 1μ – 1.8μ , forming chains. Gram stain is positive and there are no spores nor capsules.

Gelatine colony.—Round, umbonate, contoured, lobed, white. Litmus gelatine colonies are strongly alkaline and transparent.

Gelatine stab.—Needle growth and a raised surface growth.

Agar streak.—Filiform, thin, smooth, white to yellow, and later a salmon or pink color.

Fermentation tubes.—No acidity, gas, nor closed arm growth in any sugar bouillon.

Bouillon.—A flocculent sediment, a membranous pellicle and a slight turbidity.

Milk.—Becomes alkaline, but shows no other change.

Potato.—Luxuriant, thick, contoured, flesh color or pink.

Grows well at 20°; slightly at 37°. Aerobic.

This was found by Harding in a specimen of green butter.

Bact. lactis aureum I. An orange, non-acid Bacterium. Perhaps *B. lactericus* of Adametz.

Morphology.—Rods which do not form chains. Size, $.7\mu-.9\mu \times 1\mu-3\mu$. No spores are produced, a capsule is evident and Gram stain is positive.

Gelatine colony.—Round, flat, contoured, lobed, orange to yellow color. Litmus gelatine colonies are of a red-brown color.

Gelatine stab.—A needle growth and a thin, reddish surface growth.

Agar streak.—Luxuriant, deep orange-brown color, tough and tenacious, sometimes dull, and aggregated in colonies.

Fermentation tubes.—No acidity, gas, nor closed arm growth in any sugar bouillon.

Bouillon.—A turbidity, sediment, and a pellicle.

Milk.—No action except a slight orange color at the surface.

Potato.—Growth scanty or absent; when present, of an orange color.

Grows better at 20° degrees than at 37°. Aerobic.

Found in Middletown and in New York city.

Bact. lactis citreum II. n. s. A yellow, non-acid Bacterium.

Morphology.—A rod occasionally forming chains. Size, $.5\mu-.7\mu \times .7\mu-1.4\mu$. No spores nor capsules, and Gram stain negative.

Gelatine colony.—A round, opaque bead, 1.5 mm. in diameter; usually white and later turning yellow.

Gelatine stab.—A scanty needle growth and an irregular, dry, white surface.

Agar streak.—Luxuriant, at first white, but soon lemon-yellow.

Fermentation tubes.—No acidity, gas, nor closed arm growth in any sugar bouillon.

Bouillon.—Turbidity, sediment, and a flaky scum.

Milk.—No action.

Potato.—Luxuriant, yellow, thick, sometimes wrinkled.

Grows both at 20° and 37°. Aerobic.

Variety A.—This has a small lemon-yellow colony on gelatine which is lemon-yellow even when viewed under the microscope. It has also a lemon-yellow surface growth on the gelatine stab and a lemon-yellow growth on potato.

Variety B.—Produces a yellow pigment, but not lemon-yellow, both in gelatine colony and on potato. It is slightly viscous on agar, and has no pellicle on bouillon. Potato is slightly discolored, and one culture failed to grow on potato. All from milk.

The white non-acid bacteria are very numerous and show many slight variations. We have tried to separate them into groups, but they are all more or less connected by intermediate forms and our grouping is not very satisfactory.

Bact. lactis myceloidium. *A mycoloid, non spore-bearing Bacterium*. We have studied two cultures of this type, one from this locality and one sent us by Weigmann, from Kiel, as *Bact. mycoides*. The Kiel culture is not *mycoides*, for it fails to produce spores and does not liquefy gelatine. This raises the question whether it may not be a cultural variety of *B. mycoides*, having lost these two properties. As tested in our laboratory, it had the following characters:

Morphology.—Long filaments, the individual elements of which are $.7\mu \times 2\mu-3.5\mu$. The Gram stain is irregular and there are no spores.

Gelatine colony.—A myceloid colony, 2 cm. in diameter, spreading rapidly; largely under the surface.

Gelatine stab.—A needle growth and a surface growth; a layer of threads is seen extending horizontally, a short distance below the surface, to the sides of the tube.

Agar streak.—A luxuriant, moderately thick, slightly yellowish growth.

Fermentation tubes.—No acidity, gas, nor closed arm growth in any sugar bouillon.

Bouillon.—A sediment and turbidity, but no pellicle.

Milk.—Becomes slightly acid, but does not curdle, even when heated, and shows no other change.

Potato.—A scanty growth, thin, white.

Grows better at 20° than at 37° . Aerobic.

We have found a local variety of the above, agreeing with it in most respects. It is shorter, $.9\mu$, does not show the peculiar horizontal growth in gelatine stab, and produces no acidity in milk.

Bact. lactis arborescens I. n. s. *An arborescent, non-acid Bacterium*.

Morphology.—Size, $.9\mu \times 1.2\mu-1.4\mu$. It has no spores nor capsules, forms no filaments, and Gram stain is negative.

Gelatine colony.—Round, raised, smooth, entire, white colony. On litmus gelatine brownish and not acid.

Gelatine stab.—An arborescent needle growth and a surface growth.

Agar streak.—Scanty, thin, white to cream color, slightly viscous.

Fermentation tubes.—No acidity, gas, nor closed arm growth.

Bouillon.—A sediment, ring-formed pellicle, and a slight turbidity.

Milk.—Is rendered alkaline and slimy. After some days it becomes slightly transparent, indicating a slight digestion.

Potato.—Scanty, raised, grayish-brown color; potato discolored.

Grows at both 20° and 37° . Aerobic.

This organism was sent us from Michigan by Marshall, but we have not ourselves found it. Its slight digestion of milk and its slight pit in gelatine stab suggest an intermediate step toward a liquefying form. If regarded as a slow liquefier it is not unlike *Bact. arborescens* Frankland, found in water.

Bact. lactis viscosum Adametz. Under this name are included several slimy milk bacteria described by different observers. The first was described by Adametz, others by Ward, Harrison, Freudenreich, and Marshall. We have had an opportunity of studying all of these except that of Adametz, from original cultures from the authors. We have also received a similar culture from New York city milk. These have all been carefully studied by Harrison who regards them as a single type and calls them group 1. We are in agreement with him in recognizing this as a logical group. Its general characters are as follows:

Morphology.—Size, $.5\mu-1.2\mu \times .5\mu-2.5$. Frequently narrower at the ends. Forms filaments, 15μ in length. The slime seems to be produced from a capsule, but this is not always seen. Gram stain negative.

Gelatine colony.—Flat colonies, with irregular edges, or lobate. 3 mm.—6 mm. in diameter. Later viscous.

Gelatine stab.—A good needle growth, which may be separated into granules. Sometimes arborescent, but not always so. A thin, shiny, gray surface growth, lobate.

Agar streak.—Usually luxuriant, viscous, white, not very thick.

Fermentation tubes.—No acidity, gas, nor closed arm growth in any sugar bouillon.

Bouillon.—Turbidity, pellicle, and sediment. (The organisms of Ward, Harrison, and Freudenrich show no pellicle.)

Milk.—Becomes alkaline, does not curdle, but is very slimy.

Potato.—A thick, uneven, dirty gray, becoming brown or yellow; slimy.

Grows at both 20° and 37° . Aerobic.

We retain the name of Adametz, changing the word *Bacillus* into *Bacterium*. I see no good reason for separating the above mentioned organisms even as varieties. Many cases of slimy milk infections in dairies are produced by this type of bacterium, which is probably the most common cause of such troubles.

Bact. lactis acidi. *Var. E*. This organism is very similar to *Bact. acidi lactici* (see page 134). In all of its characters, except one, it agrees with that type. It shows the same unwillingness to grow in common culture media in the laboratory, and little or no surface growth, but it produces no acidity in sugar bouillons and no acidity in milk. Inasmuch as *Bact. acidi lactici* shows great variations in the power of producing acids, we regard this as an extreme variety of that type. No further description here is necessary.

Bact. lactis Connii Chester. *A white non-acid Bacterium*. Large numbers of common white bacteria have been found without striking characters. They are common in all milk. We have found them more or less constantly here and in New York milk. They are also common in cheese, and constitute the chief bacterium in the red slime of Camembert cheese. In our former report we recognized two or three different types among them, but at present we believe they should all be grouped together under one head. We have retained the name given by Chester. It is parallel with *Galactococcus versicolor* among the cocci; it may be identical with the latter.

Morphology.—A bacterium ($.5\mu$ – $.7\mu \times 1.4\mu$), forming short chains. It has no spores nor capsules and the Gram stain is negative.

Gelatine colony.—Round, raised, smooth, entire, white or cream color. *Litmus gelatine* shows a non-acid, white, not characteristic surface colony.

Gelatine stab.—A good needle growth and a white surface.

Agar streak.—Luxuriant, filiform, raised, smooth, white, opaque.

Fermentation tubes.—No acidity, gas, nor closed arm growth in any sugar bouillon.

Bouillon.—A sediment, ring-formed pellicle, and slight turbidity.

Milk.—Rendered slightly alkaline, is not curdled, and shows no digestion. In one case milk became pasty in about three weeks.

Potato.—Luxuriant, convex, smooth, white, potato discolored.

Grows both at 20° and 37° . Aerobic.

Among the numerous cultures which we have studied we can recognize two sub-varieties.

Variety A.—A less vigorous organism than the one described. Size, $1.4\mu \times 1.2\mu$, forming rather long filaments. Bouillon sometimes shows no pellicle and milk is not rendered alkaline. Potato growth is rather scanty. The potato shows no discoloration, but the growth itself is slightly yellowish.

Variety B.—Stains by the Gram method; agar streak is dry and wrinkled; dextrose slightly acid; no discoloration of potato.

This seems to be identical with *B. lactarius* of Adametz and a *Bacterium* described by Burri and Dugelli, mentioned as having a "dog odor" appears also to be the same.

II. Acid in Dextrose or other Sugars.

Bact. rudensis Connelli. *A red, acid Bacterium*. This organism was isolated by Harding from cheese vats, and regarded by him as the above species. Four cultures were sent to us by him after he had kept them in stock for a year. Of these four cultures two came originally from Canada; two of them had entirely lost their power of producing red pigments, and two still gave a slight red color to milk. All were said to produce red pigment originally. The cultures which I received had also totally lost their flagella and motility, and in this condition were not distinguishable from *B. lactis acidii*, except by showing a better growth on potato. These cultures, when studied by us, had the following characters.

Morphology.—A short rod, $1\mu \times 1.8\mu$; forming no chains, showing no spores, and staining by the Gram method (originally motile).

Gelatine colony.—A small colony, mostly under the surface, quite dense. On *litmus gelatine* it is intensely acid.

Gelatine stab.—A needle growth, but no surface growth (originally a thin surface).

Agar streak.—No visible growth, or sometimes an extremely thin transparent growth.

Fermentation tubes.—Acidity and closed arm growth in all sugar bouillons, but no gas.

Bouillon.—A sediment and slight turbidity, but no pellicle.

Milk.—Becomes acid and curdles promptly at both 20° and 37° A rusty precipitate appears in some cultures.

Potato.—Scanty, reddish-brown color (originally showing yellowish colonies that become red).

Grows at both 20° and 37°. Facultative anaerobic.

If this colorless variety should be met incidentally in milk, it would never be thought to be *Bact. rudensis*, and would doubtless be placed with *Bact. lactis acidi* group, and perhaps be regarded as identical with that organism. This raises the question naturally whether other members of that group may not be albino types of pigment-producing bacteria. So far as we know we have never found this species, although we have no proof that some of our white types are not such albino varieties.

Bact. lactis catenensis n. s. *Yellow spore-bearing Bacterium*.

Morphology.—Size, $.7\mu \times 1.2\mu$; producing chains. Gram stain is negative, and small spores are produced. In old cultures the rods may be much smaller, $.5\mu$.

Gelatine colony.—Round, raised, smooth, homogeneous, transparent, and of a yellow to white color. On *litmus gelatine* the color is yellow to gray, not acid.

Gelatine stab.—A good needle growth, and a flat orange-yellow surface.

Agar streak.—Orange to yellow, thin, and sometimes wrinkled.

Fermentation tubes.—Dextrose and lactose acid, saccharose not acid. No gas or closed arm growth.

Bouillon.—A sediment, turbidity, and a pellicle.

Milk.—No effect, as a rule, but one culture curdled milk, acid at 37°.

Potato.—Luxuriant, wrinkled, orange-yellow.

Grows at 20° and 37°, but better at 20°. Aerobic.

Three cultures of this general type have been studied from Middletown, Cromwell and New York. The first was orange, the second lemon-yellow, and the third brown-orange on agar, and white on potato and gelatine. The last did not show a wrinkled growth on agar, although it did on potato.

Bact. lactis aureum II, n. s. *An orange, acid-forming Bacterium*.

Morphology.—Size, $.8\mu$ – $1.2\mu \times 1.2\mu$ – 1.8μ . No chains, no spores. Gram stain negative.

Gelatine colony.—Round, convex, smooth, entire, orange-yellow. On *litmus gelatine* it is thin and not acid.

Gelatine stab.—A needle growth and a thin surface growth.

Agar streak.—Moderately luxuriant, filiform, thin, smooth, orange color, moist.

Fermentation tubes.—Dextrose acid, lactose and saccharose slightly acid; no gas nor closed arm growth.

Bouillon.—No visible growth.

Milk.—Very slightly alkaline, no curdling and no digestion.

Potato.—Spreading, thin, smooth, yellow.

Grows better at 20° than at 37°. Aerobic.

This organism was sent me by Gorini from Italy. Another, which seemed to be identical, was found in milk here, the only points of difference being a luxuriant potato growth and a slight sediment in bouillon. A very similar culture was sent by Harding, differing only in having a somewhat dry, wrinkled growth on agar. We regard the three as identical.

Another culture is very closely related to this, but differing in enough particulars to lead us to regard it as a separate variety.

Variety A.—This organism was found here on Camembert cheese and another culture was sent us by Gorini. It differs from the above described type only in the following points: Size, $.9\mu-1.5\mu \times .5\mu$. Gram stain positive, grows well in bouillon, with a sediment. Milk distinctly alkaline. Color of a lemon instead of an orange yellow.

Variety B.—This organism, sent by Harding, differs from the type in producing a rather more orange color, a turbidity and sediment in bouillon, and in making milk distinctly acid, but not curdling it.

Bact. lactis synxanthum.—A culture of this organism, several years old, was sent me by Harrison. It had completely lost its power of producing yellow pigment. Below are given its characters as made out by us, and in brackets the characters as originally described where they differ from those observed.

Morphology.—Size, $.8\mu-.9\mu \times 1.2\mu-2\mu$. No spores, no chains. A capsule is evident. Gram stain is negative. [Gram stain positive, motile.]

Gelatine colony.—Round, capitate, smooth, homogeneous, entire, opalescent, gray, moist. [Luxuriant, gray, yellow.] On *litmus gelatine* transparent, white colonies.

Gelatine stab.—A needle growth and a raised surface growth.

Agar streak.—Luxuriant, filiform, raised, smooth, opaque, porcelain white. Slightly viscous. [A yellow pigment, soluble in water.]

Fermentation tubes.—All three bouillons are acid, but there is no gas nor closed arm growth.

Bouillon.—A sediment, slight turbidity, and a ring-formed pellicle.

Milk.—Becomes acid but does not curdle nor digest. [Alkaline, and digests to a bright yellow color.]

Potato.—Filiform, raised, contoured, sebaceous, gray, luxuriant; potato discolored.

Grows at both 20° and 37°. Aerobic.

These organisms show how decidedly characters which are relied upon to distinguish types may disappear after long cultivation, and naturally throws doubt upon all classifications based upon physiological properties. If an organism originally isolated because of its power of producing red pigment lose this power absolutely, and if one which produces a brilliant yellow color loses this property, we naturally ask whether any physiological properties are constant.

Bact. seifge Milch Weig. This organism from soapy milk we have had an opportunity of studying from a culture sent by Weigmann. The culture had been under observation in his laboratory for some years before it was sent and did not agree in all its characters with his original description. The description below is of the culture which we have studied and where it differs from the original description, the latter is indicated in brackets.

Morphology.—Size, $1\mu \times .5\mu$. No spores, no capsules. Gram stain negative.

Gelatine colony.—Round, capitate, smooth, homogeneous, entire, translucent, gray-white. On *litmus gelatine* it grows chiefly below the surface and is strongly acid. After some days the acid reaction changes to alkaline.

Gelatine stab.—A needle growth and a raised surface growth, which later becomes a dry pit [slowly liquefying].

Agar streak.—Filiform, thin, smooth, opalescent, gray, moist, not luxuriant. [Of a yellow color.]

Fermentation tubes.—All three sugar bouillons become acid, but there is no gas and no closed arm growth.

Bouillon.—A sediment and turbidity, but no pellicle.

Milk.—Becomes amphoteric and a slight yellow scum appears around the rim. [Originally this produced a soapy taste in milk, but did not do so when studied by us.]

Potato.—Filiform, thin, smooth, brownish, moist, luxuriant [yellow].

Grows at both 20° and 37° [best at 10° according to Weigmann]. Aerobic.

Bact. lactis Isignii n. s. This bacterium has the unusual character of completely digesting milk into a transparent liquid without previous curdling, but not liquefying gelatine. It is the only species we have found showing this character. It was found upon an Isigny cheese and constituted about 44% of the bacteria on the cheese.

Morphology.—A small rod, not forming chains. Size, $.5\mu$ – $.3\mu$. It forms no spores nor capsules, and the Gram stain is negative.

Gelatine colony.—Round, raised, smooth, homogeneous, entire, opaque, yellowish, moist.

Gelatine stab.—A filiform needle growth, and a flat surface.

Agar streak.—Luxuriant, filiform, raised, smooth, translucent, yellowish, moist.

Fermentation tubes.—After three days an acidity in all three bouillons, but no gas and no closed arm growth.

Bouillon.—A sediment, slight turbidity, but no pellicle.

Milk.—Becomes slightly acid and digests. After two months the digestion is complete.

Potato.—Spreading, flat, smooth, cream-white, luxuriant.

Grows at both 20° and 37° . Aerobic.

Bact. lactis non-acidi n. s. This organism belongs to the lactis series (see below), but the departure from the central type becomes so great as to properly demand a separate name. The surface growth is very abundant and the acid production very feeble. The dextrose alone is rendered acid. Milk is never acidified nor curdled. The complete description is as follows:

Morphology.—Size, $1.8\mu \times 5\mu$ – 1.2μ . Sometimes long chains are formed. There are no spores and the Gram stain is irregular, commonly negative, but in two cultures was positive.

Gelatine colony.—Small, round, moderately thick, entire or irregular margin, white. *Litmus gelatine* colony is not acid and sometimes distinctly alkaline. Sometimes green. The colonies of different cultures show considerable variation which may indicate different varieties. Our data, however, at present do not warrant us in separating them.

Gelatine stab.—A needle growth and a spreading, moderately abundant surface.

Agar streak.—Moderate to luxuriant, linear, smooth, white, moist.

Fermentation tubes.—Dextrose acid, and sometimes lactose and saccharose.

Bouillon.—A sediment and turbidity, and frequently, though not always, a pellicle.

Milk.—Alkaline, or no change in reaction, no digestion nor other change.

Potato.—Scanty to luxuriant, white, spreading. One culture was wrinkled.

Grows at both 20° and 37° , though better at 20° . Aerobic.

The variations given above are large and we feel that this group should be broken up. But the different variations mentioned cross each other so much that we have as yet been unable to divide them into any definite varieties. As more cultures accumulate and give us more information we believe it will be possible to recognize some sharply distinct types, but at present we leave them together. The variations which we have found fail to group themselves, and if we should try to make varieties we should be obliged to recognize nearly as many as we have isolated individual cultures.

This type of lactic bacteria is very common. It appears constantly in milk in this vicinity as well as in New York state, and in cheese. It does not seem to be, however, so vigorous as the typical lactic organism, and is apparently not the cause of ordinary sour milk.

Bact. lactis ubiquitum. This organism we have not especially studied since its description in 1899. We reinsert here the description then given:

Morphology.—Size, 1.2μ – $1.4\mu \times .8\mu$. Long chains are formed; spores are developed and also a capsule.

Gelatine colony.—Round, capitate, smooth, entire, white, the outer edge thinner and lighter.

Gelatine stab.—A needle growth and a rather thick irregular surface growth.

Agar streak.—A luxuriant, white surface growth, developing irregular frost-like or feather outgrowths. Moist, smooth.

Fermentation tubes.—Not determined, but probably acidity is produced, at least in dextrose and lactose, and there is no gas.

