A STUDY OF GREEN FLUORESCENT BACTERIA FROM WATER

BY

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY FRED WILBUR TANNER

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Introduction.

Bacteriology as a science demands that systematic studies be constantly made in order that old knowledge may be better arranged and that new knowledge may be incorporated into any existing system. If this is not done, bacteriology may not be considered as a separate science. The present status of these studies of bacteria is their arrangement into groups. This paper is concerned with the study of one of these groups.

Historical.

The early investigators in bacteriology were impressed with the newness of the field and devoted more of their time to the isolation of new forms. Classification, at this time, received less attention. Of course, some general arrangement had to exist to serve as a standard for determining whether a new form was distinct from any of those previously mentioned. They examined all sorts of substances and described many shapes and sizes. The conditions for working, also made it difficult to secure accurate data and this is essential in all systematic work. They could not work with pure cultures and their microscopes approached in no way the perfection of the modern instruments. Under such conditions it remained for them as for the pioneers in any field to produce the facts upon which the later workers could build. Consequently the arrangement of bacteria into groups or related masses has been left for the bacteriologists of a later day.

One of the most apparent things which deviated attention from classification studies was the idea of pleomorphism. The

early conception of this theory has been well stated by Fischer.[1] "The pleomorphists maintained that a coccus did not necessarily remain a coccus all its life long, but it could, under certain conditions, stretch itself and assume the shape of a bacillus, that this again could become curved and change into a vibrio, to return again later on to the coccus form that it commenced with. Words like Micrococcus, Bacillus, Vibrio, Spirillum, which we know now to have a definite taxonomic value, were in the eyes of the pleomorphists worthless designations of transient changes of shape." Such a conception of bacterial forms easily prevented or inhibited attempts at the arrangement of the described forms into a classification. This idea probably had its origin in the early decades of biology or botany. In chemistry, it was one of the earliest objects of the science, to change copper into gold.

In bacteriology, this theory has constantly received some attention. Buchner revived it when he reported that he had changed Bacillus subtilis into the bacterium specific for anthrax. More recently Rosenow[2] has reported that he has changed the streptococcus into the pneumococcus. He was able to repeat this several times. The serum reactions for the new strain possessed all of those of the true pneumococcus. In turn, he was able to change these serum reactions. This raises the question with regard to what characters may be depended upon for constancy. Should others find the same results as Rosenow, it will be neces-

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1. Fischer. The structure and Functions of Bacteria. 1900 Oxford.
2. Rosenow, Transmutations within the Streptococcus-pneumococcus.Group. Jour. Inf. Dis. 14 (1914)1.

sary to start over again in classification. The term involution form is now used to designate those changes in cell shape which differ from the normal form.

Anton van Leewenhoek in 1683 was probably one of the first to report different kinds of bacteria. From our present knowledge it is doubtless true that he had a mixture of bacteria and protozoa. This is quite probable since at that time the methods of securing dilutions were very crude. In fact, few attempts were made at this time to secure pure cultures since their necessity in bacterial work was unknown. Van Leewenhoek recognized curved and straight forms and presented drawings with his paper. This may be considered as a classification only in a most general way and even then only to the extent that different shapes were observed. It is not certain that he saw the bacteria by means of his crude apparatus.

O. F. Müller in 1786 studied the bacteria from a zoological standpoint and reported a classification which has no value. He, also, really observed protozoa with possibly a few bacteria.

In 1828, Ehrenburg began his contributions to systematic bacteriology. A resume of this investigator's work is given by Smith.[1] Ehrenburg founded the genus bacterium and mentioned many organisms which belonged to it. This genus he later divided into several species. In 1838, he published his work "Die Infusionsthierchen" and therein described the genus bacterium as being "Die quergetheilten gehören zu den Zittertheirchen (Vibrionen) den langsgetheilten zu den Stabtheirchen (Bacillarien). Ehrenberg gave us several terms which have been retained but to which dif-

1. Smith. Bacteria in Relation to Plant Diseases. Vol. 1. 1905, Washington. Page 166-68.

ferent meanings have been given.

In 1852, Perty published his classification which is merely of historical interest.

Cohn[1] in 1872 put out the next classification and his system was used for some time. He divided bacteria into the following four groups:

I Sphaerobacteria

Species 1. Micrococcus

II Microbacteria

Species 2. Bacterium

III Desmobacteria

Species 3. Bacillus

Species 4. Vibrio

IV Spirobacteria

Species 5. Spirillum (Ehrenberg)

Species 6. Spirochaete (Ehrenberg)

Cohn's classification, of course, is not used now. He did not believe in pleomorphism and made no provision for it in his arrangement. Thus he immediately opposed himself to Klebs, Buchner and Lister. It was about this time that many bacteriologists thought that they were changing one kind into another. Buchner had reported his work on changing B. subtilis into B. anthrax. The publications of Cohn and Koch on spores in B. subtilis and B. anthrax which appeared in 1876, proved the constancy of this characteristic and struck a forceful blow at pleomorphism. The principle objectors to Cohn's system were the medical men,

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1. Untersuchungen über Bakterien. Cohn's Beitr. zur. Biol. der Pflanzen Bd I. Hefte 2 (1872) 127-224.

but after the articles of Cohn and Koch on spore formation, they were more careful. Pasteur and Koch did no work on classification. They were busy with the pathogens and working out the etiology of certain diseases.

Lister[1] was one of the most opposed to Cohn's classification. He transferred a drop of sour milk in which he had observed cocci, to sterile broth and then found that long chains were present. Migula[2] has summed up Lister's attitude toward the classification which Cohn had put out in the following: Für ihn war in Gegenteil mit dieser Untersuchung der Beweis erbracht, dass sich durch Veränderung der ausseren Bedingungen aus einer Form alle möglichen Morphologisch und selbst pyhsiologisch verschiedene Formen entwickeln können.

Zopf[3] in 1884 published his classification. His system was very flexible and any organism could be made to fit into it. He recognized different species but allowed a great variation in form.

De Bary[4] in 1884 divided all bacteria into two classes, those forming endospores and those forming arthrospores. He used spore formation in a different sense than we now use it. Whenever he saw a cell different from the ordinary cell, he called it

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1. Lister: On the Germ Theory of Putrefaction and Other Fermentative Changes Nature, July 10, and 17, 1872.

A Further Contribution to the Natural History of Bacteria and the Germ theorie of Fermentative Changes. Quarterly Journal of Microscopical Science. 1873 Page 380.

2. Migula. Ibid.

3. Die Bakterien I. Aufl. 1884. II. Aufl. 1885.

4. De Bary - Comparative Morphology and Biology of the Fungi, Mycetozoa and Bacteria. 1888.

an arthrospore. His discussion on the formation and germination of spores is quite complete.

Flügge[1] in 1886 published his system which was based on Cohn's classification. He recognized the constancy of characters. His book was used mostly by the medical men. He collected all described forms and worked them into his system.

Eisenberg[2] in 1891 for the first time attempted to use physiological characters in distinguishing bacteria. He divided bacteria into pathogenic and non pathogenic kinds. The pathogens were again divided with regard to their effect on man and animals. No attention was given to spore formation or flagellation.

Migula in 1897 put out his system which is generally used by bacteriologists. His classification is open to many objections but it is as good a working basis as any now available. This system is so well known that no further mention need be made at this time. Many text books in bacteriology contain an outline of Migula's system.

Alfred Fischer[3] in 1897 put out a system of classification and nomenclature in which the root of the generic name expressed the shape of the cell and the ending the arrangement of the cilia as follows:

Baktron = rod	inium = moriotorichous
Kloster = spindle	illum = lopotrichous
Plectron = drum stick	idium = peritrichous

1. Die Mikroarganismen II Aufl. 1886.
2. Bakteriologische Diagnostik III Aufl. 1891.
3. Fischer: Vorlesungen über Bakterien. Translated by Ward. The Structure and Functions of Bacteria. 1900 Oxford at the Clarenden Press.