Bouillon.—A sediment and turbidity, but no pellicle.

Milk.—Rendered acid and is curdled after several days. No digestion.

Potato.—A luxuriant growth, transparent, spreading, white, glistening.

Grows well at both 20° and 37° . Aerobic.

WHITE, ACID-PRODUCING BACTERIA.

These are immensely numerous. They are the common cause of the souring of milk, and since this phenomenon is practically universal, it follows that these bacteria are equally widely distributed. This group contains the dairy bacteria par excellence. By this is not meant that these are most abundant around the barn or in fresh milk. As pointed out elsewhere, they are not common in the udder of cows, and are usually present only in small numbers in freshly drawn milk. But they are so much better adapted to life in milk that they soon become more abundant than all other bacteria put together. Thus in older samples of milk they are by far the most common.

We have studied hundreds of cultures belonging to this general type, obtained from all over this country, as well as from several localities in Europe. Among this large series of samples we have found endless variations. There is hardly a characteristic which does not show wide variations in the numerous cultures studied. They include such widely different types that it is hardly proper to put them into one group, but to arrange any satisfactory subordinate grouping is almost an impossibility. This might be done by selecting almost any of the characteristics of the type and arranging the different cultures according to its variations. The most satisfactory arrangement as appeared to us is to divide them according to their power of producing a surface growth on various media. This is not a sharp character, but it presents at least two extremes which may be clearly separated.

Heinemann (Cent. f. Bac. u., Par. II., XVI., p. 538, 1896) doubts whether the common lactic organisms should be called by the name *Bacterium*, believing that they are really all *Streptococci*. We are inclined to doubt the correctness of this view, and prefer to retain the long accepted nomenclature.

Bact. lactis acidi Leichmann. This is the common cause of sour milk. Among the many scores of organisms of this type which we have studied we have tried to recognize some groups worthy to be called varieties. Whether this is possible is uncertain, since all are connected by slight intermediate gradations. We recognize, however, the following:

Bact. lactis acidi, type.

Morphology.—A bacterium. Size, $.7\mu$ – 1.2μ x $.5\mu$ – $.8\mu$. Sometimes so short as to be described as a streptococcus, and some cultures are very clearly cocci. (See p. 114.) There is no motility, no spores, and no long chains. Gram stain is positive.

Gelatine colony.—Colonies are small points, rather opaque, not characteristic. They are almost wholly under the surface, and never typically grow on the surface. In *litmus gelatine* they are rather dense, strongly acid, and frequently, though not always, surrounded by minute, irregular spines on the edge. This type of colony can usually be detected with a little experience, and is the most characteristic feature of the type.

Gelatine stab.—A granular or linear needle growth, and no surface growth.

Agar streak.—There is no growth, or one that is scarcely visible. On milk agar it grows rather better, but at best it is very scanty.

Fermentation tubes.—All three sugar bouillons are rendered acid and there is commonly a closed arm growth, but never any gas.

Bouillon.—Frequently there is no sign of growth, but there is commonly a slight sediment.

Milk.—Milk is rendered strongly acid and promptly curdled in from six hours to two days. The curd is smooth and hard, without gas bubbles, and never shows any digestion.

Potato.—Usually no growth, but sometimes a thin, transparent film.

Grows at 20° and 37°, but better at 20°. Facultative anaerobic, growing better without oxygen, and hence curdling milk at the bottom first.

The most characteristic features of this organism are the peculiar *litmus-gelatine colonies*, the *absence of surface growth*, and the *smooth, hard, acid curd in milk*.

Variety A.—This differs from the common type simply in its extremely minute colony, which is invisible to the naked eye, is merely transparent and does not show the characteristic spines. In our previous list this was called No. 202, but we now think that it is only a less robust form of the type. In all other respects the two agree perfectly. The difference of the colonies on gelatine, however, is usually very striking, that of the variety A being not more than 1-10 the size of the type. It is less frequently found, also, than that of the type.

Both the type and variety A show great variability in their acid-producing power. Sometimes they curdle milk in as short a time as six hours (at 37°), other cultures in twenty-four hours, others, again, in two or three days, and finally, some, identical in other respects, fail to curdle it at all, although they make it strongly acid. These variations in acid-producing power do not seem to us to be sufficient to warrant us in recognizing them even as varieties. They are certainly subject to modification in the same cultures. Cultivation in milk noticeably increases this power. Cultures, when first isolated, show a weak growth and a weak curdling power, but after a few days' growth in milk this power is very greatly increased. A gelatine plate made from fresh milk shows weak colonies, growing slowly, with a weak acid production. A plate made from the same milk after two days shows not only more numerous colonies but colonies much larger, more acid, and growing much faster. It appears thus that this organism adapts itself to milk, which seems to be its most favorite medium for growth.

This is the organism which, in previous papers (Annual Rep. Storrs Exp. Sta., 1901), we have shown to outgrow all other bacteria in milk at 20°, and commonly to comprise 99% or more of the bacteria in milk kept at 20°. We have found this organism all over the United States from the Pacific to the Atlantic, and it has been sent to us from Europe as one of the most common lactic bacteria there. It is present in practically every cheese which we have studied, including both hard and soft cheeses. It has been many times studied and been given very many different names. It appears to be the same organism which has received the following names by different authors: *Lactic bacterium* of Kozai, *Lactic bacterium* of Utz, *Strept. acidi lactici* of Marpmann, *Bact. lactis acidi* Leichmann, *B. lactis acidi* Günther and Thierfelder, *B. acidi paralactis* a of Freudenreich (also several others of his cultures are nearly identical), *Bacillus*

acidi lactici Esten. Of these various names that of Leichmann, *Bact. acidi lactici*, is preferable. The name given by Günther and Thierfelder has the priority, but since this organism is not a bacillus, that name cannot be retained.

Variety B.—This shows a dense, coarse, granular surface colony. It is very small, only $.4\mu$ – $.7\mu$. It does not curdle milk, though it makes it acid and gives it an astringent taste. It was isolated by Harrison from astringent milk.

Variety C.—This differs from the type in the following points: The colony shows a surface growth, and there is also a surface growth in gelatine stab. There is abundant growth in bouillon, with a pellicle. This variety is evidently more aerobic than the type. One culture of this, from Camembert cheese, shows a capsule.

Variety D.—This variety never curdles milk though it makes it acid. It does not stain by the Gram method. It has a thick lobate or moruloid surface colony, or sometimes smooth. There is a spreading surface on gelatine stab and a scanty opaque growth on agar streak. Its acid production is feeble and there is no acidity in saccharose bouillon. In ordinary bouillon there is a sediment, turbidity, and usually a pellicle. Its growth on potato is scanty. We have found this several times in different cheeses, and it has also been isolated from all milk. *Milch bacterium I.*, of Koning seems to belong to this type. (Milchw. Zent. II. 1906, p. 316.)

At this place should be mentioned a series of milk organisms which are called "acid fast," *i. e.*, they are not decolorized, by NNO_3 after being stained with carbol fuchsin. In this respect they agree with the *tuberculosis bacillus*, and hence may sometimes be confused with them in an ordinary microscopic study of milk. For this reason they are of some considerable significance. At least nine of them have been described by different authors as follows: *B. phlei*, *Mist bacillus*, *Grass bacillus*, No. 2, and *Milk bacillus*, all described by Moeller; the *Butter Bacillus* of Grassberger; the *Butter Bacillus* of Binot; the *Butter Bacillus* of Rabinowitsch; the *Butter Bacillus* of Coggi; the *Bacillus Freiburgensis* of Koon; also *B. Freiburgensis*, No. 2, and *Butter bacilli*, Nos. 1, 2, 3, 4 and 5, of Tobler. We have not had an opportunity of studying any of these, and the descriptions given of them are altogether too inadequate for classification according to our scheme. Some of them are acid producers and others are not. Some are white, others orange-yellow or reddish. They form a miscellaneous lot of bacteria whose relations can not be determined by the characters given. We find it quite impossible to place them in our scheme at the present time. It is quite possible that some of the bacteria which we have described are also "acid fast," for we have not used this method of staining in our routine tests.

THE GENUS BACTERIUM, LIQUEFYING.

I. No acid in dextrose or other sugars.

Bact. lactis chromatium n. s. A lemon-yellow, spore-bearing Bacterium.

Morphology.—Size, $3\mu \times 1.5\mu$. Chains are formed and spores produced, but there is no capsule.

Gelatine colony.—A liquefying pit, full of threads. There is a central nucleus with coarse granular masses around it.

Gelatine stab.—A deep, dry pit is first formed which, after several days, shows liquefaction.

Agar streak.—A luxuriant, moist, yellow to white growth.

Bouillon.—A sediment, turbidity, and pellicle. The liquid later becomes clear.

Milk.—Becomes alkaline and curdles. It subsequently digests into a clear liquid, with a tenacious scum.

Potato.—Luxuriant, dry, rough, wrinkled, with a brilliant yellow color.

Grows at both 20° and 37°. Aerobic.

Bact. lactis arborescens II. *An arborescent spore-bearing Bacterium*. This organism, originally found in 1896, has appeared once or twice subsequently, although the later organisms differed slightly from the original. One culture, sent by Weigmann, differed in points inclosed in brackets.

Morphology.—Rods with square ends. Size, 2μ – $4\mu \times 1\mu$ – 1.8μ , forming long chains. Spores are produced but no capsules. Gram stain negative.

Gelatine colony.—A very peculiar felted mass of fibers extending through the gelatine, and a ground glass-like felted surface, on a liquefying disk. The appearance is variable, but the fibers are characteristic. [Myceloid.]

Gelatine stab.—An arborescent needle growth, liquefying slowly, infundibuliform, with a folded, ground glass-like scum.

Agar streak.—Widely spreading, filamentous and somewhat cotton-like on the surface and extending into the agar; luxuriant, wrinkled, dull. [Cretaceous.]

Fermentation tubes.—[Dextrose and saccharose acid, lactose not acid, no gas, closed arm growth in lactose and saccharose.]

Bouillon.—A flaky turbidity, a sediment, and a ground glass-like scum.

Milk.—Alkaline, curdled and digested. After digestion it may be amber-colored or colorless. One culture showed a ground glass-like scum.

Potato.—A luxuriant growth, with a white cotton-like surface, extending below into the potato.

Grows at both 20° and 37°. Aerobic.

In the specimens found later and regarded as the same as the above the arborescent needle growth was not always found, and the scum on the milk was lacking.

Bact. lactis filiformis, formerly described, is very similar to the last and may be the same. It has not been found since the original description. It differs only in the following points: No arborescent growth in gelatine. The ground glass-like appearance is lacking. The agar streak shows a dry, white, lobate growth. Bouillon shows a scum of tangled fibers, and on potato it forms a thick, slimy growth, yellowish, covering the whole surface of the potato.

Bact. lactis truncatum. *A Bacterium with proteus or curled colonies*. Described by us in a previous report and named by Chester. The following is a description of a culture studied more recently:

Morphology.—Size, 1.2μ – $2.5\mu \times .8\mu$ – 1 , forming long chains of square-ended rods. Spores are produced but no capsules.

Gelatine colony.—An opaque colony, $\frac{3}{4}$ inch in diameter in two days; curled, i. e., made of twisted threads. In some cases the colony is proteus-like, with threads in parallel rows.

Gelatine stab.—Liquefies, stratiform, with a tough, white, mold-like skin, and, later, with a complete liquefaction and a yellowish scum. The rapidity of liquefaction is very different in our two cultures.

Agar streak.—A luxuriant, whitish-yellow, rough growth, with an irregular, lobate or feathery edge.

Bouillon.—A tough, felted scum, without turbidity or sediment.

Milk.—Rendered alkaline, curdled in three days, and digested, showing a thick, folded scum. In twelve days a translucent surface with a curd below.

Potato.—Luxuriant, white, velvety or powdery dry growth.

Grows at 20° and 37°. Aerobic.

Bact. lactis Michiganii n. s. A white, spore-bearing *Bacterium*. This organism, sent by Marshall, does not agree with any that we have found, although closely allied to the last. The most striking difference is in the colony, but this may be due to the greater vigor of liquefaction. At present, however, we keep it as distinct.

Morphology.—Size, $1.8\mu \times .9\mu$, forming chains. Spores developed. Gram stain negative.

Gelatine Colony.—A rapidly liquefying colony, with a uniformly cloudy liquid.

Gelatine stab.—Needle growth beginning to liquefy in one day, infundibuliform; liquefaction complete in three days.

Agar streak.—Spreading widely, moderately thick, wrinkled, opaque, white, not luxuriant.

Fermentation tubes.—No acidity, no gas, no closed arm growth.

Bouillon.—A sediment, turbidity, and a wrinkled pellicle.

Milk.—Alkaline, curdled and completely digested. At 37° slightly pinkish, but not at 20°.

Potato.—Luxuriant, filiform, thick, alveolate, opaque, gray-brown, dry, wrinkled.

Grows better at 37° than at 20°. Aerobic.

Bact. lactis Genevum n. s. A white, spore-bearing *Bacterium*, sent us by Harding.

Morphology.—Size, $3\mu-8\mu \times 1.4\mu$. No chains. Spores formed chiefly at the ends of the rods. Rods with square ends. Gram stain positive. (One culture negative.)

Gelatine Colony.—A rapidly liquefying colony that may be a smooth liquid mass or may be cloudy. On *litmus gelatine* there may be a wrinkled film. Colony not acid.

Gelatine stab.—A needle growth and a stratiform liquefaction, beginning in one to three days.

Agar streak.—Spreading, thin or raised, smooth, whitish or creamy, moist or sometimes dull, luxuriant.

Fermentation tubes.—No acidity or gas in any sugar bouillon; closed arm growth usually seen. (A slight acidity may appear for a day or two in dextrose, but it then disappears.)

Bouillon.—A sediment, turbidity and pellicle.

Milk.—Is rendered alkaline, curdled and digested completely, with a prominent odor.

Potato.—Spreading, thin or raised, smooth, opaque, cream colored, luxuriant; discolored.

Grows at 20° and 37°. Facultative anaerobic.

Bact. lactis erythrogenes Grotenfeld. *Bacterium with pink fluorescence*. We have several times found bacteria that belong probably to this well known type. None of them produce much red color in milk, although some render it of a pinkish color, and one turned it deep red after several weeks' growth. All of them, except variety D, produce a peculiar pinkish fluorescence in agar. We give below the characters of one of the cultures isolated, with the others as varieties.

Morphology.—Size, $1.2\mu \times .9\mu-1\mu$. Not forming chains. It forms no spores, stains by the Gram method and shows a capsule.

Gelatine colony.—Round, raised, smooth, homogeneous, entire, translucent, yellowish, shining, later liquefying.

Gelatine stab.—Begins to liquefy in three days, stratiform.

Agar streak.—Filiform, raised, smooth, translucent, flesh color or pink, shining, luxuriant. The agar shows a pink fluorescence.

Fermentation tubes.—Dextrose and saccharose show growth in closed arm, but not lactose. No acidity nor gas produced in any sugar bouillon.

Bouillon.—A flocculent sediment, a membranous pellicle, and a decided turbidity.

Milk.—No change in reaction. The milk may curdle in ten days and begin to digest. The digestion is nearly complete in three weeks and the liquid is of a pinkish color, with a slight odor.

Potato.—A luxuriant growth, white, with discolored potato. There is no pink color shown.

Grows both at 20° and 37° Facultative anaerobic.

This organism was from a yellow slime on the surface of cheese.

Variety A.—Differs from the above in showing no closed arm growth, a yellowish growth on agar, no curd or digestion in milk, and a scanty growth on potato.

Variety B.—Shows yellow growth on agar. No pellicle on bouillon. Milk becomes alkaline and digests into a red liquid, which later becomes very red. Growth on potato scanty.

Variety C.—Size, $.3\mu \times .5\mu-.6\mu$. No pellicle on bouillon. Milk digests; pinkish. Potato scanty. Growth on agar not yellow.

Variety D.—This fails to produce a pink fluorescence, but has a yellow growth and is wrinkled. The milk is rendered pink, and the growth on the potato is yellow and scanty. This variety is, perhaps, *Bact. erythrogenes* of Dyar (Trans. N. Y. Acad. of Sci., 1895). Probably the same as *Bact. luteum* of Zimmermann. (See p. 142.)

Bact. lactis rubrum. *Non spore-bearing, pink Bacterium.* This has not been found since its original description in 1899. Its characters, as then given, are as follows:

Morphology.—Size, 2μ – $4\mu \times .9\mu$. Forming chains. No spores, no capsule.

Gelatine colony.—A bead-formed colony, .7 mm. in diameter, with a granular edge. Liquefies with a nucleus and a clear zone.

Gelatine stab.—A slow liquefier, stratiform, producing a clear liquid with a scum and a sediment.

Agar streak.—Luxuriant, wrinkled, dull orange-yellow or pinkish.

Bouillon.—A sediment, but no pellicle nor turbidity.

Milk.—Becomes alkaline and curdles after several days at 37° . It digests into a dirty liquid.

Potato.—Glistening, smooth, pink or salmon-colored, luxuriant.

Grows at both 20° and 37° .

Variety A.—Found later. Agrees with the above, except that it is of an orange rather than a pink color.

Bact. lactis Burri n. s. *A reddish, bitter milk organism*, described by Burr and Dugelli. As described by them its characters are as follows: (Cent. f. Bac. II., XV., p. 709.)

Morphology.—Size, 1μ – $3\mu \times .7\mu$. No chains; no spores. Gram stain negative.

Gelatine colony.—Surface colony in a liquefying area, $\frac{1}{8}$ mm. in diameter. In fourteen days it is $\frac{1}{2}$ mm. in diameter, of a clear brown color.

Gelatine stab.—Begins to liquefy, in four days; infundibuliform.

Agar streak.—Luxuriant, smooth, lobed, reddish.

Fermentation tubes.—No acidity, gas, nor closed arm growth.

Bouillon.—A turbidity, but no sediment nor pellicle.

Milk.—Becomes acid, but does not curdle or digest. It becomes a rusty red, with a cheesy smell and, later, a bitter taste.

Potato.—No growth.

Grows at 20° but not at 37° . Aerobic.

Bact. lactis citronis n. s. *A non spore-bearing, lemon-yellow Bacterium.*

Morphology.—Size, $1\mu \times .6\mu$, forming chains. No spores are produced, and no capsules.

Gelatine colony.—Small pits are produced, with a nucleus and a lighter outer zone, which may be variously streaked.

Gelatine stab.—A slow liquefier, producing a cratiform liquefaction, with a dense sediment and a yellow liquid. At first clear, but later cloudy.

Agar streak.—A luxuriant, thick, folded growth, at first greenish-yellow, and later lemon-yellow.

Bouillon.—A turbidity and a sediment.

Milk.—Becomes alkaline and digested into an amber-colored or pale yellow liquid. Sometimes it curdles before digestion.

Potato.—Thick, smooth, flesh-colored, and later lemon-yellow; or sometimes lemon-yellow from the start.

Grows at both 20° and 37° . Aerobic.

Variety A.—This variety, also found in milk in Middletown, agrees with the above in most respects, differing in the following points: Size, $3\mu \times 1.5\mu$. Spores are produced. The gelatine forms a pit full of threads. Milk is curdled and after digestion shows a clear liquid and tenacious scum. The potato growth is wrinkled.

Variety B.—This organism forms no chains nor spores. Its colony is lemon-yellow, even when viewed under the microscope, a very unusual character; later it liquefies and sends processes into the gelatine. Its gelatine stab is infundibuliform, or a dry pit. Its growth on agar is lemon-yellow and very thin.

It is doubtful whether these three varieties should be grouped together, and certainly they should not if it is true that two of them do not form spores. We have lost these cultures and are unable to verify the characters above given. Therefore we insert them in their original form.

Bact. lactis minutissimum. *A very slender, orange Bacterium.* We have not found this since the original description in 1899. The following are the characters as then described.

Morphology.—Size, $1.5\mu \times .4\mu$. Forming long chains, and not producing spores.

Gelatine colony.—Surface colony irregularly branching; deep colonies burr-like, with a yellow centre and irregular processes, or sometimes simply lobed. Rays extend from a liquefying pit into the gelatine.

Gelatine stab.—Begins to liquefy in two days with an infundibuliform or cratiform liquefaction, and a brilliant yellow sediment.

Agar streak.—A luxuriant, widely spreading, orange growth, covering the whole surface.

Bouillon.—A sediment, turbidity and a pellicle.