Using Fischers nomenclature a peritrichous rod would be a "bactronidium". Fischer's system met with little approval and is not used by bacteriologists.

In 1899, Lehmann and Neumann[1] published their classification which was different than any other heretofore published. Their system is largely used by the medical bacteriologists and has the following divisions:

I Coccacae

Streptococcus	Divides into 1 plane
Sarcina	" " 3 "
Micrococcus	" " 2 "

II Bacteriacae

Bacterium	Spores
Bacillus	No spores

III Spirillaceae

Spirilum	Many flagella
Spirochaete	Crooked shape
Vibrio	One flagellum

Their method of classification changes with the genus.

Orla Jensen[2] in 1909 made an attempt to group bacteria according to their natural development. His three divisions are as follows:

I Those bacteria which need no organic nitrogen or organic carbon.

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1. Lehmann and Neumann: Atlas and Principles of Bacteriology. Translated in 1901. Philadelphia.
2. Die Hauptlinien des naturlichen Bakteriensystems. Cent. f. Bakt. II 22 (1909) 305.
   The Main Lines of the Natural Bacteria System and the Bacteriological nomenclature. VII. Intern. Cong.Appl. Chem. Part VI f. Fermentation 176-181
   Jordan. General Bacteriology 1912. p. 107-109.

II Those bacteria which need organic carbon but are able to dispense with organic nitrogen. These forms use carbohydrates, NH_3, N, or NO_3

III Those forms which require both organic nitrogen and carbon. Inorganic substances are of little use.

Jensen has tried to give us a classification which would show the relationship of forms. Reasoning from the synthetic processes Jensen has constructed a family tree for bacteria. His methanomonas is the first bacterium on earth and this he considers the beginning of all life. His method of nomenclature is cumbersome and so far his suggestions are of little more than theoretical interest.

The present day system of classification is of course the result of all the past work. Fischer in his book previously mentioned has pointed out that the very factor contributing mainly to the progress of bacteriology, the number and variety of its students, has been a great hinderance. There are pathologists, chemists, brewers, botanists, etc. manufacturing species. Fischer does not claim that to any one class of investigators should be given the privilege of systematizing the science but that established principles should be followed.

A student reviewing the systems which have been proposed in the classification of bacteria realizes that the problem is a difficult one. The number of systems which have been proposed is an example of this. Smith[1] has said in a discussion of nomenclature and classification that no harm will come to anyone if

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1. Bacteria in Relation to Plant Diseases. Washington 1905 p. 177.

all of these perplexing questions are not settled definitely within his own generation.

Classification of Water Bacteria.

The water bacteriologist has been primarily interested in the presence of certain bacteria which are of sanitary significance. They have done very little work on the other bacteria that may be in the water with which they are dealing but have confined their attention to some ten organisms which indicate pollution. Only after the other branches of bacteriology had become well advanced did the water bacteriologists give serious attention to classification.

This may have been due to the fact that the flora of water is heterogenous and that a few bacteria are probably secured from each material with which the water comes in contact. The environment of a water determines its bacterial flora. A water flowing from an unpolluted water shed will have quite another flora than a water flowing in a river which is made to carry the wastes of domestic and industrial life. Each of these examples cited would have constant flora but one characteristic for the case mentioned.

With the exception of possibly ten bacteria or groups whose presence is supposed to indicate pollution, very little attention is given to the large number of bacteria which are present in a sample of water. More accurate deductions might be made with regard to the sanitary character of a sample of water, could more be known concerning its bacterial flora.

This question has been summed up on page 106 of Standard Methods of water Analysis.[1] "In certain cases the determination of species may play directly a useful part in water analysis. It sometimes happens that the bacteria present in a filtered water are different in character from those found in the raw water and unless the facts are known erroneous inferences may be drawn as to the efficiency of the filters. The determination of particular species is sometimes of importance in proving the identity of water from a particular source. At times also the presence of certain species in water may be indicative of pollution."