Milk.—Rendered alkaline and becomes somewhat thick and dark colored, but no visible digestion.

Potato.—Luxuriant, deeply orange.

Grows better at 20° than at 37° . Aërobic.

Variety A.—A second culture, found later, may be a variety of the same, although it is not so small. It is $.6\mu$ in diameter, and does not appear to form chains. Its colony is brilliant yellow, smooth and translucent, but does not show the irregular processes. Milk does not become thick. The color on potato is bright yellow rather than orange.

Bact. lactis Marshalli n. s. *A slimy milk, yellow Bacterium.* This is the organism with which Marshall has worked and which was shown by him to have a hastening action upon lactis bacteria (Cent. f. Bact. II.). The following characters were determined by us from cultures sent by him, and agree essentially with those determined by him:

Morphology.—A rod, not forming chains. Size, $1.2\mu \times .3\mu$. (Marshall's measurements, $1.7\mu-5.25 \times .8\mu-.875\mu$.) It produces no spores, has no capsules and Gram stain is negative.

Gelatine colony.—A slowly liquefying, granular colony, which may later become large, irregular and slimy.

Gelatine stab.—Begins to liquefy in two to three days, infundibuliform. In nine days it liquefies $\frac{1}{4}$ inch. Upon *litmus gelatine* it forms a rough, red-brown colony, with a dark center. Not acid.

Agar streak.—A luxuriant, viscous colony, filiform, raised, smooth, cream-white or gray, but later lemon color.

Fermentation tubes.—No acidity, gas, nor closed arm growth in any bouillon.

Bouillon.—A sediment and turbidity, and a pellicle formed around the edges of the tube.

Milk.—Becomes alkaline and digests, but does not normally curdle. Has a prominent odor, and is slimy.

Potato.—A luxuriant, filiform, effused, smooth, lemon-yellow growth.

Grows both at 20° and 37°. Aerobic.

We have found essentially the same organisms in Middletown, and Harding has sent us one from New York which failed to digest milk, but agreed in other respects. This latter one may, perhaps, be called *variety A*.

Variety B.—An organism isolated from Camembert cheese, sent us direct from France, really belongs here, although differing in the following characters: Its colony is white. It is not viscous. It forms no pellicle. It does not digest milk. Its growth on potato is orange. We have found the same on Camembert cheese from New York markets.

Bact. lactis Limburgii n. s. A non spore-bearing, orange-yellow Bacterium, This organism was isolated from milk and described by Burri and Dugelli as follows. (The name is our own):

Morphology.—Size, $1.5\mu-3\mu \times .5\mu$. Forming no chains and no spores.

Gelatine colony.—A round, brownish colony, 1 mm. in diameter. After six days a yellow disk is seen in its cloudy liquid.

Gelatine stab.—A needle growth and a surface growth, but after six days liquefaction begins; stratiform.

Agar streak.—Luxuriant, smooth, glistening, dirty yellow.

Fermentation tubes.—Not described, but no acidity is produced, and probably no gas in sugar bouillons.

Bouillon.—Liquid becomes turbid, but shows no pellicle.

Milk.—No change in reaction, and no curdling. The milk becomes digested and has a Limburger smell.

Potato.—Scanty yellow, glistening. Potato not discolored.

Bact. lactis luteum. This name was given by Zimmerman (Cent. f. Bac. II., XI., p. 200,) to a type isolated by him from the udder. Several of our cultures agree with it as closely as can be determined from his incomplete descriptions. It agrees closely with *Bact. erythrogenes*, except in lacking a pink fluorescence, and in not making milk red or pink. The characters given below are from our own cultures, to which we have given Zimmerman's name. We have found the organism in Camembert cheese from the markets, probably imported from France.

Morphology.—A rod, forming no chains. Size, $1.2\mu \times .8\mu$. No spores no capsules. Gram stain positive.

Gelatine colony.—A slowly liquefying colony, with a dense central growth and a clear liquid.

Gelatine stab.—A slow liquefaction; crateriform.

Agar streak.—A luxuriant, filiform, raised, rugose, lemon-yellow growth; dull and wrinkled.

Fermentation tubes.—No gas, no acidity, and no closed arm growth in any bouillon.

Bouillon.—A sediment, membranous pellicle and a slight turbidity.

Milk.—Becomes alkaline, but no other visible change.

Potato.—A luxuriant, spreading, thick, contoured growth, opaque, white or brownish-yellow.

Grows at 20° and 37° . Aerobic.

The color of this organism varies from a brilliant yellow to a dull yellow. Some cultures do not appear to grow on potato and show a slight digestion of milk.

Variety A.—One culture we regard as distinct enough to be recognized as a variety. It was obtained from milk rather than from cheese. Its size is $.8\mu \times .3\mu$, and it does not stain with Gram. Its colony is round, moderately thick, smooth and yellow, and then forming a pit. Upon *litmus gelatine* it is pale and thin. It produces no pellicle on bouillon, but curdles and digests milk, turning it slightly yellow. Its growth on potato is dry, luxuriant and lemon-yellow.

Bact. lactis Ashtonii n. s. *A non spore-bearing, yellow Bacterium*.

Morphology.—Size, 1.2μ – $3.5\mu \times 1.2\mu$. No chains, no spores nor capsules. Gram stain irregular.

Gelatine colony.—A slow, liquefying pit, with a cloudy liquid, tinged with yellow, *litmus gelatine* is not acid.

Gelatine stab.—A needle growth and a napiform liquefaction, beginning in three days.

Agar streak.—Filiform, raised, smooth, yellow, moist, viscous.

Fermentation tubes.—No acidity or gas in any bouillon, closed arm growth in all. (Saccharose is slightly acid after several days.)

Bouillon.—Sediment, turbidity and pellicle.

Milk.—Is rendered alkaline, curdled and digested completely, with a yellowish surface and a strong odor.

Potato.—Filiform raised, smooth, yellow, luxuriant; no discoloration.

Grows at 20° better than at 37° . Facultative anerobic.

Bact. lactis album n. s. *A white, liquefying Bacterium, not acid*. Found in the udder at Storrs. It may be the same as *Bact. luteum*, without pigment. Its characters are as follows:

Morphology.—Rods, forming no chains. Size, 1μ – $3\mu \times .7\mu$ – $.9\mu$. It forms no spores, no capsules, and Gram stain is positive.

Gelatine colony.—A very slowly liquefying colony, not characteristic; sometimes the gelatine dries before liquefaction takes place.

Gelatine stab.—Begins to liquefy in three days, and is about $\frac{1}{3}$ liquefied in twenty-one days. Napiform and stratiform.

Agar streak.—Filiform, raised, smooth, opaque, cream-white, shining, luxuriant, viscous.

Fermentation tubes.—No acidity, gas, nor closed arm growth.

Bouillon.—A sediment, turbidity and pellicle.

Milk.—Becomes alkaline, is curdled, or sometimes digests without curdling. The digested milk is very slimy and of a slight straw color (in one culture, pink).

Potato.—A very abundant, spreading, convex, smooth, brown growth; potato discolored.

Grows at 20° and 37° abundantly. Aerobic.

Variety A.—An organism found in the pink slime on Camembert cheese closely resembles this, but differs in the following: It is not viscous on agar, nor does it make milk slimy. It produces no pellicle in bouillon, and neither curdles nor digests milk.

II. Acid in dextrose or other sugars.

Bact. lactis musci. An arborescent bacterium with myceloid colonies. This was found in milk in Middletown, in cheese, and one culture was sent from Kiel isolated from Mazoon. Perhaps identical with *Bact. mycoides* (Flügge).

Morphology.—Long filaments made up of rods $3\mu \times 1\mu$. Central spores no larger than the rods. Gram stain positive.

Gelatine colony.—Myceloid, branching, radiating colonies before liquefaction. Commonly forming a thin, white, velvety, or ground-glass surface upon the liquefying gelatine. *Litmus gelatine* is not acid.

Gelatine stab.—There is at first an arborescent needle growth, but liquefaction begins in one day, and a wrinkled glass-like surface is formed.

Agar streak.—Luxuriant, thin, white, wrinkled. In one culture threads grow down into the agar, and the agar turns dark colored.

Fermentation tubes.—Growth is somewhat variable. In one culture dextrose is rendered acid with a closed arm growth in all sugar bouillons. In another culture dextrose and saccharose are acid, and there is no closed arm growth. No gas.

Bouillon.—A turbidity, sediment and pellicle, except in one culture where the pellicle is wanting.

Milk.—Curdles with amphoteric reaction and subsequently completely digested.

Potato.—Luxuriant, thin, whitish, sometimes wrinkled; potato may be discolored.

Grows at 20° and 37°. Aerobic.

Variety A.—One culture was sufficiently different to be separately tabulated. The differences are as follows: Size, $2.5\mu \times 1.4\mu$. Gram stain negative. The gelatine colony shows masses of bacteria hanging together. They are arborescent under mica. A dry pit formed in gelatine stab. Agar growth not wrinkled. No closed arm growth. The reaction of milk is alkaline. Here belong Nos. 14 and 16 of Adametz.

Bact. lactis cretaceum n. s. A non-arborescent, spore-bearing *Bacterium*.

Morphology.—Size, $3-5\mu \times 1.4\mu$. No chains nor capsules. The Gram stain is positive. Spores are produced, no larger than the rods.

Gelatine colony.—Not characteristic; a rather slowly liquefying pit forms, without any distinguishing marks. In *litmus gelatine* it is not acid.

Gelatine stab.—No needle growth. In one day it begins to liquefy; stratiform or sometimes infundibuliform.

Agar streak.—Filiform, raised, smooth, cretaceous, white or flesh-colored, moist, luxuriant.

Fermentation tubes.—Acidity and closed arm growth in dextrose and saccharose; closed arm growth, but not acid in lactose. No gas.

Bouillon.—A sediment, a slight turbidity and, later, a slight pellicle.

Milk.—Becomes alkaline, curdles promptly and completely digests, with a prominent odor.

Potato.—Spreading, raised, smooth, cretaceous, white, luxuriant; potato discolored.

Grows at 20° and 37° . Facultative anaerobic.

Bact. lactis lobatum n. s. An orange, acid liquefier. This organism was found in stable dust and in milk direct from the udder. The two cultures differ slightly in color and bouillon growth.

Morphology.—Size, $.8\mu-1\mu \times .5\mu$. No chains, no spores, no capsules. Gram stain positive.

Gelatine colony.—A round, raised, smooth homogeneous colony, sometimes yellowish, with a lobate center and a clear ring when liquefying. *Litmus gelatine* shows an acid, liquefying colony.

Gelatine stab.—Slow liquefier, at first saccate, and then stratiform. The liquid is cloudy.

Agar streak.—A smooth, raised, thin, orange-colored colony, luxurious.

Fermentation tubes.—All three sugars are rendered acid, and there is growth in the closed arm, but no gas.

Bouillon.—A sediment, turbidity, and pellicle. In one culture there is no pellicle.

Milk.—Rendered acid, but not curdled. It is digested and turned amber-color or reddish, with a jelly-like sediment.

Potato.—Thick, opaque, orange-colored, luxurious.

Grows well at 20° , hardly at all at 37° Facultative anaerobic.

Bact. lactis cloacae n. s. This organism appears much like *B. cloacae* of Jordan, differing slightly in its growth in bouillon and on potato. Its liquefying power is also less. It was found only once in milk, in Middletown.

Morphology.—Size, $.7\mu-.8\mu \times 5\mu$. No chains, no spores. Gram stain negative. It shows a capsule.

Gelatine colony.—A round, thick, smooth, homogeneous colony, 1 mm. in diameter; white, acid on *litmus gelatine*.

Gelatine stab.—A dry pit is first formed, which later liquefies, infundibuliform.

Agar streak.—A narrow, raised, smooth growth; opaque, whitish, dull, fairly luxuriant.

Fermentation tubes.—Acidity, closed arm growth and gas in all sugars.

Bouillon.—A sediment and turbidity, but no pellicle.

Milk.—Acid and curdled, showing a clear whey, but there is no visible digestion. A sour odor.

Potato.—A scanty, white growth, with a discolored potato.

Grows at 20°, and moderately at 37°. Aerobic.

Bact. lactis liquaerogenes n. s. *A gas-producing, liquefying Bacterium*.

Morphology.—Size, $1\mu-1.6\mu \times .7\mu$. No chains, no spores, and Gram stain negative.

Gelatine colony.—A non-characteristic, rapidly-liquefying colony.

Gelatine stab.—Begins to liquefy in two days; complete in nine days.

Agar streak.—Not luxuriant, spreading, thin, smooth, white.

Fermentation tubes.—Dextrose and saccharose show acidity, gas and closed arm growth. Lactose shows closed arm growth but no acidity or gas.

Bouillon.—A turbidity, sediment and a pellicle.

Milk.—Is curdled without change of reaction, and is digested with an odor of Brie cheese.

Potato.—Not luxuriant, spreading, smooth, thin, white; potato discolored.

Grows at 20° and 37°. Facultative anaerobic.

Bact. visco fucatum Harrison and Barlow. *A slimy milk Bacterium with blue pigment*. This organism, isolated and described by Harrison and Barlow, from oily butter, seems to be new. (Trans. R. S. of Canada, XI., 1905-6.) It was not the cause of the oiliness of the butter, but is peculiar in showing a wide range of color. Its characteristics are given below in condensed form.

Morphology.—Size, $1\mu-1.8\mu \times .6\mu-.9\mu$. No long chains, no spores. Gram stain positive, and an evident capsule. Branched involution forms in all cultures.

Gelatine colony.—A slimy colony, yellowish-green, with crystals in the gelatine. When carbohydrates are present, the gelatine is blue or green.

Gelatine stab.—Liquefies in ten days, complete in two months; cratiform. The liquid is of a dark inky color above and rusty below.

Agar streak.—A slow, smooth growth, viscous, no pigment.

Fermentation tubes.—Not stated, but probably acid without gas.

Bouillon.—A sediment and turbidity, but no pellicle. The liquid is alkaline, slimy.

Milk.—Rendered acid and curdled after several days; later digested, with a greenish-blue color, and becomes slimy.

Potato.—Yellowish-white, slimy, luxuriant. The potato becomes light-blue and, later, amber-colored.

Grows at both 20° and 37°. Aerobic.

Bact. lactis brevis n. s. *A white liquefier*. This has been found several times in milk and cheese here and elsewhere. It seems to be identical with one sent by Freudenreich and is apparently a widely distributed species.

Morphology.—Size, .7 μ -.9 μ x .5 μ -.6 μ . No chains, no spores. Gram stain irregular; no capsule.

Gelatine colony.—A round, thin colony, lobed, whitish. It may show acidity in litmus gelatine, and it slowly liquefies.

Gelatine stab.—Begins to liquefy in from one to two days, and may be completely liquefied in from three to twelve days. Stratiform.

Agar streak.—Fairly luxuriant, smooth, white, not characteristic.

Fermentation tubes.—All three sugars are acid, and there is usually growth in the closed arm, but no gas.

Bouillon.—A sediment, but no turbidity, and no pellicle.

Milk.—Rendered acid and curdled, and later, is partly digested.

Potato.—Barely visible; thin and white.

Grows at 20° and 37°. Facultative anaerobic.

Found many times, the numerous varieties showing some differences. The Freudenreich organism is longer, 1 μ in length. It makes milk acid and bitter. This is apparently *Milch. Bacterium II.* of Koning (Milchw. Zent. II., p. 317, 1906).

Variety A.—This organism, sent by Harding, differs in the following points: Size, 2-3 μ x 1.2 μ . Liquefaction of gelatine tube never complete. A pellicle in bouillon. Rather more luxuriant on potato.

Bact. lactis fluorescens n. s. *A fluorescent Bacterium*. This organism, being non-motile, seems to be different from the common fluorescent form. We have found it but once, in New York city milk. It may be only a non-motile variety of *B. fluorescens*, but we have been unable to detect any motility in our cultures.

Morphology.—Size, 1.4 μ -2.5 μ x .7 μ -.9 μ . No chains, no spores, no Gram stain and no capsule.

Gelatine colony.—A slow liquefier, forming a peculiar lace-like colony in a pit, with a dense centre.

Gelatine stab.—A needle growth, stratiform; liquefaction beginning in one day.

Agar streak.—Filiform, capitate, smooth, translucent, white, luxuriant, with a green fluorescence.

Fermentation tubes.—Dextrose and saccharose are rendered acid; lactose is not; no closed arm growth; no gas.

Bouillon.—A sediment, turbidity and a pellicle.

Milk.—Rendered alkaline and curdled at 20°, but not at 37°. Digestion occurs later and the milk has a strong odor.

Potato.—A filiform, raised, smooth, white, luxuriant growth.

Grows at 20° and only slightly at 37°. Aerobic.

Bact. lactis plicatum n. s. *A non-acid, white, liquefying Bacterium*. Isolated from milk by Harding.

Morphology.—Size, 3 μ –5 μ x .8 μ –.9 μ , growing in long chains. No spores, no capsules, and Gram stain irregular.

Gelatine colony.—A slow liquefying colony, showing a peculiar folding.

Gelatine stab.—A needle growth, beginning to liquefy in one day, and about half liquefied in ten days; infundibuliform.

Agar streak.—Filiform, thick, smooth, opaque, whitish, moist, moderately luxuriant.

Fermentation tubes.—All three sugar bouillons are slightly acid, but there is no closed arm growth, and no gas.

Bouillon.—A sediment and slight turbidity, but no pellicle.

Milk.—Rendered alkaline, curdled in three days and subsequently digested.

Potato.—Spreading, thick, mottled, wrinkled, white, luxuriant, with discolored potato.

Grows at 20° and 37°. Aerobic.

Variety A.—Differs in the following points: No chains observed. Colony without the peculiar folding. Digestion of milk not noticeable. Growth on potato scanty and not wrinkled.

Bact. lactis Gorinii n. s. *A non-acid, white, liquefying Bacterium*. The original of this organism was sent from Italy by Gorini. Two very similar cultures were found by us, one in New York and one in Connecticut. The original culture had the following characters:

Morphology.—Size, 1.5 μ –2.5 μ x 1 μ . Rods with square ends. No chains, no spores. Gram stain positive.

Gelatine colony.—A slow liquefier, producing a large pit with irregular mottled clusters of bacteria.

Gelatine stab.—Begins to liquefy in two days and is $\frac{3}{4}$ complete in three weeks. Infundibuliform.

Agar streak.—Spreading, raised, smooth, opaque, white, moist, luxuriant, viscous.

Fermentation tubes.—Dextrose and saccharose are acid; lactose not acid. No closed arm growth; no gas.

Bouillon.—A sediment, turbidity, and a pellicle.

Milk.—Made strongly alkaline, curdled and completely digested at 20°.

Potato.—Spreading, thick, smooth, opaque, moist, luxuriant; potato discolored.

Grows well at 20° and 37°. Aerobic.

Variety A.—Isolated from milk in Connecticut. Is not quite so large. Gram stain negative. Saccharose is not acid. Produces iridescent brown color. Does not grow well at 20°. Milk has a peculiar color.

Variety B.—(From milk in New York city.) Saccharose is acid. There is no pellicle on bouillon. No digestion of milk. Growth on potato is scanty.

Variety C.—(From Brie cheese.) Has a simple, granular, rapidly-liquefying colony. Shows growth in closed arm of fermentation tubes, and a pellicle on bouillon. The milk develops the odor of Brie cheese.

Varieties A and B are probably cultures of the same organism, one of which is more vigorous than the other. Here evidently belongs *Bact. C.* of Müller (Arch. f. Hyg. LXVII., p. 127), which agrees in all points, but shows a blue fluorescence in gelatine, and gas in dextrose bouillon.

Bact. lactis magnum n. s. *A non-acid, white, liquefying Bacterium.*

Morphology.—Size, $3\mu \times 1.5\mu$. Chains produced; no spores or capsules. Gram stain positive. Rods with square ends.

Gelatine colony.—A fairly rapidly liquefying pit, which may be filamentous and with ciliated edge. On *litmus gelatine* it is not acid.

Gelatine Stab.—A needle growth, which may be arborescent and later liquefying; stratiform. Liquefaction begins in one to three days; complete in three weeks.

Agar streak.—Filiform or spreading, thick, punctate, opaque, white, moist, luxuriant.

Fermentation tubes.—Acid in dextrose only. No closed arm growth and no gas.

Bouillon.—A sediment, a turbidity, and a pellicle.

Milk.—Becomes alkaline, curdles after three days and digests into a brownish liquid, and a prominent odor.