Bacterial control of filter operations is very important when the health of the public depends upon this factor. A knowledge of the general flora of the raw water might at certain times be very valuable information. This would tell whether the bacteria found in the treated water were passing through the filters from the raw water or whether they were growing in the underdrains. Certain bacterial standards have been formulated which state that a filtered water must not contain more than 100 bacteria per c.c. The character of bacteria present in filter effluents which contain more than 100 cells per c. c. would be interesting and important in making deductions with regards to the efficiency of the filters.

In order to lay down some definite plan for describing cultures the Committee of the American Public Health Assn. suggested the following scheme. These have been well summed up by

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1. American Public Health Assn. Standard Methods for the Examination of Water and Sewage. New York. 1913.

Savage[1] and the following outline is taken from his book.

I Source and habitat

II Morphological characters

Form. Manner of grouping. Dimensions, staining reactions. Motility. Spores. Capsules. Involution forms.

III Cultural characteristics

Mode of growth in and upon: nutrient broth, gelatin plates, gelatin tubes, agar plates, agar tubes.

IV Biochemical Reactions.

Action upon milk, carbohydrates, nitrates.

Production of indol

Relation to free oxygen

Temperature relations

Pigment formation

Liquefaction of gelatin

Such a scheme as outlined above tends toward greater uniformity in the examination of bacteria in water. It seems to be the general tendency at the present time to arrange bacteria in groups and this is probably the best method of handling the flora of a material like water.

Wyatt Johnston[2] early called attention to the arranging of bacteria into groups. His paper discussed the tests which should be used to separate bacteria into clearly marked groups.

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1. The Bacteriological Examination of Water Supplies. P. Blakiston's Sont& Co., Philadelphia, 1906.

2. On the Grouping of Water Bacteria. Amer. Pub. Heath. Assoc. Proc. 20 445-449. 1894.

He analyzed 235 described organisms and came to the following conclusions:

I Present descriptions of bacteria do not permit grouping. Points of difference are given more attention.

II Too much attention is given to tests which can not be answered by "yes" or "no."

III There is a great difference and lack of uniformity in describing species.

IV For grouping, a single strongly marked character peculiar to a few species is of more value than a number of minor details, but the members of any group should not differ unduly in regard to minor points.

About this time the American Public Health Association[1] realized the necessity of uniform procedures in the study of bacteria and appointed a committee for that purpose. The committee reported in 1897 and recommended the following tests:

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1. Procedures Recommended for the Study of Bacteria with Special Reference to Greater Uniformity in the Description and Differentiation of Species. Being the Report of a Committee of Bacteriologists to the Committee on the Pollution of Water Supplies of the American Public Health Association. Amer. Pub. Health Assoc. Proc. 1898. 60-100.

Necessary Information.

I Source and Habitat

II Morphological characters

Form, dimensions, grouping, staining, capsule, flagella, spores, pleomorphism, involution forms.

III Biological Characters

A. Cultural growth in or on nutrient broth, gelatin plates, gelatin tubes, agar plates, agar tubes, potato, milk, blood serum.

B. Biochemical

Temperature relations, relation to oxygen, acidity of medium, action on gelatin, protein, carbohydrates, nitrates, indol, pigment, odor.

C. Pathogenesis

Optional Tests.

I Morphological

Staining reactions, study of flagella by special stains permanency of characters.

II Physiological

A. Cultural

Growth - litmus gelatin, blood serum, synthetic media, photograph.

B. Biochemical

Maximum, minimum and optimum temperature of growth, growth in special gases, limit of acid and alkali, chemical properties of pigments.

C. Pathogenesis

Inoculation, toxins, etc.

At the end of the report are given charts which may be used to record descriptions of bacteria. This is one of the first attempts in which the organized efforts of a society were brought to bear in systematizing methods for the study of the bacterial cell.

Marshall Ward studied the bacterial flora of the Thames River in 1897 and arranged his bacteria into the following groups:

I Forms identical with Bacterium ureae

II Violet bacteria

III B. fluorescens liquefaciens group

IV B. fluorescens non-liquefaciens

V Typical B. coli communis

VI Series of forms centering around B. protens

VII Like VI except that a yellow pigment is formed

VIII Bacteria between VII and IX. Characters changed

IX Golden yellow liquefying forms were put here

X Yellow non-liquefying forms. Probably related to IX

XI Colorless capsulated forms

XII Yellow capsulated forms

XIII B. prodigiosus type

XIV Rapid liquefyers, colorless, pronounced putrefactive tendencies

XV B. subtilis-like group

XVI Yellow Sarcina-like bacteria

XVII Rose-colored micrococci

XVIII Fungur forms

XIX White micrococcus. (M. candicans)

XX Lemon yellow gelatin liquifiers

XXI Short oval cocii forming a red pigment

This classification of water bacteria is artificial and some of the groups are so closely connected that the question arises whether such a separation should have been made. Ward[1] realized this, for he says "My work goes to show not that species can not be made out but that the limits of the species are, in most cases, far wider than is assumed in descriptions - in other words, that many so-called species in books are merely variated forms, whose characters, as given, are not constant but depend on treatment. How far this is true for any given case will have to be tested on the particular form in question."

Fuller and Johnson[2] in 1899 made an important contribution to systematic bacteriology. They arranged bacteria into groups using some prominent character as a basis for separating their groups. For instance, the first group which they mention is the fluorescent group which is separated from the other groups solely by means of this one character. They realized weaknesses in their arrangements and state that too much importance was given to morphological data and too little to cultural studies. Difficulty was also experienced in separating short rods from cocci. This study has formed the basis for much later work in systematic bacteriology. The thirteen groups are given on the subsequent page.

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1. Fifth Rept. to the Royal Society Water Research Committee Proc. Roy. Soc. London. 61 (1897) 415-423.

2. Fuller and Johnson: On the Differentiation and Classification of Water Bacteria. Jour. Exp. Med. 4 (1899) 609-26.

   Some points on the Differentiation and Classification of Water Bacteria. Amer. Public Assn. Proceedings 25 (1899) 580-6.

I Fluorescent forms

II All red chromogenic forms

III All orange " "

IV All yellow " "

V All violet " "

VI All non-fluorescent, non-chromogenic, gelatin liquefying bacteria, forming protens - like colonies on gelatin.

VII All non-fluorescent, non-chromogenic, gelatin liquefying bacteria, forming subtilis-like colonies on gelatin.

VIII All non-fluorescent, non-chromogenic, non-protens like, non-subtilis-like, bacteria which liquefy gelatin and ferment carbohydrates with production of gas.

IX All bacteria conforming to specified characteristics of VIII, except that fermentation of carbohydrates takes place without formation of gas.

X All bacteria conforming to group VIII except that no fermentation of carbohydrates occurs.

XI All non-fluorescent, non-chromogenic, non-gelatin liquefying bacteria which ferment carbohydrates with the production of gas.

XII All bacteria confirming to specified characteristics of XI except that carbohydrates are fermented without gas.

XIII All bacteria conforming to specified characteristics of Group XI except that no fermentation of carbohydrates occurs.

E. O. Jordan[1] studying 543 cultures isolated from the Missouri, Mississippi and Illinois rivers worked out the following seventeen groups:

I B. coli communis

II B. lactis aerogenes

III B. protens

IV B. enteriditis

V B. fluorescens liquefaciens

VI B. fluorescens non-liquefaciens

VII B. subtilis

VIII Non gas formers, non fluorescent, non spore formers bacilli which liquefy gelatin and acidify milk.

IX Similar to Group VIII, save that milk is rendered alkaline.

X Similar to Group VIII, save that gelatin is liquefied.

XI Similar to Group IX, save that gelatin is not liquefied.

XII Similar to Group XI, save that the reaction of milk is not altered.

XIII Chromogenic bacteria not included above.

XIV Chromogenic staphylococci

XV Non chromogenic staphylococci

XVI Sarcinae

XVII Streptococci

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1. The Kinds of Bacteria Found in River Water. Jour. Hyg. 3 (1903) 1-27

Cornwall[1] in 1914 classified the bacteria in water from a sanitary standpoint. He attempted to include the excretal forms which may be detected in water. He makes the following divisions:

I Glucose fermenters forming white or transparent colonies.