Potato.—Spreading, thick, contoured, translucent, white; potato discolored. Grows at 20° and 37°. Aerobic.

Bact. lactis flocculus n. s. *An acid, non-curdling, liquefying Bacterium.* From Camembert cheese.

Morphology.—Size, $1\mu-2\mu \times 1\mu$. No chains, no spores. Gram stain positive.

Gelatine colony.—A slowly liquefying colony, which is lobate or moruloid.

Gelatine stab.—A needle growth and a surface growth, which begins to liquefy in ten days.

Agar streak.—A filiform, raised, smooth, opaque, white colony, rather scanty.

Fermentation tubes.—Dextrose is acid, but no other sugar bouillon. No closed arm growth, no gas. One culture is acid in all sugars.

Bouillon.—A sediment, turbidity, and a pellicle. One culture shows no growth.

Milk.—Acid but not curdled, and shows no digestion. Has a prominent odor.

Potato.—Spreading, thin, contoured, white, moist, luxuriant; potato discolored.

Grows better at 20° than at 37° Aerobic.

THE GENUS PSEUDOMONAS.

1. Non-liquefying.

Ps. lactis Middletownii n. s. *A Pseudomonas, producing gas, but no acidity.*

Morphology.—Size, $1.4\mu \times .8\mu-.9\mu$. No chains, no spores, no capsules. Gram stain negative.

Gelatine colony.—A round, raised, smooth, gyrose, entire, gray-white colony. On *litmus gelatine* the colony is coarsely granular, looking like a colony of yeast.

Gelatine stab.—A dry pit is formed, with needle growth.

Agar streak.—Filiform or spreading, smooth, thin, gray-white, moderately luxuriant, moist.

Fermentation tubes.—No acidity in any sugar bouillon, but closed arm growth and gas produced in all.

Bouillon.—A sediment, turbidity and a pellicle.

Milk.—Rendered acid and curdled after several days, and subsequently slightly digested.

Potato.—A luxuriant, spreading, thick, gray-white growth; potato discolored.

Grows at 20°, but hardly at all at 37°. Aerobic, or facultative anaerobic.

Ps. fluorescens aurea Weigmann. *A fluorescent, non-liquefying Pseudomonas.* This culture was sent me by Weigmann, but had lost its fluorescent character when received by us. Its characteristics, as given below, were made in our laboratory. This is perhaps *Ps. convexa* of Wright. We have several times found a similar organism in milk, but unfortunately at the time we did not make a study of their flagella; but we have no doubt that they belong with this organism of Weigmann. We list one of our own organisms as variety A.

Morphology.—Size, $2.5\mu \times .9\mu$. Short chains. There are no spores nor capsules and the organism does not accept the Gram stain. [Our organisms were usually shorter.]

Gelatine colony.—A round, raised, contoured, grumose colony, of a brownish-red color. [A green halo around the colony is common.] On *litmus gelatine* it is much folded.

Gelatine stab.—A filiform, needle growth and a raised, surface growth. [The surface growth is thick, and the gelatine may show a green color.]

Agar streak.—A filiform, smooth, thin growth of a yellowish color, moderately luxuriant. It was probably originally fluorescent, but this character has been lost. Our own cultures always showed the fluorescence.

Fermentation tubes.—No acidity, gas, nor closed arm growth in any sugar bouillon.

Bouillon.—A red sediment, a ring-like pellicle, and slight turbidity are formed. [Greenish.]

Milk.—Rendered slightly alkaline, or there is no change in the reaction. No curdling nor digestion occurs, and there is no change in color.

Potato.—A filiform, raised, contoured growth, of a brownish-yellow to an orange color; luxuriant, and with the potato discolored. [Yellow to brown, and sometimes folded.]

Grows at both 20° and 37° Aerobic.

Variety B.—Isolated from milk here, is a similar, non-liquefying fluorescent monotrich, which we regard as the same. It is quite common and agrees closely enough with the above to be called by the same name. It commonly makes the gelatine green, and has a strong fluorescent action on agar. Milk is also turned green, but is otherwise unchanged. On agar the growth is rather scanty, but is luxuriant on potatoes, of a brown color to white. On potato it may be folded.

Ps. lactis Estenii n. s. *A Pseudomonas with a smoky fluorescence*. This organism is quite common in milk. Another one practically identical agrees in all points except that it does not produce the smoky fluorescence.

Morphology.—Size, $.8\mu-1.2\mu \times .4\mu$. No chains are formed, no spores nor capsules, and the organism does not accept the Gram stain.

Gelatine colony.—A round, smooth, capitate, contoured, homogeneous colony, of a cream-white to a gray color. On *litmus gelatine* it forms a pale, thin surface colony.

Gelatine stab.—A filiform, needle growth, and a raised, dry, not spreading, surface growth.

Agar streak.—Filiform, raised, smooth growth, translucent; gray in color; luxuriant and slightly viscous. The agar becomes smoky.

Fermentation tubes.—No acidity, gas, nor closed arm growth in any sugar bouillon.

Bouillon.—A sediment and flocculent turbidity are formed, but no pellicle.

Milk.—No action on milk.

Potato.—A filiform, thin, smooth growth; gray in color; moderately luxuriant.

Grows both at 20° and 37°. Aerobic.

Variety B differs from A in being slightly larger, $1.8\mu-3\mu \times .7\mu$, and in producing no fluorescence.

Ps. lactis filiformis n. s. *A yellow, non-liquefying Pseudomonas*. Found once in New York milk.

Morphology.—Size, $2.5\mu-3.5\mu \times .8\mu-.9\mu$. No capsule, and no Gram stain. Spores are produced which are frequently seen as long chains. One long flagellum.

Gelatine colony.—A round, convex, smooth, entire colony of a creamish or yellow color. On *litmus gelatine* the colony is red-brown, non-acid.

Gelatine stab.—A filiform needle growth, and a flat surface.

Agar streak.—A filiform, raised, smooth, yellowish growth, not very luxuriant, moist.

Fermentation tubes.—Dextrose and saccharose bouillons are rendered acid. There is no gas nor growth in the closed arm in any bouillon.

Bouillon.—A sediment, a ring-formed pellicle, and a flocculent turbidity.

Milk.—Rendered acid, but no other change produced.

Potato.—A beaded, thick, punctate growth, of a yellow color, not very luxuriant. Potato discolored.

Grows better at 20° than at 37°. Aerobic.

Ps. pseudo tuberculosis Klein. This organism, isolated by Klein from London milk, was sufficiently well described by him to be inserted here as follows:

Morphology.—A rod, forming long chains; individual elements 1.2μ–1.8μ x .4μ–.5μ. Gram stain positive. No spores nor capsules described.

Gelatine colony.—A white surface colony, somewhat granular, and resembling that of *B. coli*. No gas is produced, however.

Agar streak.—Resembles *B. coli*, but is less luxuriant.

Fermentation tubes.—Not described but, doubtless, no acidity nor gas is produced.

Bouillon.—A turbidity and a slight pellicle, but no sediment.

Milk.—No action on milk.

Potato.—A thin, crenate, faintly brownish growth, with a whitish-yellow margin.

This is found in 8% of London milk, and is said to be pathogenic for Guinea pigs.

Ps. lactis viridis n. s. *A Pseudomonas, turning gelatine green.* This organism has been found only once, in milk from Colchester.

Morphology.—Size, .9μ–1μ x .4μ–.5μ. Spores are produced, and there is no capsule. There are no chains, and the Gram stain is negative.

Gelatine colony.—A round, raised, smooth, homogeneous colony, entire, yellowish, moist.

Gelatine stab.—A needle growth with raised surface. The gelatine is turned green.

Agar streak.—A filiform, raised, smooth, translucent, white growth, quite luxuriant, moist; no fluorescence.

Fermentation tubes.—Dextrose rendered acid and shows growth in closed arm. There is no gas. In the other sugar bouillons there is no acidity, gas, nor closed arm growth.

Bouillon.—A flocculent sediment, a pronounced turbidity, but no pellicle.

Milk.—Very slightly acid, but no further change.

Potato.—A filiform, thin, smooth, creamish-colored, moist growth; not very luxuriant. Potato discolored.

Grows at both 20° and 37° Facultative anaerobic.

Ps. sapolactica Eichholz. An acid, non-liquefying *Pseudomonas* (soapy). This organism was sent by Weigmann and was stated by him to produce soapy-tasting milk. When studied by us this latter character was not apparent. We have found an identical organism (not soapy) in New York city milk, and twice in Middletown. The Middletown culture was found in great quantities in some milk that had been preserved at 1° for several weeks. A very closely allied organism was sent us from Geneva by Harding, which we have called variety A.

Morphology.—Size, $.8\mu$ – 1.7μ x $.7\mu$ – $.8\mu$. No chains, no spores, no capsules. Gram stain negative.

Gelatine colony.—A round, raised, smooth, homogeneous, entire colony, of a gray color. In *itmus gelatine*, in some cases, the colony becomes acid.

Gelatine stab.—A filiform needle growth, and a flat surface.

Agar streak.—Filiform, thick, smooth, opaque, white, moist, moderately luxuriant.

Fermentation tubes.—Dextrose only is rendered acid. There is no gas or closed arm growth in any sugar bouillon.

Bouillon.—A sediment, decided turbidity, and a ring-like pellicle.

Milk.—Is rendered alkaline, at least after several days, but there is no other change.

Potato.—A slight growth, linear, thin, contoured, brownish in color, moist. Potato not discolored.

Grows rather better at 37° than at 20°. Aerobic.

Variety A.—Is acid in all three sugar bouillons, curdles milk and shows no potato growth.

II. Liquefying.

Ps. lactis anana n. s. This was originally described in 1899. Several cultures from milk in Middletown, and New York, and Storrs, have been found agreeing with this in all points except in the presence of the banana smell upon potato. The following more complete description was from a culture isolated more recently from milk in Middletown:

Morphology.—Size, $.8\mu$ – 1.2μ x $.5\mu$. Forms chains, no spores and no capsules. Gram stain negative.

Gelatine colony.—A very rapid liquefier, producing a granular pit; not characteristic.

Gelatine stab.—Liquefies rapidly; either stratiform or infundibuliform.

Agar streak.—Spreading, flat, smooth, creamish to brown color, rather luxuriant, and sometimes slightly viscous.

Fermentation tubes.—No acidity nor gas in any sugar bouillon. One culture showed growth in the closed arm, but the others did not.

Bouillon.—A granular sediment, and a decided flocculent turbidity, but no pellicle. The liquid is sometimes but not always greenish.

Milk.—Is curdled without change in the reaction, or slightly alkaline, and is afterwards digested. After digestion it is colorless and transparent, and sometimes jelly-like. It may have a greenish tinge.

Potato.—A luxuriant growth, widely spreading, convex, smooth and moist, of a brownish color. Potato not discolored.

Grows at both 20° and 37° Aerobic.

Probably the culture that produces the green color deserves to be called a distinct variety.

Ps. lactis Eurotas n. s. A brown, fluorescent, liquefying *Pseudomonas*. This organism has been found in milk directly from the udder and in New York city milk, the latter differing from our organism in the points indicated by brackets.

Morphology.—Size, $.9\mu$ – $1.5\mu \times .3\mu$. No chains, no spores, no capsules, and Gram stain negative.

Gelatine colony.—A round, convex, smooth, punctate, entire colony, of a gray-brown color. On *litmus gelatine* it is alkaline and slowly liquefies.

Gelatine stab.—Liquefaction is slow; stratiform.

Agar streak.—A luxuriant growth, linear, flat, smooth, gray [yellow], moist. The agar shows an amber-colored fluorescence.

Fermentation tubes.—All three bouillons are rendered alkaline, and show closed arm growth, but no gas.

Bouillon.—A sediment, a decided turbidity, and a flocculent pellicle.

Milk.—Is rendered alkaline, is curdled both at 20° and 37°, and is completely digested. [The milk does not curdle.]

Potato.—A luxuriant, linear, smooth growth, of a brown color [yellowish].

Grows at both 20° and 37°. Facultative anaerobic.

Ps. lactis nigra Gorini. A black pigment forming *Pseudomonas*. This organism was received from Gorini, and the characters as given below were determined by him. It clearly resembles *B. lactis niger* A.

Morphology.—Size, 2μ – $3.5\mu \times 1\mu$. No chains, no spores, no capsules. Gram stain negative.

Gelatine colony.—A pit, liquefying slowly, and with an irregular centre.

Gelatine stab.—Liquefaction begins in twelve hours; infundibuliform.

Agar streak.—A filiform, raised, rugose growth; opaque, cream-white. Luxuriant, with a dull surface, and sometimes wrinkled. The agar shows a brown fluorescence.

Fermentation tubes.—No acidity nor gas in any sugar bouillon. A very slight growth in the lower quarter of the closed arm.

Bouillon.—A sediment and slight turbidity, with a wrinkled pellicle, which may sink in flakes to the bottom.

Milk.—Rendered acid, curdled, and subsequently digested. The digestion is complete and a slight sliminess is produced.

Potato.—Luxuriant, spreading, convex, smooth, grayish-brown. Potato chocolate-colored.

Grows well at 20° and 37°. Aerobic.

Ps. lactis contorta n. s. *A polyipiform, spore-bearing, monotrich.* This organism has been found only once, in milk from New York.

Morphology.—Size, $1.5\mu \times .8\mu$. It apparently produces spores and has a single flagellum, although it was lost before the study was completed.

Gelatine colony.—A slow, liquefying pit, at first umbonate. On *litmus gelatine* it is highly lobed and contorted, and does not liquefy in four days.

Gelatine stab.—A filiform needle growth, with a flat surface growth, which later becomes a dry pit. True liquefaction does not appear.

Agar streak.—A moderate growth, filiform, convex, smooth, opaque, of a cream-white color.

Fermentation tubes.—No acidity nor gas in any sugar bouillon, but growth in closed arm.

Bouillon.—A sediment, slight turbidity, and a granular pellicle.

Milk.—Rendered alkaline, but not curdled. At 20° there is a slight digestion and the milk is slightly slimy.

Potato.—Luxuriant growth, filiform, convex, smooth, opaque, of a gray color.

Grows better at 20° than at 37° Facultative anaerobic.

Ps. lactis minuta n. s. *A very small spore-bearing monotrich.* This organism has been found only once, in milk directly from the udder.

Morphology.—Size, $.6\mu-.8\mu \times .3\mu$. A very short rod, not forming chains. No spores, no capsules. Gram stain positive.

Gelatine colony.—Round, raised, smooth, entire colonies, of a brownish-yellow color, which, later, liquefy.

Gelatine stab.—A slow liquefaction, crateriform.

Agar streak.—Luxuriant, filiform, raised, translucent, porcelain-white, moist.

Fermentation tubes.—No acidity, gas, nor closed arm growth in any sugar bouillon.

Bouillon.—A flocculent sediment and a slight turbidity, but no pellicle.

Milk.—Rendered acid, is not curdled or digested, and no other change is noticeable.

Potato.—No visible growth.

Grows at both 20° and 37° Aerobic.

Ps. lactis mina n. s. *A Gram-staining, spore-bearing, monotrich.* This organism has been found only once, in New York city milk. It is somewhat similar to the last, but differs in some important points.

Morphology.—Size, $1.4\mu-1.8\mu \times .6\mu$. A slender rod. No spores, no capsules. Gram stain negative.

Gelatine colony.—A slow liquefying pit, or sometimes a dry pit without liquefaction, but with a dense colony at the bottom.

Gelatine stab.—No true liquefaction, but a deep, dry pit is formed.

Agar streak.—A luxuriant growth, filiform, raised, smooth, opaque, white, moist.

Fermentation tubes.—No acidity, gas, nor closed arm growth in sugar bouillon.

Bouillon.—A granular sediment and decided turbidity, with a membranous pellicle.

Milk.—No change except a slight alkalinity.

Potato.—A nodose, convex, contoured growth, gray in color, moist, and not luxuriant. The potato is discolored.

Grows better at 20° than at 37°. Aerobic.

Ps. lactis Robertii n. s. *A white, non-acid, liquefying monotrich.* This organism was found in Middletown and New York city milk. A second variety, found at Storrs, differed in points shown in brackets.

Morphology.—Size, $2\mu \times .7\mu-.9\mu$ [$1\mu \times 3\mu$]. Large rods with square ends. No chains. Gram stain positive. No capsules and no spores.

Gelatine colony.—A rapidly liquefying colony, with a greenish-orange pigment. [Brownish.]

Gelatine stab.—A rapid liquefier, stratiform, with a clear yellow liquid. [Slow liquefier.]

Agar streak.—Luxuriant, raised, smooth, moist, white. [Dry and wrinkled.]

Fermentation tubes.—No acidity or gas in any bouillon, but growth occurs in closed arm in all cases.

Bouillon.—A sediment, decided turbidity, and membranous pellicle.

Milk.—Rendered alkaline, curdled and digested. It has an odor and a greenish color. [Greenish color and developed.]

Potato.—Moderately luxuriant, filiform, flat, smooth, brown. [Spreading flesh color.]

Grows at both 20° and 37°. Facultative anaerobic.

The Storrs type should, perhaps, be called variety A. The culture from New York did not produce greenish milk.

Ps. lactis aurea n. s. *A yellow, liquefying monotrich.* Found only once, in New York city milk.

Morphology.—Size, $1.4\mu \times 1\mu$. No spores, no capsules, no chains. Gram stain positive.

Gelatine colony.—A slow liquefier, with a dark-ringed colony; round. On *litmus gelatine* it is lobed at the edge and not acid.

Gelatine stab.—A filiform needle growth, with a flat surface. Liquefaction begins in one day; stratiform.

Agar streak.—Luxuriant, filiform, raised, papillate, of a lemon-yellow color.

Fermentation tubes.—No acidity, gas, nor closed arm growth in any sugar bouillon.

Bouillon.—A sediment, slight turbidity, and ring-formed pellicle.

Milk.—Rendered alkaline, but no other change noticed.

Potato.—A luxuriant growth, spreading or beaded, contoured; of a lemon-yellow color, moist.

Grows better at 20° than at 37° Aerobic.

Ps. lactis aerogenes A. u. s. A gas-producing, liquefying monotrich. This was found in milk which had been kept at 1° for several weeks. Possibly this is *Ps. coadunata* of Wright.

Morphology.—Size, $1\mu-1.2\mu \times .7\mu-.9\mu$. A short rod, with no chains. No spores, no capsules, and Gram stain negative.

Gelatine colony.—Round, raised or flat, smooth, homogeneous, with wavy edge. Cream-white to brownish color. On *litmus gelatine* the colony is large, moist and acid, like a typical *B. aerogenes*.

Gelatine stab.—A very slow liquefaction. There is a beaded needle growth, a moderately thick surface, rather rough, and, after a long time, a liquefaction.

Agar streak.—Thin, whitish, extending over the whole surface of the agar; not characteristic.

Fermentation tubes.—Acid, gas, and closed arm growth in all sugar bouillons.

Bouillon.—A sediment, slight turbidity and a membranous pellicle.

Milk.—Rendered acid and promptly curdled. A very slight digestion, with a strong odor. The digestion not always apparent.

Potato.—Usually scanty, thin and of a porcelain-white color.

Grows better at 20° than at 37° Facultative anaerobic.

Ps. fluorescens Gorini. A fluorescent monotrich. This organism, received from Gorini, had the following characters when studied by us. A similar organism from Colchester milk differed in points shown in brackets.

Morphology.—Size, $1.2\mu-1.8\mu \times .7\mu$. No chains, no spores, no capsules. Gram stain negative.

Gelatine colony.—A moderately transparent, liquefying colony, granular and cloudy. It liquefies the whole plate in time. *Litmus gelatine* not acid.

Gelatine stab.—It begins to liquefy in one day, infundibuliform, with a tenacious scum. [Stratiform.]

Agar streak.—Luxuriant spreading, raised, smooth, opaque, gray. [Thin and flat.] The agar shows a green fluorescence.

Fermentation tubes.—Dextrose bouillon is acid, but the others are not. No gas in closed arm growth in any case.

Bouillon.—A sediment and a decided turbidity, but no pellicle. [Pellicle is formed.]

Milk.—Becomes alkaline at 20°, is curdled and completely digested, but not at 37°. Shows a green color and strong odor. [Curdles and digests at 37°, without the green color.]

Potato.—A luxuriant, spreading, thin, white growth. Potato discolored. [Thick.]

Grows better at 20° than at 37° Facultative anaerobic.

Ps. lactis varians n. s. *An acid-producing, white monotrich.* This organism, incompletely described in 1899, is now more completely described from recently isolated cultures that are probably the same. They are quite common and have been found in abundance in milk that had been preserved for several weeks at 1°.

Morphology.—A rod, forming chains; the individual elements $1\mu-1.4\mu \times .8\mu$. No spores, no capsules. Gram stain negative.

Gelatine colony.—A round, flat or umbilicate, smooth, rugose colony, of a slight brown, yellow or cream-brown color, producing a slow liquefaction.

Gelatine stab.—Stratiform or infundibuliform. The liquefaction is slow, and one culture produced a dry pit without liquefaction.

Agar Streak.—Filiform, raised, smooth, opaque, white and moderately luxuriant.

Fermentation tubes.—Usually acid in dextrose but not in other sugar bouillons. No gas nor closed arm growth in any bouillon.

Bouillon.—A sediment, an abundant turbidity, and a membranous pellicle.

Milk.—Rendered slightly acid and curdled at 20°, but not at 37° No visible digestion, but a prominent odor.

Potato.—Somewhat variable. It may be thin, spreading, smooth and gray-brown, or more luxuriant, varying from white to brown.

Grows better at 20° than at 37°, though there is a slight growth at 37°. Aerobic.

Variety A.—One culture of this organism differs from the above description in the following points: Size, $1\mu \times .5\mu$. Liquefies rapidly, beginning in one day. The colony forms a granular liquefying pit. Saccharose bouillon acid and shows growth in closed arm. Milk rendered alkaline, curdled, and digested. A luxuriant, thick growth on potato.

Here belong, probably, Gorini's *B. acidificans presamigenes casei* and Grüber's *Ps. fragagariae* II. (Cent. f. Bact. II., XIV., 122), neither of which is thoroughly described. Grüber's organism is not sufficiently described for identification. Gorini's has the following characters:

B. acidificans presamegenus casei Gorini. (Rev. gen. d'lait, III., 505.)

Morphology.—Size, $8\mu-10\mu \times 2\mu$ (?) Produces spores and stains by the Gram method. Though not distinctly stated, this is probably monotrichic.

Gelatine colony.—A round, white, filamentous, irregular colony, which liquefies gelatine.

Agar streak.—A luxuriant, white growth.

Fermentation tubes.—Dextrose bouillon is rendered acid and shows closed arm growth.

Bouillon.—A sediment, turbidity, and pellicle are produced.

Milk.—Rendered acid, curdled, and digested at 20°, without change in color.

Potato.—Grows well.

Grows at 20° and 37°.

Ps. lactis granula n. s. *A non-curdling, liquefying monotrich.* Found in New York city milk and also sent by Harding.

Morphology.—Size, $.7\mu \times 2\mu$. A rod, forming chains. There are no capsules, and the Gram stain is negative. Spores are produced.

Gelatine colony.—A rapidly liquefying pit is formed, which is uniformly coarsely granular, and has a ciliated margin.

Gelatine stab.—A spiny needle growth and a napiform pit, which later is stratiform. Liquefaction begins in one day.

Agar Streak.—A filiform, raised, smooth, grayish, moderately luxuriant growth.

Fermentation tubes.—Acidity is produced in all three bouillons, but no gas nor closed arm growth.

Bouillon.—A sediment, an abundant turbidity, and a membranous pellicle.

Milk.—Rendered slightly alkaline, but no other change seen.

Potato.—Shows no growth.

Grows hardly at all at 20°, but abundantly at 37°. Aerobic.

THE GENUS BACILLUS, LOPOTRICHIC.

B. syncyanus (Ehrb.) Migula=*cyanogenes* Flüge. *Bacillus of blue milk.* We have not found this species, but a culture was sent to us by Duckwall. Its characters, as determined by us, are as follows:

Morphology.—Size, $1.2\mu-2\mu \times .5\mu$. Short chains; spores produced, but no capsules. Gram stain irregular. It possesses a tuft of flagella at one end.

Gelatine colony.—A round, raised, smooth, entire colony, of a grayish color, .5 mm. in diameter, in three days.

Gelatine stab.—A filiform, needle growth, and thin surface, which does not spread. After a few days the gelatine turns a dark color at the surface, but does not liquefy.

Agar streak.—Luxuriant, spreading, thin, smooth growth, translucent, white, and showing a dark, smoky fluorescence.

Fermentation tubes.—Dextrose and saccharose, rendered alkaline, without gas or closed arm growth, and no change in color. Lactose becomes very slightly acid, without gas or closed arm growth, and turns a deep blue-black.

Bouillon.—A black sediment, and dark-colored turbidity, with a membranous pellicle.

Milk.—Rendered slightly alkaline without curdling, but develops an odor, and after a few days becomes distinctly blue.

Potato.—A very luxuriant, spreading, thick growth, translucent or opaque, brownish in color.

Grows at both 20° and 37°. Aerobic.

B. lactis olivaceus n. s. *A greenish Peretrich.* This organism has been found in milk directly from the udder.

Morphology.—Size, $1.5\mu-2\mu \times .4\mu$. A small rod. No chains, no spores, no capsules. Gram stain negative. It has a tuft of flagella at one end.

Gelatine colony.—A round, convex, smooth, homogeneous, entire colony, of a reddish color below the surface. The surface colony has an irregular outline, and a reddish or a greenish color.

Gelatine stab.—No liquefaction, but a needle and surface growth.

Agar streak.—Luxuriant, filiform, raised, smooth, greenish in color.

Fermentation tubes.—No acidity, gas, nor closed arm growth in any sugar bouillon.

Bouillon.—A sediment, an abundant turbidity, and a granular pellicle formed.

Milk.—Rendered alkaline, becomes greenish, and develops a strong odor without curdling.

Potato.—A luxuriant, filiform, flat, smooth growth, brownish-yellow.

Grows both at 20° and 37° . Aerobic.

B. lactis minutus n. s. *A yellow Lophotrich.* This organism was isolated from milk directly from the udder.

Morphology.—Size, $.5\mu \times .4\mu$. An extremely minute rod, which forms short chains. It produces no spores, no capsules, and does not accept the Gram stain. Several flagella at one end.

Gelatine colony.—A round, convex, smooth, homogeneous colony, of a red color. On *litmus gelatine* it is not acid.

Gelatine stab.—A needle growth, but no surface growth.

Agar streak.—Filiform, raised, smooth, translucent, yellow, moist, luxuriant.

Fermentation tubes.—No acidity, nor closed arm growth in any sugar bouillon.

Bouillon.—A sediment and turbidity, but no pellicle.

Milk.—No action.

Potato.—Very scanty growth, yellowish.

Grows at both 20° and 37° . Aerobic.

B. lactis molecularis n. s. *A white, non-liquefying Lophotrich.* Found in New York city milk.

Morphology.—Size, $1.4\mu \times .7\mu$. A lophotrichic rod with flagella at both ends. No spores, no capsules. Gram stain negative.

Gelatine colony.—An opaque bead, smooth, entire, white. On *litmus gelatine* an extremely diffused growth, made up of microscopic dots, appearing to the naked eye simply as a cloud extending over the plate. The individual colonies are visible only under the microscope.

Gelatine stab.—A filiform needle growth and a flat surface, without liquefaction.

Agar streak.—A filiform, flat, smooth growth, of a gray-white color, rather scanty.

Fermentation tubes.—Dextrose is rendered acid, but there is no acidity on other sugar bouillons, and there is never any gas or closed arm growth.

Bouillon.—A sediment, turbidity and a pellicle.

Milk.—Rendered alkaline, and is curdled at 37°. There is no digestion, but an unpleasant odor.

Potato.—A scanty, flat growth, moist, smooth, of a brownish color.

Grows better at 20° than at 37°. Aerobic.

B. lactis Isignii n. s. A brown, fluorescent *Lophotrich*. Found in Isigny cheese, comprising 18% of the bacteria in the centre of the cheese.

Morphology.—Size, $.6\mu \times .9\mu$. A rod, occasionally forming chains. Spores are developed, capsules are present, and Gram stain is positive. Usually one flagellum is present, though sometimes a tuft is seen at one end.

Gelatine colony.—An extremely rapid liquefier, without any distinct characteristic.

Gelatine stab.—Begins to liquefy in two days, and is complete in twelve days. Sacchate or stratiform.

Agar streak.—Spreading, thin, smooth, luxuriant, translucent white. At 37° the agar becomes brownish, and at 20° it becomes at first flesh color and, later, brown.

Fermentation tubes.—Acidity, gas and closed arm growth in all sugar bouillons.

Bouillon.—A sediment and decided turbidity, but no pellicle.

Milk.—Rendered acid and curdled at both 20° and 37°, but no subsequent digestion noticeable.

Potato.—A luxuriant, thick, smooth growth, of a yellowish color, and a discolored potato.

Grows at both 20° and 37°. Aerobic.

II. Liquefying Lophotrichic Bacilli.

B. lactis fluorescens I. n. s. A lophotrich producing a smoky fluorescence and a green color.

Morphology.—Size, $8\mu-1.6\mu \times .4\mu-.6\mu$. A lophotrichic rod with two or three polar flagella. No chains. Spores developed in the ends of the rods. Gram stain negative, and no capsules.

Gelatine colony.—A round, gray, smooth, white colony, which liquefies into a uniformly granular pit.

Gelatine stab.—Liquefies rapidly, infundibuliform, with a cloudy liquid, full of flakes.

Agar streak.—Luxuriant, filiform, smooth, raised, gray, moist. The agar is smoky.

Fermentation tubes.—Dextrose bouillon is rendered acid, but there is no gas nor closed arm growth, nor is there acidity in any other sugar bouillon. In all cases the liquid is turned green.

Bouillon.—A granular sediment and turbidity, but no pellicle. The bouillon is green after a few days.

Milk.—No change in the reaction, but the milk is curdled and digested at both 20° and 37°, and becomes green in color, with a strong odor.

Potato.—Scanty, filiform, flat, smooth, brownish-yellow.

Grows at both 20° and 37°. Aerobic.

B. lactis fluorescens II. n. s. *A green fluorescent lophotrich.* This is a common form. The culture from which the following description was made was isolated from Camembert cheese. Some variations are shown in the points in brackets.

Morphology.—A lophotrichic rod, sometimes forming chains. Individual elements, 1μ – 1.4μ x $.6\mu$ – $.9\mu$. No spores nor capsules. Gram stain negative.

Gelatine colony.—A very rapid liquefier, forming a cloudy pit. [A bead and a granular pit.] On *litmus gelatine* it is strongly alkaline.

Gelatine stab.—Begins to liquefy in about three days, napiform. [Infundibuliform.] The liquid shows fluorescent green, and there is a surface membrane.

Agar streak.—Luxuriant, filiform or spreading, raised, smooth, of a white or brownish color. The agar turns green. The depth of the green color varies in different cultures. [Growth on agar in one culture is green, as well as the agar itself.]

Fermentation tubes.—Dextrose bouillon is acid, but no other sugar bouillon, and there is no gas, nor closed arm growth. [Dextrose not acid.]

Bouillon.—A sediment, a decided turbidity, and a flocculent pellicle. The liquid is green.

Milk.—Is alkaline and curdled at 20° and subsequently digested, green. Has a pleasant odor.

Potato.—Scanty, flat, smooth, brownish. [Luxuriant.]

Grows at both 20° and 37°, but better at 20°. Aerobic, or facultative anaerobic.

B. fluorescens minutissimus. *A fluorescent lophotrich.* This organism, described in 1899, is probably identical with the last. It shows slight variations and its description is reinserted here.

Morphology.—An extremely small rod. Size, $.5\mu$ – $.7\mu$ x $.5\mu$. No chains, no spores, no capsules. Flagella were not made out.

Gelatine colony.—A smooth, liquefying pit, without a nucleus, but granular.

Gelatine stab.—Liquefies stratiform, with a cloudy liquid, which is not green.

Agar streak.—Luxuriant, soft white growth, with a green fluorescence.

Bouillon.—A sediment and turbidity are formed, with a pellicle. In two days the bouillon is very cloudy, but not green.

Milk.—The milk is curdled with a slight green color at the top, but there is no apparent digestion.

Potato.—A luxuriant, white to brownish growth, with no discoloration.

Grows at both 20° and 37°, but the green color does not appear at 37°. Aerobic.

B. lactis fluorescens III. n. s. *A green, non-fluorescent lophotrich.* This organism does not produce a fluorescence, but the fact that it turns milk green suggests the relationship to the last organisms, and hence we class it here.

Morphology.—A slender rod.* Size, $1.5\mu-3\mu \times .4\mu-.7\mu$. No chains, no spores, no capsules. Gram stain negative.

Gelatine colony.—A very rapidly liquefying colony, uniformly granular, but not characteristic.

Gelatine stab.—Begins to liquefy in two days, stratiform.

Agar streak.—Luxuriant, spreading, flat, smooth growth, gray-brown, moist.

Fermentation tubes.—Acidity and closed arm growth in dextrose and sometimes lactose, but not in saccharose. No gas produced. The liquid is usually, though not always, green.

Bouillon.—A sediment, slight turbidity, and a membranous pellicle.

Milk.—Becomes alkaline, curdles, and digests. The digested liquid is green or yellow in color, and has a strong odor.

Potato.—Growth scanty, but the potato is discolored.

Grows at both 20° and 37°. Facultative, anaerobic.

B. lactis moruloideus n. s.

Morphology.—Size, $1\mu-1.5\mu \times 1.2\mu$. No chains, spores, or capsules. Gram stain irregular, frequently showing only a single flagellum.

Gelatine colony.—A slowly liquefying pit, which is lobed and moruloid, with a putrefactive odor. In *litmus gelatine* it is not acid.

Gelatine stab.—A needle growth, and a stratiform liquefaction, beginning in one day, and never complete.

Agar streak.—Filiform, raised, smooth, rather opaque, white, moist, not luxuriant.

Fermentation tubes.—Dextrose is acid but no other sugar bouillon. Occasionally growth in closed arm, but no gas.

Bouillon.—A sediment, turbidity, and a pellicle.

Milk.—Is rendered acid and curdled with a subsequent digestion, with a strong odor and a yellowish color.

Potato.—Scanty, thin, smooth, white, moist; no discoloration.

Grows at 20°; very slightly at 37° Facultative anaerobic.

* Frequently appearing monotrichic, from the breaking away of some of the flagella.

THE GENUS *BACILLUS*, PERITRICHIC, NON-LIQUEFYING.

I. No acid in dextrose or other sugars.

B. lactis nigroferous n. s. *A black bacillus*. This bacillus was obtained from milk in New York city, and is distinctly characterized by its *blue-black, nearly jet-black color*. *B. niger* of Biel, and a species isolated by Gorini, produces black colonies on gelatine, but not on agar. We have seen only one culture of this remarkable bacillus, which is, so far as we know, the only bacterium described of a jet-black color.

Morphology.—A peritrichic rod. Size, $.9\mu-1\mu \times .9\mu$. There are no spores, and the organism does not accept the Gram stain. No chains are formed.

Gelatine colony.—A round, thin, smooth, moist colony, at first white in color, but later a very deep blue-black. On *litmus gelatine*, black; in a pit, not acid.

Gelatine stab.—A needle growth and a good surface growth, slightly arborescent at the top, becoming black.

Agar streak.—A moderately thick, smooth, moist growth, which becomes jet-black.

Fermentation tubes.—There is growth in the closed arm in all three sugar bouillons, but no acid is produced, and no gas. An indigo blue scum appears.

Bouillon.—An abundant sediment, a turbidity, and a blue-black pellicle.

Milk.—No change, except the formation of a thick, black scum. Later the milk becomes black.

Potato.—A thick, spreading, moist, very luxuriant growth, of a deep blue-black color; potato discolored.

Grows at 37° and 20° , but not well at 37° . Aerobic.

We have several times isolated from milk bacilli that seem essentially identical with *B. Zenkeri* (Hauser). They have been found in milk from several localities in Connecticut. This essential agreement is indicated by the name we have given it. The culture as studied by us has the following characters:

B. lactis Zenkeri n. s. (Hauser). *A rhizoid or proteus-like bacillus*.

Morphology.—Size, $2\mu-3\mu \times 1\mu$. Frequently in chains. It produces no spores, and does not accept the Gram stain.

Gelatine colony.—A *peculiar rhizoid colony* is formed, with lateral extensions of variable character.

Gelatine stab.—A prominent needle growth and a lobate or polypiform surface.

Agar streak.—A thick, luxuriant, white growth, with radiating fibres from a ragged edge. Its surface is dull. Sometimes viscous.

Fermentation tubes.—No acidity, gas, nor growth in closed arm in any sugar bouillon.

Bouillon.—A sediment, but no pellicle, and usually no turbidity.

Milk.—Unchanged, except for a slight alkalinity. Some specimens, however, show a slight sliminess.

Potato.—A moderately thick, dirty white or brown growth, which is apt to be rough and dry. Sometimes it is yellow, and the potato is discolored.

Grows both at 20° and at 37°, but better at 37°. Aerobic.

One culture of this organism which we have found, spread over the surface of a gelatine stab as a thick felt, but on potato it produced no growth. The characteristic rhizoid colony, however, leads us to place it here.

B. lactis Colchesterii n. s. A yellow, rhizoid, peritrichic bacillus.

Morphology.—Size, $1\mu-2\mu \times .7\mu-.9\mu$, forming short chains. It accepts the Gram stain, and has an evident capsule, but produces no spores.

Gelatine colony.—A rhizoid colony is produced that looks exactly like a mold.

Gelatine stab.—There is a needle growth and a surface growth.

Agar streak.—Mold-like colonies are formed which extend under the surface of the agar. The growth is luxuriant and of a yellow color. Frequently with an iridescence.

Fermentation tubes.—No acidity, gas, nor growth in closed arm in any sugar bouillon.

Bouillon.—An abundant sediment, a slight turbidity, but no pellicle.

Milk.—No action on milk.

Potato.—A thin, yellow growth, not widely spreading.

Grows at both 20° and 37°. Aerobic.

Only a single culture of this organism has been found in milk, from Colchester, Conn. It is readily distinguished from other bacilli by its mold-like colony, and its yellow color.

B. lactis nebulus n. s. A smoky bacillus.

Morphology.—A very small rod. Size, $.8\mu \times .3\mu$. It forms chains, is actively motile, produces no spores, and does not accept Gram stain.

Gelatine colony.—A thick, contoured, smooth colony of a yellow color. On litmus gelatine a plain white colony is formed, not characteristic.

Gelatine stab.—An abundant needle growth and a transparent surface growth.

Agar streak.—A luxuriant, rather thick, smooth growth, opaque, white. The agar shows a smoky fluorescence.

Fermentation tubes.—Neither acidity, gas, nor growth in closed arm in any sugar bouillon.

Bouillon.—An abundant, amorphous sediment, and a slight turbidity, with a pellicle on the surface.

Milk.—No action.

Potato.—A thin, scanty, white growth.

Grows better at 20° than at 37°. Aerobic.

Two cultures have been found which we have carefully studied and regarded as probably the same. One of them was isolated in 1895 and the other in 1903. The former did not produce the smoky fluorescence in agar, nor did it produce a pellicle on bouillon. In other respects they were the same.

II. Acid in Dextrose or other Sugars.

B. lactis citreus n. s. *A yellow, non-liquefying peritrich.*

Morphology.—Our observations were incomplete when the culture was lost. It is a peritrichic (?) rod, $.8\mu \times .5\mu$, which forms no chains or spores.

Gelatine colony.—A white, opaque colony, that later becomes yellow, 2 mm. in diameter.

Gelatine stab.—A needle growth and a lemon-yellow surface growth; umbilicate.

Agar streak.—A luxuriant, lemon-yellow growth, smooth, moist.

Fermentation tubes.—Probably acid without gas.

Bouillon.—A sediment, a turbidity, and a pellicle, the latter sinking to the bottom.

Milk.—Becomes acid and curdles into a hard curd, with a layer of liquid on top.

Potato.—A luxuriant growth which is at first white and then lemon-yellow.

Grows at 20° and 37°. Aerobic.

B. lacto rubifaciens. Gruber. *A red pigment bacillus.* This culture was sent us from Kiel by Weigmann. The culture when received and studied in our laboratory, produced no color except a slight pinkish tint in milk.

Morphology.—An active rod, $2\mu-3\mu \times .7\mu$. No chains are formed; it does not accept Gram stain. Spores are formed, but no capsule.

Gelatine colony.—Thick, contoured, gyrose, white. On *litmus gelatine* it becomes 5 mm. in diameter, is acid and lobed, with gyrose and with a mottled surface.

Gelatine stab.—A good needle growth, villous, with a spreading surface.

Agar streak.—Linear, moderately thick, white.

Fermentation tubes.—All three sugars develop acidity, and show growth in the closed arm, but no gas is produced.

Bouillon.—A flocculent sediment, a turbidity, and a ring-formed pellicle.

Milk.—Rendered acid and curdled into a gelatinous mass at 20°, but not at 37°. When heated the jelly becomes a hard curd, with a jelly-like whey. The milk has an odor of the barn, and a pinkish color.

Potato.—Rather thick, white, luxuriant. Potato discolored.

Grows at both 20° and 37°, though better at 20°. Facultative anaerobic.

B. lactis sulcatus n. s. *A non-gas-producing, acid bacillus, without spores.*

This organism has been found twice at intervals of three years. In one case it came from market milk, and in the other directly from the udder. There were slight differences in the colonies of the two cultures, and one culture failed to curdle milk even when heated. They seem to agree fairly well with a culture from cream described by Severin (Cent. f. bact. II., XI., 1903, p. 202). We name it from its resemblance to *B. sulcatus*.

Morphology.—An active rod. Size, $2\mu-2.5\mu \times .6\mu$. No chains nor spores. It accepts the Gram stain.

Gelatine colony.—A large [6-8 mm.], spreading, white colony, with a rough, irregular, contoured surface, outline indented. On *litmus gelatine* the colony is acid.

Gelatine stab.—A needle growth and a very thin surface growth.

Agar streak.—Thin, linear, white, rather scanty, and not characteristic.

Fermentation tubes.—All three sugar bouillons show acidity and growth in the closed arm, but no gas.

Bouillon.—A flocculent sediment, but no turbidity nor surface pellicle.

Milk.—Becomes acid but does not curdle unless heated. * No other change.

Potato.—Thin, scanty, moist, white.

Grows both at 20° and 37° Facultative anaerobic.

B. aromaticus lactis Grimm, (Cent. f. Bac. u. Par. II., VIII., 584, 1902) seems to belong here.

For peritrichic gas-producing acid bacilli see p. 182.

B. dysenteriae Shiga. This organism, regarded as the cause of some forms of dysentery and some cases of summer complaint, we insert here, although, so far as we know, it has not actually been found in milk. It is strongly suspected, however, that it is sometimes distributed by milk, and we have thought it well to include it in our list. The characters as given below are described by Vedder and Duval.

Morphology.—A peritrichic rod, $1\mu-3\mu$ in length, and very slender. Sometimes it is extremely short, almost a coccus. It produces no chains nor spores. It does not accept the Gram stain.

Gelatine colony.—Practically identical with the colony of *B. coli communis*. A thin, slightly spreading, white colony, which is acid in litmus agar.

Gelatine stab.—There is a needle growth and a slight surface growth which does not spread.

Agar streak.—A luxuriant, uneven, rather thick, cream-white growth, which later shows a feathery edge.

Fermentation tubes.—All sugar bouillons are rendered acid but no gas is produced.

Bouillon.—A sediment and turbidity are formed, and occasionally a thin pellicle; later the liquid is clear.

Milk.—At first acid, but later alkaline. No curdling nor other change.

Potato.—A luxuriant, rough, thick, spreading, yellowish growth.

Grows better at 37° than at 20° . Produces indol, and is pathogenic.

This is very similar to *B. coli*, but grows less rapidly at 37° . They are not easy to separate from each other, special culture methods being necessary

B. lactis fragariae (Weig.). This culture was sent me by Weigmann, labeled *Pseudomonas fragariae*. The culture we have received is a peritrichic bacillus rather than a *Pseudomonas*, and does not produce any peculiar odor in

milk. Whether this is due to a contamination and thus to a loss of the original culture, or whether it has changed its characters, there was no way of determining. The culture which we received shows the following characters:

Morphology.—A bacillus $1.3\mu-1.5\mu \times .7\mu-.9\mu$. It forms no chains nor spores, and does not accept the Gram stain.

Gelatine colony.—A round, thick, smooth, homogeneous, entire colony of a white color. On *litmus gelatine* it is nearly transparent, mottled, .5 mm. in diameter, and gives an odor of ammonia.

Gelatine stab.—A needle growth, and thin surface growth.

Agar streak.—A scanty, thin, smooth, moist, white, growth.

Fermentation tubes.—Acidity is produced in dextrose, but no gas and no closed arm growth. No effect upon other sugar bouillons.

Bouillon.—A flocculent sediment, decided turbidity, and a pellicle.

Milk.—Is rendered alkaline and slightly transparent, but no other change. A slight odor is produced.

Potato.—A scanty, thin, smooth, moist, white growth.

Grows at 20° and at 37° . Aerobic.

THE GENUS *BACILLUS*, PERITRICHIC, LIQUEFYING.

I. Producing Pigment.

B. prodigiosus (Ehrb) Flügge. This well-known organism we have found many times in milk. So far as we have seen, it never produces any trouble in the dairy. Its characters are well known, but we insert them here for completeness' sake.

Morphology.—Size, $5\mu-1\mu \times .5\mu$, with chains and coccoid forms. No spores.

Gelatine colony.—Round, oval, entire, reddish-brown, with translucent borders. Surface irregular and liquefying with production of a red pigment.

Gelatine stab.—Saccate liquefaction, with reddish sediment.

Agar streak.—White, becoming red.

Fermentation tubes.—Glucose is not acid, gas production variable.

Bouillon.—Turbid, a reddish sediment, and a pellicle.

Milk.—Acid coagulated and subsequently digested, with more or less of a pink color.

Potato.—Rose-red, moist, becoming dark-red to purple.

Grows best at $20^{\circ}-25^{\circ}$. Aerobic.

B. butyri rubri, Stadling and Poda. (Milch. Zent. II., p. 97, 1906.) *A red, liquefying bacillus*. This bacillus, recently isolated from red butter by Poda, has been carefully studied by him and is quite similar to *B. prodigiosus*. It differs, however, very decidedly in its gelatine colony and in its power of producing red pigment, which is much less than in *B. prodigiosus*. It produces red spots in butter. Poda has given it the above name and regarded it as a distinct type with the following characters:

Morphology.—A peritrichic rod. Size, $1\mu-1.5\mu \times .7\mu-.8\mu$. It forms chains but no spores nor capsules, and does not accept Gram stain.

Gelatine colony.—Round to oval, brown or yellow colonies, with a central colony in a liquid pit. The colony is opaque and granular, 4 mm. in diameter in 48 hours, and liquefies at the edge; with a cheesy smell.

Gelatine stab.—A needle growth and a shallow liquefying pit, with no color. It then becomes infundibuliform, but no red pigment develops.

Agar streak.—A luxuriant, opaque growth, with a central wine-red streak, and a colorless peripheral zone. Condensation water, dark colored.

Fermentation tubes.—Not given, but probably produces acid and gas.

Bouillon.—A sediment and turbidity. There is no pellicle, but there is a rose-red color near the surface.

Milk.—Made acid, and curdled with a cheesy smell and a rose-red color. It is subsequently digested into a yellow liquid with a cheesy smell.

Potato.—A luxuriant, carmine red growth, the pigment appearing at 37° as well as at 20° .

Grows at 20° and 37° , but the color is not so well developed at the higher temperature.

B. lactis citreus n. s. A lemon-yellow, peritrichic bacillus. This we have found but once, in milk fresh from the udder.

Morphology.—Peritrichic bacillus. Size, $1.5\mu \times .8\mu$. It forms no spores, accepts the Gram stain and has evident capsules. No chains are formed.

Gelatine colony.—A round, convex, smooth, homogeneous, entire, white colony, which at liquefaction shows radiation.

Gelatine stab.—Liquefies, infundibuliform.

Agar streak.—Filiform, flat, smooth colony, lemon-yellow color, luxuriant.

Fermentation tubes.—Lactose is rendered acid, but the other sugars are not acid. Growth in the closed arm shows in all cases, and no gas is produced.

Bouillon.—A sediment and an abundant turbidity, but no pellicle.

Milk.—Becomes acid and is curdled at both 20° and 37° . The milk is subsequently digested with an odor, but no color.

Potato.—Spreads over the potato, thin, lemon-yellow, luxuriant.

Grows both at 20° and 37° . Facultative anaerobic.

B. lactis Harrisonii n. s. A slimy milk, yellow bacillus. Isolated by Harrison and described by him. (Rev. gen. d'Lait, 1906). I have ventured to name it after him.

Morphology.—Somewhat irregular. Size, $.25\mu-.75\mu \times .3\mu-.3\mu$. No chains nor spores, and no capsules. It stains by the Gram method.

Gelatine colony.—Irregular, lobulate, slimy, becoming umbonate. Sinks in a pit 3-7 mm. in diameter, from which the whole colony can be removed by a needle.

Gelatine stab.—Outgrowths from the needle track. In two weeks it sinks into a pit 4 mm. deep, and there is no further change.

Agar streak.—Luxuriant, viscous and dull, at first shiny, later dry; citron yellow; spreading.

Fermentation tubes.—Not described, but apparently no acid and no gas.

Bouillon.—A turbidity, sediment, and a ring pellicle.

Milk.—Rendered alkaline, but neither curdled nor digested. It is turned yellow and becomes slimy.

Potato.—A luxuriant growth, spreading and intensely yellow.

Grows at 20° and at 37°. Aerobic.

B. lactis fluorescens IV. n. s. *A fluorescent, liquefying bacillus*.

Morphology.—Size, 2.5μ – 3.3μ x $.9\mu$ – 1.5μ . Forming chains. It produces central spores, accepts the Gram stain, and shows an evident capsule.

Gelatine colony.—A granular colony with a central nucleus, liquefying almost immediately.

Gelatine stab.—Liquefying rapidly, infundibuliform.

Agar streak.—Filiform, flat, contoured, opaque, yellowish, moist, and later, wrinkled. The agar shows a yellow-green fluorescence.

Fermentation tubes.—All three tubes show growth in closed arm; dextrose alone being acid. No gas produced.

Bouillon.—A flocculent sediment, turbidity, and pellicle. The liquid is yellow at the top.

Milk.—Rendered alkaline, is curdled at 37°, and is subsequently completely digested, both at 20° and 37°. It becomes yellow to orange in color.

Potato.—Spreads completely over the potato, rather thin and yellow, luxuriant.

Grows both at 20° and at 37°. Is facultative aerobic.

Variety A.—Found in milk in Colchester. Differs from the last in the following points. Size, 1.5μ x $.6\mu$. Gram stain is negative. The flagella are extremely long and numerous, and protrude from very thick capsules. Gelatine stab shows a greenish growth, which later becomes yellow. Yellow pigment is not produced on agar or on potato. There are no wrinkles on agar and no growth in the closed arm of fermentation tubes.

The differences between these two varieties are considerable, and are perhaps sufficient to require recognition under separate names. At present, however, we leave them together.

B. lactis niger (Gorini) n. s. *A black liquefying bacillus*. This organism, which was sent us from Geneva by Harding, is almost identical with *Ps. lactis niger* of Gorini. See p. 154. The latter, however, is monotrichic, while this is peritrichic. The other differences are very slight.

Morphology.—Size, 2μ – 3.5μ x $.9\mu$. Long chains are produced. The organism stains with the Gram method, produces no spores and shows no capsules.

Gelatine colony.—Not characteristic. A slow liquefier, forming a pit which is at first clear and then cloudy. No acid is produced upon litmus gelatine.

Gelatine stab.—Begins to liquefy on the first day, infundibuliform. In ten days the gelatine is about $\frac{3}{4}$ liquefied.

Agar streak.—Spreading, thin, smooth, opaque, white, moist, and later wrinkled. Quite luxuriant.

Fermentation tubes.—There is a very slight acidity in all three sugars. No gas and no closed arm growth.

Bouillon.—After three days there is a sediment, a turbidity, and a membranous pellicle.

Milk.—Is rendered alkaline, curdled, and subsequently digested both at 20° and at 37°

Potato.—Spreading, flat, irregular surface or wrinkled, and becoming blue-black.

Grows better at 37°, very little at 20° Aerobic.

B. lactis arborescens II. *An arborescent spore-producing bacillus*. This organism was found originally in 1899 and a second culture was isolated from dust in 1904. The original culture was kept in the laboratory for four years and then tested again. It was found to agree with the original description except that it no longer produced the arborescent growth in gelatine.

Morphology.—Size, 1.5 μ –4 μ x .8 μ . No chains nor capsules; spores, sometimes in the ends of the rods and sometimes in the center; Gram stain positive.

Gelatine colony.—Filamentous, 1 cm. in diameter, of radiating knotted fibers and sometimes showing secondary radiations from the knots; slowly liquefying. In *litmus gelatine* deep dry pits with radiating filaments.

Gelatine stab.—A dry pit, which later liquefies. Needle growth arborescent.

Agar streak.—Very thin, scarcely visible, covering the whole surface, white.

Fermentation tubes.—No acidity, gas, or closed arm growth in any sugar bouillon.

Bouillon.—A flocculent sediment, a slight turbidity and a tough scum.

Milk.—No action.

Potato.—Thin, not luxuriant, diffuse gray or brown.

Grows at 20° and 37°. Aerobic.

B. lactis rhizoides n. s. *A rhizoid, non-arborescent bacillus*.

Morphology.—Size, 3 μ x .8 μ . No chains, no spores, no capsules, Gram stain negative.

Gelatine colony.—A myceloid colony, slowly liquefying. On *litmus gelatine* the colony is proteus like.

Gelatine stab.—A needle growth and a saccate liquefaction, beginning in three or four days.

Agar streak.—Spreading, smooth, thin, transparent, white, luxuriant, moist.

Fermentation tubes.—No acidity, gas, nor closed arm growth in any sugar bouillon.

Bouillon.—A slight sediment and turbidity, but no pellicle.

Milk.—No action.

Potato.—A very scanty white growth, with discolored potato.

Grows at 20° and very slightly at 37°. Facultative anaerobic.

B. lactis mycoides n. s. *Rhizoid, spore-bearing bacilli*. This type of bacillus we have found very frequently. There are some variations in the characters of the various cultures. The one described below, which we take as a type, was from Dr. Maher, and has been used by him in numerous inoculation experiments against certain diseases. The variations which we have found in other cultures are indicated in brackets.

Morphology.—Size, $1\mu-4\mu \times .6\mu-1.2\mu$. Long chains produced, spores present, no capsule, and Gram stain positive.

Gelatine colony.—A small burr like, rhizoid colony, soon liquefying and forming a pit with a nucleus. [The rhizoid character is not always found, and the liquefaction may be slow. The color is sometimes yellowish; it sometimes shows tangled threads like anthrax.]

Gelatine stab.—An arborescent needle and a crateriform liquefaction. [Infundibuliform without arborescence.]

Agar streak.—Luxuriant, dull, wrinkled, white, tough. [Yellowish.]

Fermentation tubes.—No acidity, gas, nor closed arm growth in any sugar bouillon.

Bouillon.—A sediment, turbidity, and a pellicle.

Milk.—Rendered alkaline and curdled after a few days. Digested into an amber colored or yellowish liquid.

Potato.—Luxuriant, velvety, dry, wrinkled, white.

Grows at 20° and 37°. Aerobic or facultative anaerobic. [A ground-glass like appearance, with liquid under the folds. Sometimes of a pasty consistency.]

B. subtilis. Is extremely common in milk, though never in great numbers. While it will grow in sterilized milk, it does not usually thrive in milk containing lactic bacteria. In old milk it is, therefore, usually overgrown by the lactic organisms. We describe a typical culture and two varieties below.

B. subtilis (Ehrb.).

Morphology.—Size, $1.5\mu-4\mu \times .6\mu-1.5\mu$, commonly forming chains. Spores are produced in abundance. The Gram stain is positive, and there is no capsule. The size is somewhat variable in different cultures.

Gelatine colony.—A rapidly liquefying colony, with irregularly distributed granular masses. The appearance of these masses is striking but not uniform, and hence not characteristic.

Gelatine stab.—Begins to liquefy in one day, crateriform and later stratiform.

Agar streak.—A filiform or spreading, raised, contoured, cretaceous, white growth, frequently wrinkled and dull. In some cultures it is quite dry.

Fermentation tubes.—No acidity, gas, nor closed arm growth, in our cultures, although Chester says dextrose is made acid.

Bouillon.—A sediment, turbidity, and pellicle.

Milk.—Rendered alkaline, curdled and digested at both 20° and 37°

Potato.—A spreading, raised, gray, dry, or moist, luxuriant, wrinkled growth. Some cultures are yellowish and thin.

Grows well at 20° and 37° Aerobic.

Variety A.—Differs in the following points from the type. Size, $2.5\mu \times .5\mu$. Gram stain negative; colony with a radiate pit which is later gyrose; agar streak shows no wrinkling; potato discolored, a bluish-black.

Variety B.—Size, $3.5\mu \times .9\mu$. Gram is negative; liquefaction is so slow that it hardly occurs on gelatine plate, the colony being round, smooth, raised, entire, translucent, yellowish; a slow infundibuliform liquefaction; acid is produced in dextrose and saccharose, and a closed arm growth in lactose and saccharose; on potato the growth is yellow.

Here belong several of the forms of *Tythothrix* of Duclaux, (*turgidus*, *filiformis*, *urophalum*,) and also *B. Bernensis*, found in Emmenthaller cheese.

At this place should be included *Ty. vergula* of Duclaux, found in cheese, and *B. mesentericus* of Flügge, neither of which is sufficiently described to be clearly identified, as equivalent to any of the organisms which we have already described.

B. lactis Cromwellii n. s. Producing a slimy jelly on potato. This has been found but once, but its peculiar potato growth demands special recognition.

Morphology.—Size, 1μ – $.6\mu$. Forming chains. Spores are developed and also a capsule. Flagella not definitely made out but probably peritrichic.

Gelatine colony.—An opaque colony, in a pit, at first somewhat lobate and then breaking into opaque granules as the liquefaction increases.

Gelatine stab.—At first a dry crateriform pit, with later a liquefaction and a scum.

Agar streak.—Luxuriant, opaque, white, with a thin edge; the whole subsequently becoming yellow.

Bouillon.—A sediment, turbidity, and a pellicle, with a tinge of reddish or brown color.

Milk.—Rendered alkaline, curdled, and digested at both 20° and 37°; may digest without curdling. The milk becomes nearly transparent in 12 days. A yellow scum sometimes forms, but soon sinks to the bottom.

Potato.—A moist, slimy, jelly develops all over the potato, extremely profuse. White or yellowish-brown color.

Grows at both 20° and 37°. Aerobic.

B. janthinus (Zopf.), *violaceus* (Macé). *A violet bacillus*. We have never found this, but it is said to occur in milk occasionally. Its description is not complete, as follows:

Morphology.—Size, 2μ – $5\mu \times .4\mu$ – $.5\mu$. It forms chains and spores and stains by the Gram method. Flagella not described.

Gelatine colony.—A rapid liquefier with a membrane on the surface, which assumes a violet tinge in some cases.

Gelatine stab.—A rapid liquefier, forming a cloudy liquid and a pellicle; violet in color.

Agar streak.—A luxuriant growth, at first white and then violet; moist, wrinkled.

Fermentation tubes.—Not given; probably no acid or gas.

Bouillon.—A turbidity and a slight pellicle; the bouillon becomes violet.

Milk.—The reaction is unchanged, or slightly acid. There is no curdling, but there forms a violet surface layer.

Potato.—The original needle track is violet, but a dark brown growth covers the whole surface of the potato. Luxuriant.

III. No pigment and no acid in dextrose or other sugars.

B. lactis circulans I. and II. White circulating bacilli. These two varieties have not been found by us since their original isolation in 1895 as described previously. The two cultures, found at different times, differ from each other in the points indicated in brackets, which refer to No. II. Both of them showed the circulation which is characteristic of this type. Their characters are as follows:

Morphology.—Size, $1.5\mu \times 6\mu$. Forming chains.

Gelatine colony.—A protruding bead or in a dry pit; then liquefying and showing a circulation in the liquid.

Gelatine stab.—Liquefaction slow; a narrow funnel is formed with a dry pit above, and a rotating axis in the center of the funnel below. [Rotating axis absent.]

Agar streak.—Luxuriant, thick, yellowish.

Bouillon.—A sediment, turbidity, and pellicle, but the liquid clears up after six weeks.

Milk.—Slightly alkaline or unchanged in reaction. Digests without [with] curdling in 25 days, into a cloudy liquid with a sediment.

Potato.—A rather scanty, thin, watery growth, white. [Reddish brown.] Potato sometimes discolored and sometimes not.

Grows both at 20° and 37° Aerobic.

B. aerolactis n. s. *A non-acid, gas-producing bacillus*. This has been isolated several times from milk. In one case it was from a sample of old milk that developed a pleasant, fruity flavor. This culture produced in milk, however, a strong odor of decay. There are slight variations in the different cultures which we have isolated, but we regard them as identical. Some of the variations are indicated by brackets.

Morphology.—Size, $1\mu-1.2\mu \times .4\mu-.8\mu$. No chains have been found. Spores are produced, sometimes at the middle and sometimes at the end. There is frequently a capsule and the Gram stain is negative. [Capsule wanting.]

Gelatine colony.—A rapidly liquefying colony, cloudy and sometimes showing a nucleus, but not characteristic.

Gelatine stab.—Begins to liquefy in one day, infundibuliform; complete in 8 to 10 days.

Agar streak.—Luxuriant, capitate, smooth or contoured, gray, moist, commonly viscous.

Fermentation tubes.—Grows in the closed arm; produces gas in dextrose and saccharose and in some cases in lactose. No acidity is developed. [One culture shows gas in saccharose only.]

Bouillon.—A sediment, a strong turbidity, and a ring-formed pellicle.

Milk.—Either unchanged in reaction, or rendered slightly alkaline; is curdled both at 20° and 37°; is always digested more or less completely and always shows the presence of gas bubbles. The odor is variable, in some cases being that of decay and in others that of cheese.

Potato.—Spreading, raised, contoured, gray, moist, luxuriant, with a discolored potato.

Grows at 20° and abundantly at 37° Facultative anaerobic.

We have isolated this organism from milk from several sources. It is quite similar to *B. megatherium* of Du Bary.

B. lactis tetragenesis n. s. *A rhizoid liquefying bacillus*.—The original culture of this organism was sent me by Weigmann and isolated from cheese. The characteristics below were determined by us from Weigmann's culture. Later we found apparently the same organism in milk here. The points where our new culture differed from that of Weigmann are indicated in brackets.

Morphology.—A large rod, not forming chains. Size, $3\mu \times .7\mu$ [$2.5\mu \times 1.4\mu$]. No spores, no Gram stain, but a capsule is evident.

Gelatine colony.—A rhizoid or proteus-like colony, of large size, slowly liquefying. It is not acid in *litmus gelatine*.

Gelatine stab.—Begins to liquefy in one day [three days], liquefaction stratiform. At first an arborescent needle track. [Not arborescent.]

Agar streak.—A filiform, flat or raised, smooth, gray growth, moderately luxuriant, moist.

Fermentation tubes.—No action upon any sugar bouillon.

Bouillon.—A slight turbidity and a tenacious pellicle is formed, but no sediment. [Sediment without pellicle.]

Milk.—No change in reaction, or a slight alkalinity. Milk is curdled at the bottom, at 37°, and is subsequently slightly digested, with a faint pinkish color. [Yellowish.]

Potato.—A luxuriant, wide spreading, thin, contoured growth, of a gray color, with a discolored potato. [Growth is slight.]

Grows at 20° and 37° [Growth at 37° slight.] Aerobic.

B. lactis distortus n. s. This resembles *Ty. distortus* of Duclaux.

Morphology.—Size, $3\mu \times .7\mu$. Chains are formed, but no spores or capsules. The Gram stain is positive.

Gelatine colony.—A slowly liquefying colony, with a uniformly granular liquid. On *litmus gelatine* it is not acid.

Gelatine stab.—A slow, stratiform, liquefaction, with a cloudy liquid and a scum.

Agar streak.—Filiform, raised, smooth, translucent, white, moist, not luxuriant.

Fermentation tubes.—No acidity, gas, nor closed arm growth in any sugar bouillon.

Bouillon.—A turbidity and a thick, wrinkled scum, but no sediment.

Milk.—No change in reaction, or amphoteric; the milk is curdled slowly and is later digested.

Potato.—A luxuriant, white, much folded, dry or pasty growth.

Grows at 20° and 37°. Aerobic.

B. lactis gelatinosus n. s. *A bacillus producing jelly-like milk*. One culture of this organism was isolated from milk here and a second was sept from Geneva. Where the latter organism differs from ours the differences are indicated by brackets.

Morphology.—Size, $.8\mu \times .6\mu$. [$1.8\mu \times .6\mu$] No chains, no spores, no capsule, and Gram stain negative.

Gelatine colony.—A round, smooth, white colony, which slowly liquefies [rapid liquefaction], not characteristic.

Gelatine stab.—Slow liquefier, cratiform, white. [Rapid liquefier.]

Agar streak.—A filiform, smooth, raised, brownish or cream-colored growth, luxuriant, moist.

Fermentation tubes.—No acidity, gas, nor closed arm growth in any bouillon. [Slight acid reaction in lactose and some closed arm growth in saccharose.]

Bouillon.—A granular sediment, a turbidity and a membranous pellicle.

Milk.—Rendered acid and curdled, and upon digesting becomes a transparent jelly.

Potato.—A moderate growth, raised, smooth, brownish with a slight odor. [Spreading.]

Grows at both 20° and 37°. Aerobic. [Facultative anaerobic.]

B. lactis tenuis n. s. (Ducl.) Several cultures of *non-acid liquefying bacilli*, with no wrinkling, and no rhizoid colonies have been studied. Here would belong *Ty. tenuis*, and *Ty. Scaber* of Duclaux. We describe two varieties from our cultures to which we have given the above names, derived from Duclaux.

Morphology.—A slender rod. Size, $1.2\mu \times .5\mu$. No chains, no spores, no Gram stain.

Gelatine colony.—A rapidly liquefying colony, not characteristic.

Gelatine stab.—An arborescent needle growth and a stratiform, later an infundibuliform, liquefaction, beginning in one day, half liquefied in ten days.

Agar streak.—Luxuriant, umbonate, smooth, gray, moist, iridescent.

Fermentation tubes.—Acid, gas, and closed arm growth in dextrose. Closed arm growth in other bouillons but no acid nor gas.

Bouillon.—A granular sediment, a decided turbidity, and a flocculent pellicle.

Milk.—Rendered alkaline, digested, curdled, and subsequently digested into a gelatinous mass at both 20° and 37°, with an unpleasant odor. Later the odor is of old cheese.

Potato.—A filiform, capitate, smooth, gray growth, not very luxuriant, with potato discolored.

Grows abundantly at 20° and 37° Aerobic. Found in Camembert cheese.

Variety A.

Morphology.—Size, $2\mu-3\mu \times .5\mu-.7\mu$. No chains, no spores, a very evident capsule and Gram stain positive.

Gelatine colony.—A very slow liquefier, not characteristic.

Gelatine stab.—An arborescent needle growth, with crateriform liquefaction, beginning in one day.

Agar streak.—Filiform, flat, contoured, transparent, gray, luxuriant, moist.

Fermentation tubes.—No gas, acidity, nor closed arm growth in any bouillon. Saccharose is rendered acid. No other effect in any sugar bouillon.

Bouillon.—A slight sediment is produced, but no other effect.

Milk.—No action on milk.

Potato.—Spreading, thin, smooth, translucent, luxuriant, gray, moist. Potato discolored.

Grows at 20° and moderately at 37°. Aerobic.

B. lactis plicatus n. s. A spore bearing, liquefying bacillus with a folded scum. Found in Camembert cheese.

Morphology.—Size, $2\mu \times .8\mu-1.2\mu$. No chains, central spores produced, Gram stain positive, no capsules.

Gelatine colony.—Rapidly liquefying into a non-characteristic cloudy pit.

Gelatine stab.—At first arborescent; liquefies in one day, stratiform; about one third of the gelatine liquefies in ten days, with a white folded scum.

Agar streak.—Nodose, capitate, rugose, opaque, white, luxuriant, moist and wrinkled.

Fermentation tubes.—Dextrose is rendered acid; no gas and no closed arm growth in any sugar bouillon.

Bouillon.—A granular sediment, a turbidity, and a pellicle forms, which subsequently sinks.

Milk.—Becomes alkaline and curdles at 37°, not at 20°. Is subsequently digested.

Potato.—Luxuriant, diffused, thin, smooth, of an orange-white color. Potato is discolored.

Grows at 20° and 37°, but better at 37°. Aerobic.

B. lactis amberis n. s. *A brown fluorescent liquefying bacillus.* Found only in milk from Colchester.

Morphology.—Size, $1\mu-1.5\mu \times 3\mu-4\mu$. No chains, central spores, Gram stain is positive and a capsule is produced.

Gelatine colony.—A non-characteristic, rapidly liquefying colony.

Gelatine stab.—Liquefaction begins in one day, infundibuliform; complete in six days.

Agar streak.—Linear, raised, rugose, translucent, yellowish, moist, wrinkled. The agar with an amber colored or yellow-green fluorescence.

Fermentation tubes.—Dextrose is acid, the other two sugars alkaline. No gas, but a very slight growth in closed arm.

Bouillon.—A flocculent sediment, a slight turbidity, but no pellicle.

Milk.—No change in reaction. Curdled in one to two days, and digested both at 20° and 37° . Digestion is nearly complete in three weeks.

Potato.—Linear, raised, smooth, yellowish, dry, luxuriant; potato discolored. Grows at 20° and 37° . Facultative anaerobic.

The last two organisms are very closely related and may be identical. The amber colored fluorescence and the yellow pigment lead us to separate the two.

B. mesentericus fuscus n. s. *A brown, spore-bearing acid liquefier.* Found only once in milk, directly from the udder.

Morphology.—A peritrichic bacillus which does not form chains. Size, $1.2\mu-1.5\mu \times .4\mu-.6\mu$. It stains by Gram method and produces central spores.

Gelatine colony.—A round, convex, smooth, entire colony, of a brownish-red color. On litmus gelatine it is acid.

Gelatine stab.—A slow liquefaction, napiform.

Agar streak.—Spreading, thin, rugose, translucent, gray, luxuriant, dull, and wrinkled.

Fermentation tubes.—Dextrose and saccharose rendered acid, but no gas nor closed arm growth in any sugar bouillon.

Bouillon.—A slight turbidity is produced, although sometimes even this is wanting. No sediment, nor pellicle.

Milk.—Rendered slightly alkaline, and curdles in six days at 37° . The milk is digested, although this power of digestion was lost after long cultivation.

Potato.—A luxuriant, spreading, thin, rugose growth, of a brown-red color, wrinkled.

Grows better at 37° than at 20° . Aerobic.

B. lactis vinus n. s. *A spore-bearing, acid liquefier.* This has been found but once, in milk in Middletown.

Morphology.— $1\mu-1.2\mu \times .6\mu$. No chains. Spores are produced, no capsules, and Gram stain is negative.

Gelatine colony.—A very rapidly liquefying colony with a slight granular liquid. Not characteristic.

Gelatine stab.—A needle growth, liquefying in one day, infundibuliform; complete in eight days.

Agar streak.—Scanty, linear, thin, smooth, opalescent, gray, moist.

Fermentation tubes.—All three sugar bouillons show acidity and growth in the closed arm, but no gas..

Bouillon.—An amorphous sediment and a slight turbidity, but no pellicle.

Milk.—Becomes acid, and curdles at 20° and 37°, and subsequently digests, giving a clear whey, with a vinous odor.

Potato.—Scanty, linear, thin, smooth. Potato not discolored.

Grows at 20° and 37°, but better at 20°. Facultative anaerobic.

Close to this belong the butyric acid organisms of Prazmowski, Hueppe and Botkin. We have not studied them.

B. lactis Pruchii n. s. *A slimy milk, peritrichic bacillus.* This peculiar bacterium was sent from Geneva. Its remarkable involution forms, and its other unique characters clearly distinguish it, although it was lost before we had quite completed our work upon it.

Morphology.—A spore-producing, peritrichic bacillus, with no capsule. It does not accept the Gram stain. Involution forms, curved, club shaped, and showing other irregularities, are common.

Gelatine colony.—A rapidly liquefying pit, not characteristic.

Gelatine stab.—Liquefies in one day, stratiform, with a turbid liquid, and a reddish-yellow sediment.

Agar streak.—Round, flat, smooth, opaque, white, luxuriant and viscous. No fluorescence is seen, although milk is turned green.

Fermentation tubes.—Dextrose bouillon is rendered acid. No gas and no closed arm growth in any bouillon.

Bouillon.—A viscous sediment, a turbidity, and a flocculent pellicle.

Milk.—Is rendered acid, is curdled and digested at both 20° and 37°, with a slight yellowish color. Later it becomes quite yellow and slimy.

Potato.—Spreading, thin, smooth, brownish, luxuriant. Potato discolored.

Grows at both 20° and 37°. Anaerobic.

B. lactis fungiformis n. s. *A white, rhizoid, spore-producing bacillus.* This has been found in fresh milk and stable dust several times. The different cultures show slight variations, indicated within brackets.

Morphology.—Size, $3\mu-3.5\mu \times 1.3\mu$. No chains. Spores are developed and an evident capsule. The Gram stain is positive.

Gelatine colony.—The colony throws out fibers like a mold, but after two days this character disappears, the colonies disintegrating into a liquefying pit. On *litmus gelatine* this character is not evident.

Gelatine stab. Liquefaction begins in two days, but never becomes complete. Infundibuliform.

Agar streak. Filiform, raised, grumose, translucent, porcelain white, dull [wrinkled].

Fermentation tubes.—Dextrose is acid, the other two sugar bouillons alkaline. Growth in closed arm in all cases, and no gas. [Dextrose and saccharose alkaline; lactose acid.]

Bouillon.—A sediment, a granular pellicle, and no turbidity. [Turbidity.]

Milk.—Becomes alkaline, curdles and digests with a strong odor. [Acid, otherwise as above, except that the milk is brownish.]

Potato.—Luxuriant, rather thick, rough, white.

Grows at 20° and 37°. Facultative anaerobic.

Variety A.—Agrees in all points except that the mold-like colony is not evident, the colony liquefying very rapidly.

II. No Pigment, but Acid in Dextrose and other Sugars.

We have found at least three *liquefying gas producers*. Two of them are quite similar to *B. cloacae*, while the other is closely related to it. The characters as made out by us are as follows:

B. lactis cloacae n. s. *A gas-producing, liquefying bacillus.*

Morphology.—Size, $1\mu-1.3\mu \times .7\mu$. No chains are formed. The organism accepts the Gram stain, and has a capsule, but produces no spores.

Gelatine colony.—A slow liquefier, forming a dense granular pit. In *litmus gelatine* there is no acid.

Gelatine stab.—Begins to liquefy in one day and is completely liquefied in six days. Liquefaction deep infundibuliform.

Agar streak.—Filiform, raised, smooth, cream-white or yellowish, iridescent, luxuriant.

Fermentation tubes.—All three sugars rendered acid and show growth in the closed arm. Gas produced in dextrose and saccharose only.

Bouillon.—A sediment, turbidity, and a pellicle.

Milk.—Rendered acid, curdled, and subsequently digested at 20° but not at 37°, showing a yellow color.

Potato.—A scanty, thin, irregular, white growth.

Grows better at 20° than at 37°. Facultative anaerobic.

B. lactis cloacae A. n. s. This was found in milk in this vicinity, but an almost identical organism was sent us by Weigmann from Kiel, labeled *aerogenes*. It is clearly not that specie, since it liquefies gelatine. Its characters are as follows:

Morphology.—Size, $1.5\mu \times .5\mu-.6\mu$. Chains are formed in bouillon. It produces no spores nor capsules, and does not accept the Gram stain.

Gelatine colony.—Round, raised, smooth, grumose, with wavy edge, gray-white. On *litmus gelatine* a good sized, white, acid colony.

Gelatine stab.—A rapid liquefier, infundibuliform, and showing an abundance of gas.

Agar streak.—Filiform, flat, smooth, moist, not abundant.

Fermentation tubes.—All three sugars show acidity, gas, and closed arm growth.

Bouillon.—An amorphous sediment, a slight turbidity, and a granular pellicle.

Milk.—Is rendered acid and curdled, but with no visible signs of digestion. It develops a cheesy odor.

Potato.—The growth is very scanty and white; potato discolored.

A second culture of the same differed in showing digestion of the milk and a more luxuriant growth on potato.

Grows at both 20° and 37°. Facultative anaerobic.

Bacillus (Proteus) vulgaris (Hauser). This is not uncommon in milk. The characteristics are as follows:

Morphology.—Size, 1.2 μ –4 μ x .6 μ . Forming long chains. It shows no spores nor capsules, and does not accept Gram stain.

Gelatine colony.—Very characteristic, showing irregular amoeboid processes, the so called "proteus type"

Gelatine stab.—Begins to liquefy in twelve hours, with liquefaction complete in a few days. Saccate.

Agar streak.—Luxuriant, moist, slimy, glistening, translucent.

Fermentation tubes.—Not determined, but dextrose is doubtless acid and gas is produced.

Milk.—Rendered acid and curdles at 20°. Is subsequently digested, becoming yellowish.

Potato.—A luxuriant, yellowish-white and slimy growth.

B. lactis diffusus n. s. A pink *Bacillus*.

Morphology.—A motile rod. Size, 1 μ x .6 μ –.9 μ . No chains.

Gelatine colony.—Diffuse appearing as a faint cloud made up of microscopic colonies. To the naked eye it appears like a mold; 3mm. in diameter, then liquefying.

Gelatine stab.—A napiform liquefaction, with a cloudy pink liquid. Below the surface it is orange-red.

Agar streak.—A luxuriant, pink, moist, smooth growth.

Fermentation tubes.—Probably acid without gas.

Bouillon.—A sediment and a turbidity but no pellicle. The sediment is red.

Milk.—Becomes acid and curdles after several days. No other change.

Potato.—A luxuriant, bright pink growth. No discoloration.

Grows at 20° and 37°. Aerobic.

B. lactis cochleatus n. s. A non-gas-producing, peritrichic bacillus, without spores. This has been observed twice. One culture was sent us from Geneva by Harding and a second was found in milk in Middletown. The following description is from the Geneva culture. The points where our own differ from it are indicated by brackets.

Morphology.—Size, $1.8\mu-3\mu \times .7\mu-.9\mu$. No chains, no spores, no capsules. Gram stain positive. [Negative.]

Gelatine colony.—A curiously¹lobed colony, cochleate, rather slowly liquefying. *Litmus gelatine* is not acid. The colony is quite characteristic. [A simple lobed, slowly liquefying colony, not cochleate.]

Gelatine stab.—Begins to liquefy in three days, stratiform.

Agar streak.—Linear or spreading, thin, moist, opaque, white or gray, not luxuriant. [Yellowish, luxuriant.]

Fermentation tubes.—Dextrose and saccharose acid, lactose not acid. No gas nor closed arm growth.

Bouillon.—Sediment and turbidity, but no pellicle.

Milk.—Made alkaline and curdled at 37° . Subsequently digested, with a prominent odor.

Potato.—Very scanty, white. [Luxuriant, gray-white, with potato discolored.]

Grows better at 37° than at 20° . Aerobic.

B. lactis Robertii n. s. *A non-gas-producing, acid-forming peritrich*. Found only once.

Morphology.—Size, $1.5\mu \times .5\mu-.8\mu$. No chains, no spores, no capsule, and Gram stain negative.

Gelatine colony.—A dense, white colony, very slowly liquefying. Is not acid on *litmus gelatine*, but forms a pit colony.

Gelatine stab.—A slow liquefier, stratiform, with a clouded liquid.

Agar streak.—Filiform, thin or thick, smooth, contoured, white, luxuriant.

Fermentation tubes.—Dextrose rendered acid, but no other change in any of the sugar bouillons.

Bouillon.—A flocculent sediment, a turbidity, and a ring formed pellicle.

Milk.—Acid and curdled, but without digestion. The milk develops an odor.

Potato.—A luxuriant, thick, moist, white growth. Potato may be discolored.

Grows at 20° and 37° ; better at 20° . Aerobic.

ACID GAS PRODUCERS.

The gas-producing Bacteria and Bacilli constitute, with the exception of the *Bact. lactis acidi* group, the most important dairy organisms. To this group belong many of the most mischievous dairy bacteria. Among them are those that spoil large quantities of cheese by the production of the trouble known as swelling. Sometimes great quantities of cheese are utterly ruined by the development of gas bubbles. The gas bacteria, also, sometimes spoil butter, and they are generally undesirable. Whereas the non-acid-producing bacteria are commonly the dairyman's friends, at least so far as relates to butter and cheese making, the gas-producing bacteria are universally his enemies. In their relation to milk problems they thus form a group by themselves. In their systematic relations they belong to different divisions; some of them are Bacteria,

others are Bacilli. But because of their practical association together in dairy problems, we think it more convenient to consider them all in one group, only referring to them in their logical place in our scheme of classification. The other gas producers, clearly not related to these, are described under the different groups where they belong.

Two somewhat extensive studies of the acid-producing, rod-shaped bacteria have been made besides our own. One of these was by Harrison, who studied fifty-six different cultures (Cent. f. Bac. II., XIV. 359, 1905), and the second by Gruber who carefully studied thirty-seven cultures (Cent. f. Bac. II., XVI. 654, 1906). Although these differ in some slight respects, the general conclusion from their study is in essential agreement with our own. There exists a long series of these forms that show slight variations, which grade into each other in such a way as to make it, at present at least, out of the question to arrange them all in any logical scheme even if it were worth while. Both Harrison and Gruber agree that all of these types may be arranged between two extremes, represented by *Bact. lactis aerogenes* and *B. coli communis*. The essential differences of the extremes are as follows: *B. lactis aerogenes* is non-motile, produces no indol, and has a thick colony on gelatine; *B. coli communis* is motile, produces indol, and a thin colony. But even these primary characters cross each other more or less, especially those of indol production and the type of colony, so that they cannot be regarded as especially characteristic. Indeed, almost any combination of the above characters as well as others can be found among the many cultures that have been studied.

Whether, under these conditions, it is worth while to attempt any classification may well be doubted. Harrison does divide them into a series of groups without, however, implying that his groups have any diagnostic significance. In doing this he recognizes all the variations he can find between the different cultures studied, with a result of making a confusing series of types that clearly have no very great value.

Gruber endeavors further to divide these organisms into groups by their power of fermenting a long series of carbohydrates, and finds it possible to recognize four types. But each of the four is found among both the *aerogenes* and the *coli* type, so that the plan is not particularly useful, quite independent of the fact that no other observer has made test with this long list of sugars.

The sum and substance of the matter is, that there is no means at our command at present by which we may satisfactorily group these types into definite subdivisions. The plan we have adopted is very simple. We recognize, first, the typical *aerogenes* type, under which we have referred to a number of the variations that are known to occur. Then we recognize a type with flagella, but with the typical luxuriant *aerogenes* colony. Third, a type with the typical *coli* characters but monotrichic, and lastly, the typical *coli* form with its peritrichic flagella.

Gruber states that in the *coli* types studied by him, the flagella were always monotrichic and that the name Bacillus should be changed to Pseudomonas. In our own work we have found both the monotrichic type and the peritrichic type. The peritrichic type has appeared far more frequently than the monotrichic type, but the latter has been found quite a number of times. Whether these modifications are the same or really two different types of *coli*, we do not

venture to determine at present. We think the best course to pursue is to recognize the two as different, and we have consequently done so in our classification.

The following classification is based upon the work of Harrison and Gruber, with the aid of such additional data as we have ourselves obtained.

BACT. AEROGENES TYPE.

This organism has appeared in literature under a long list of names. The organisms of the following list appear to be identical with each other and are of this type. *B. pyogenes* (Albarran), *Bact. aceticum* (Baginsky), *Bact. theloideum* (Gassner), *Bact. ubiquitous* (Jordan), *Bact. candicans* (Frankland), *Bact. zurianum* (List), *B. capsulatus* (Smith), *B. chologens* (Stern), *B. acidi laevolactici* (Kozai). It is by no means certain that these organisms are identical, but the descriptions given of them agree so closely as to lead to the conclusion that they are essentially the same. The general characters of this type are as follows:

Bact. lactis aerogenes (Esch.). *The non-motile, acid, gas producers.*

Morphology.—Size, $1.4\mu-5\mu \times 1\mu-1.5\mu$. There are no chains, no spores, and no flagella. A capsule is frequently found and the Gram stain is variable.

Gelatine colony.—Large colonies, 2 mm. in diameter, thick, round, smooth, moist, sometimes viscous. On *litmus gelatine* they are essentially the same, and very strongly acid. They frequently show bubbles of gas under the surface of the gelatine.

Gelatine stab.—A good needle growth, and a thick white surface. If sugar is present, gas bubbles may appear.

Agar streak.—Luxuriant, moist, gray-white, smooth.

Fermentation tubes.—All sugar bouillons show acidity, closed arm growth, and an abundance of gas.

Bouillon.—A turbidity and a sediment, and commonly a pellicle.

Milk.—Becomes strongly acid, and curdles, bubbles of gas being commonly evident. This curd is usually very different in appearance from that produced by *Bact. lactic acidi*.

Potato.—Luxuriant, of a dirty-white to straw color.

Grows at both 20° and 37° , but better at 37° . Aerobic. Indol is not produced.

Twenty-three of Harrison's organisms belong to this general type, and we have ourselves found it extremely common.

Among the varieties of this typical form which appear among the many cultures studied, we make special reference to the following:

Variety A.—Produces indol. Among the different cultures belonging here some produce the typical thick, aerogenes colony, and some the thinner umbonate colony with a smooth center.

Variety B. Produces no indol, but has a thick colony more like that of coli. Among them are some which produce no acidity in saccharose, and do not curdle milk, and others that ferment all of the ordinarily used sugars.

Varieties C. and D. These two differ from common aerogenes bacteria in making *milk bitter*. They also show some other rather peculiar characters, although they plainly belong to the aerogenes type. Variety C was isolated by ourselves from milk in Connecticut and differs from the type in the following points: the litmus gelatine is not acid; a brown fluorescence is produced on agar; dextrose is acid, but no gas is produced; milk is curdled at 37° only, and is very bitter; potato growth is very luxuriant, white, and the potato is discolored.

Variety D, sent us by Harding, produces gas in dextrose only, but all three sugar bouillons become slimy; there is no closed arm growth, no pellicle on bouillon; milk bitter; potato growth is luxuriant and the potato is discolored.

THE COLI COMMUNIS TYPE.

The rest of the gas producers found in milk are flagellates. Among them we recognize some with thick colonies, like *aerogenes*, and others with thinner, spreading colonies, of the *coli* type. In most cases the flagella are peritrichic, but there is one type found by us several times that is monotrichic. Beginning first with the types most similar to *aerogenes*, we recognize the following varieties:

B. coli aerogenes n. s.

Morphology.—Size, $1\mu-3\mu \times 1\mu-1.4\mu$. There are no chains nor spores; Gram stain is negative, and the bacilli are peritrichic.

Gelatine colony.—Prominent, thick, moist, smooth, large, surface colonies.

Gelatine stab.—A needle growth, and a thick, white surface growth.

Agar streak.—Filiform, raised, smooth, opaque, cream-white to brown.

Fermentation tubes.—All three sugars are rendered acid, gas is produced, and there is growth in the closed arm. The amount of gas is not very great.

Bouillon.—A sediment and turbidity, and usually a pellicle.

Milk.—Rendered strongly acid and curdled with gas bubbles.

Potato.—White to straw color, luxuriant.

Grows at both 20° and 37°, better at 37° Aerobic. Indol is produced.

Some twenty of Harrison's organisms would belong here. They differ among themselves very much in their gelatine colonies, and somewhat in their growth on agar. There are also differences in their powers of fermenting different sugars. We do not see in these points any good reasons for separating them. One variety, however, may be properly recognized in accordance with the plan adopted above.

Variety A.—Produces no indol.

Here apparently belongs *B. Schafferi*.

B. coli communis (Esch.) This differs from the last chiefly in producing a thinner colony on gelatine, which is umbonate, and has a granular, lobate edge. Its agar growth is smooth and white. Upon potato it does not usually grow luxuriantly, and indol is produced.

B. coli is very common in milk, as would be expected from the frequency of fecal contamination. The distinction between this and the last type is not sharp, and perhaps should not be recognized as marking separate types. The organisms which we have called *aerocoli* seem to be in a measure intermediate between *aerogenes* and *coli*, a fact further suggested by the study of some cultures sent me by Weigmann and labeled *aerogenes*. These were distinctly motile and peritrichic when studied by us, suggesting that either the presence of flagella has not always been regarded as diagnostic for separating *coli* and *aerogenes*, or that a non-flagellate type may later develop flagelli. The typical characters of this group are as follows:

B. coli communis.

Morphology.—Size, $1\mu-1.6\mu \times .4\mu-1\mu$. No chains, no spores, no capsules. Gram stain negative. Flagella peritrichic.

Gelatine colony.—A rather thin, spreading colony, umbonate, with a smooth center, granular edge, lobate. *Litmus gelatine* shows a dense white colony, which is decidedly acid, and may show gas bubbles.

Gelatine stab.—A filiform needle growth, with a spreading, moderately thick surface growth.

Agar streak.—Filiform, raised, smooth, sometimes lobate, opaque, white, moist.

Fermentation tubes.—All three sugars are rendered acid and develop gas. They also show growth in closed arm. The amount of gas is somewhat variable, and the proportion of hydrogen and carbon dioxide is approximately two to one, but quite variable.

Bouillon.—An abundant turbidity, a sediment, and commonly a ring-formed pellicle.

Milk.—Is rendered acid and curdled. The curdling is not absolutely constant, however. Sometimes the milk does not curdle till after it is boiled. There is never any digestion, but a whey may separate from the curd.

Potato.—A moderate, smooth, gray-white growth, sometimes luxuriant.

Grows both at 20° and 37° , but better at 37° . Aerobic. Indol is produced.

Variety A. Agrees with the type except that it fails to produce gas in saccharose or lactose.

Variety B. This differs from the typical *coli* chiefly in its action on milk, which it turns acid with a viscous coagulum. This is extremely slimy. Its colony is umbonate and tenacious, and can only be removed from the gelatine as a whole. In other respects it agrees with *coli*. Harrison regards it as *aerogenes*, but since it is motile, it must be grouped here.

Ps. coli communis n. s. A gas-producing, non-liquefying *Pseudomonas*. This organism and two sub-varieties were all found in cheese made at this place. They are probably physiological varieties of the same organism. Varieties C and D came from the same colony and are interesting, therefore, as showing a possibility of physiological variations from the same culture.

Morphology.—Size, $1\mu-1.5\mu \times .8\mu-.9\mu$. No spores, no chains, no capsules; Gram stain negative.

Gelatine colony.—A round, thick, smooth, or contoured, auriculate colony, of a gray color. On *litmus gelatine* a large surface colony is produced, strongly acid, and with a gas bubble. Resembles *B. lactis aerogenes*. In some cultures it is thinner and of the coli type.

Gelatine stab.—A filiform growth, and an umbonate surface, with a bluish sheen over the surface.

Agar streak.—Not luxuriant, linear, raised, smooth, gray.

Fermentation tubes.—Acidity, gas, and closed arm growth in all three sugar bouillons.

Bouillon.—A sediment, decided turbidity, and a flocculent pellicle.

Milk.—Rendered acid and curdled at 37°. No digestion, but a prominent odor.

Potato.—Moderately luxuriant, thin, spreading, gray.

Grows better at 20° than at 37° Facultative anaerobic.

Variety A differs from the above in the following points:

Variety A. Size, $1\mu \times .5\mu$. Flagellum very long. Gelatine stab, spreads from needle track below surface. Agar, luxuriant. Milk, curdled.

Variety B. Size, $1.2\mu \times .5\mu$. Gelatine colony, thin, transparent. Not acid on litmus gelatine. No acidity nor gas in lactose or saccharose, but showing closed arm growth. Potato, growth thin, cream-white.

These organisms are in nearly every respect identical with *B. coli communis*, except that they have one flagellum instead of many. This flagellum is very long and characteristic.

CLASSIFICATION OF DAIRY BACTERIA.

Bacteria that are spherical in form,	<i>Coccaceae</i> .
Bacteria that are rod-shaped,	<i>Bacilliaceae</i> .
	<i>Coccaceae</i> .
Coccus forms that do not liquefy gelatine,	108, 188
Coccus forms that liquefy gelatine,	117, 189
Sarcina type,	124
	<i>Bacilliaceae</i> .
Non-flagellate rods (Bacterium),	I.
Flagellate rods (Bacillus)	II.
Bacteria and Bacilli which produce acid and gas, ;	182
I. Bacteria that liquefy gelatine,	136, 190
Bacteria that do not liquefy gelatine,	125, 189
II. Bacilli with flagella over the whole body (Peritrichic),	164
Bacilli with a single polar flagellum (Monotrichic),	150, 191
Bacilli with a tuft of flagella at the end (Lophotrichic),	159, 191

Non-liquefying Cocci.

Liquefying Cocci.

Monotrichic Bacilli=Pseudomonas

Lophotrichic Bacilli.

Non-Liquefying Peritrichic Bacilli.

Liquefying Fermenting Bacula.

TABLE I.—*Coccaceae*.

GROUP NUMBER.	MORPHOLOGY.				CULTURAL FEATURES.										BIO-CHEMICAL.					NAME.											
	Diameter over 1 μ .	Chains.	Spores.	Motility.	Gram stain.	Broth.		Agar.		Gel. Plate.			Gel. Stab.		Po- tato.	Lique- faction.	Milk.														
						Turbidity.	Sediment.	Dull.	Wrinkled.	Chromogenesis.	Round-compact.	Proteus-like.	Rhizoid.	Filamentous.			Curled.	Funnel.	Surface growth.		Needle growth.	Abundant.	Discolored.	Grows at 37°.	Gelatine.	Casein.	Blood serum.	Curdled.	Acid.	Alkaline.	Indol.
212.33307, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M. lactis rosaceus, p. 108
212.33305, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M. lactis citreus, p. 108
212.33306, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M. lactis flavus, p. 109
212.33300, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M. lactis viscosus C, p. 109
212.33300, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M. lactis arborescens, p. 110
212.33300, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	G. versicolor, p. 110
212.22208, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S. lactis fulvus, p. 112
212.22205, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M. lactis aureus, p. 112
212.22206, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S. lactis aureus, p. 113
212.22200, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S. lactis viscosus, p. 113
212.23300, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S. lactis lacticus I. and II., p. 115
212.22200, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M. lactis gigas, p. 116
212.22200, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M. lactis acidus, p. 116
212.22200, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S. lactis lacticus, p. 114
211.33301, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M. lactis erythrogenes, p. 117
211.33307, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M. lactis rubidus, p. 117
211.33306, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M. lactis citronus, p. 117
211.33305, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S. lactis citreus, p. 118
211.33305, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	S. lactis Rogeri, p. 118
211.33305, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M. lactis minutissimus, p. 119
211.33305, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M. lactis aureus A, p. 119
221.23301, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M. lactis albus, p. 120
211.33305, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M. lactis fluorescens, p. 120
211.22205, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M. lactis varians, p. 121
211.22206, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M. lactis giganteus, p. 122
211.??706, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M. lactis rugosus, p. 122
11.22200, -	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M. lactis albidus, p. 123

TABLE 2.—*Bacterium, Non-Liquefying.*

GROUP NUMBER.	MORPHOLOGY.										CULTURAL FEATURES.										BIO-CHEMICAL.					NAME.				
	Diameter over 1 μ .	Chains.	Spores.	Motility.	Gram stain.	Turbidity.	Scum.	Sediment.	Dull.	Wrinkled.	Chromogenicness.	Protus-like.	Rhizoid.	Filamentous.	Curled.	Funnel.	Surface growth.	Needle growth.	Abundant.	Discolored.	Grows at 37°.	Gelatine.	Caseln.	Blood serum.	Curdled.		Acid.	Alkaline.	Indol.	
212.33307, -	+	+	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	Bact. lactis salmonis, p. 125	
212.33306, -	+	+	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	Bact. lactis aureum I., p. 126	
212.33305, -	+	+	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	-	+	Bact. lactis citreum, II., p. 126	
212.33300, -	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	Bact. lactis myceloidium, p. 127
212.33300, -	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	Bact. lactis arborescens, I., p. 127
212.33300, -	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	Bact. lactis viscosum, p. 128
212.33300, -	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	Bact. lactis acid E, p. 128
212.33300, -	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	Bact. lactis Connii, p. 128
212.22207, -	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	Bact. lactis rudensis, p. 129
112.22305, -	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	Bact. lactis catenensis, p. 130
212.22205, -	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	Bact. lactis aureus II., p. 130
212.22200, -	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	Bact. lactis zynxanthum, p. 131
212.22200, -	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	Bact. seifge milch, p. 132
212.22200, -	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	Bact. lactis Isignii, p. 132
212.22200, -	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	Bact. lactis non acidi, p. 132
212.22200, -	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	Bact. lactis ubiquitum, p. 133
212.22200, -	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	Bact. lactis acidi, p. 134
212.22200, -	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	Bact. lactis acid B, p. 135
212.22200, -	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	Bact. lactis acid C, p. 136
212.22200, -	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	Bact. lactis acid D, p. 136
212.22300, -	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	

TABLE 4.—*Monotrichic Rods=Pseudomonas.*[illegible]

* Gas, not acid.

TABLE 5.

Lophotrichic Rods=Bacillus.

GROUP NUMBER.	MORPHOLOGY.										CULTURAL FEATURES.										BIO-CHEMICAL.					NAME.
	Diameter over 1 μ .	Chains.	Spores.	Motility.	Gram Stain.	Broth.		Agar.		Gel. Plate.				Gel. Stab.		Po- tato.	Lique- faction.	Milk.								
						Turbidity.	Scum.	Sediment.	Dull.	Wrinkled.	Chromogenesis.	Round-compact.	Proteus-like.	Rh zoid.	Filamentous.			Curled.	Funnel.	Needle growth.	Surface growth.	Abundant.	Discolored.	Grows at 37°.		
112.23301(3),	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	B. syncyanus, p. 159	
212.33304, -	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	B. lactis olivaceus, p. 160	
212.33300, -	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	B. lactis minutus, p. 160	
212.23300, -	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	B. lactis molecularis, p. 160	
121.11108, -	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	B. lactis Isignii, p. 161	
111.23301, -	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	B. lactis fluorescens I., p. 161	
221.23301, -	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	B. lactis fluorescens II., p. 162	
211.00001, -	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	B. lactis mutissimus, p. 162	
221.00001, -	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	B. lactis fluorescens III., p. 163	
211.23300, -	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	B. lactis moruloides, p. 163	

TABLE 6.
Peritrichic, Non-Liquefying Rods—Bacillus.

GROUP NUMBER.	MORPHOLOGY.				CULTURAL FEATURES.										BIO-CHEMICAL.				NAME.								
	Diameter over 1 μ .	Chains.	Spores.	Motility.	Gram stain.	Broth.			Agar.		Gel. Plate.				Gel. Stab.		Po- tato.	Lique- faction.		Milk.			Indol.				
						Turbidity.	Serum.	Sediment.	Dull.	Wrinkled.	Chromogenesis.	Round-compact.	Proteus-like.	Rhizoid.	Filamentous.	Curdled.				Funnel.	Surface growth.	Needle growth.		Abundant.	Discolored.	Grows at 37°.	Gelatin.
212.3330*	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	B. lactis nigroferous, p. 164
212.33300,	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	B. lactis Zenkeri, p. 164
212.33305,	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	B. lactis Colchesteri, p. 165
212.33301,	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	B. lactis nebulus, p. 165
212.22205,	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	B. lactis citreus, p. 166
122.22200(7),	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	B. lactis rubifaciens, p. 166
222.22200,	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	B. lactis sulcatus, p. 166
212.22200,	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	B. dysenteriae, p. 167
212.23300,	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	B. lactis fragarie, p. 167

TABLE 7—*Peritrichic Liquefying Rods=Bacillus.*

[illegible]

* Black. † Gas but not acid.

TABLE 8.
Bacteria Producing Acid and Gas.

GROUP NUMBER.	MORPHOLOGY.					CULTURAL FEATURES.													BIO-CHEMICAL.					NAME.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																							
	Diameter over 1 μ .	Chains.	Spores.	Motility.	Gram stain.	Broth.		Agar.	Gel. Plate.			Gel. Stab.	Po- tato.	Lique- faction.			Milk.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																														
						Turbidity.	Scum.		Sediment.	Dull.	Wrinkled.			Chromogenesis.	Round-compact.	Proteus-like.		Rhizoid.	Filamentous.	Curled.	Funnel.	Surface growth.	Needle growth.		Abundant.	Discolored.	Grows at 37°.	Gelatine.	Casein.	Blood serum.	Curdled.	Acid.																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																															
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