II Those which ferment glucose and form chromogenic or diffusible pigment on agar.

III Bacilli which do not ferment any sugar.

IV Cocci

V Anaerobes

Each of these divisions is split into ten groups depending on the action on glucose, lactose or gelatin. Subgroups are made depending on some other character.

Such a classification made largely on biochemical features is probably not as accurate as one which demanded up to a certain point morphological data.

Species in Bacteriology.

From the above classification it may be easily seen that species in bacteriology has not the same meaning that it has in the other branches of biology or botany. It is a term over the definition of which, there is much discussion. It is often used with different meanings in the same discussion.

The term "species" seems to be a term used merely for convenience. The same may be said of such terms as "plant" and "animal". It is easy to define these in the large sense, but the

1. Classification of Bacteria for the Purpose of Water Analysis. Indian. Jour. Med. Res. 2 (352-368) Original not seen. Chem. Absts. 9 (1915) 228.

difficulty is apparent when one is trying to classify a form which is on the borderline between them.

Linneus was one of the first to arrange plants and animals into groups. Although he was a zoologist, his system of dividing the animal kingdom into divisions for convenience has been adopted by the other branches of science. He introduced the binomial nomenclature which was a distinct advance over the method then used. Linneus[1] divided the entire animal kingdom into classes; the classes into orders; these into genera; and the genera into species". Haeckel[2] has summed up the question when he states that the term species since that time has been the most indispensible and important idea in descriptive natural history, in zoological and botanical classification although there have been endless controversies as to its real meaning. Linneus gave us the term and then sought to define it. He accepted the story of the flood and that a pair of each of the animals were preserved on the ark. Hence his species started with the conception of that story. His species were units of creation. At this time, the investigators were interested in settling whether species were fixed quantities or were they constantly changing.

John Ray[3] early realized the importance of the term and sought to work out a meaning for the term. He did his work about a century previous to that of Darwin. "Ray reached the genetic definition when he said: for plants there is no more

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1. Hertwig. Manual of Zoology. 1912. New York. Holt.
2. Heckel. The Evolution of Man. 1906. New York. Page 23.
3. Hertwig. Ibid.

certain criterion for specific unity than their origin from the seeds of specifically or individually like plants; that is to say, generalized for all organisms; to one and the same species belong individuals which spring from similar ancestors." This is much similar to the idea that Darwin expressed much later.

Lamarck, who worked about 1800, believed that life was gradually changing. The simplest organisms were supposed to have been formed from inert matter; from these, developed the higher forms reaching eventually man. Cuvier's ideas dominated the biological world and consequently the theory of evolution was put off for about seventy-five years.

It remained thus until Charles Darwin published his theory of evolution. Previous to this time, it was believed that species were fixed. There were but a few supporters of the theory of evolution and they thought that species were gradually changing. Those who held to the idea of fixed species did allow, however, that the individuals of a species were not alike "but it was believed that the variations never transcended specific bounds." Darwin[1] gives a summary of this question and states that varieties may not be separated from species except, first, by the discovery of the intermediate linking forms, and secondly, by a certain indefinite amount of difference between them. Morphological similarity and the production of fertile offspring constituted Darwin's conception of species.

It is doubtful whether discussions from botany and

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1. Origin of Species. Summary following Chapter II

zoology with regard to species can not be applied directly to bacteriology. We must reason only by analogy. Among animals and plants, we may work with individual organisms and study the changes produced by experiments. Among bacteria in the past, this has been impossible. We have not been able to work with single organisms but have had to resort to the use of a great number. Reproduction among the higher animals is a sexual process and presents more complications than does the division of a bacterial cell by fission.

We must conclude that a sharp distinction between species and varieties does not exist but that there is an intergrading relation of one to another. Hertwig states that species are varieties which have become constant and varieties are incipient species. It would probably be better for us to assume if we must assume a definition for species that they are varieties which have become more constant. Granting that varieties will ultimately become species, we can easily see how closely some species may be related.

Such a conception of species and varieties makes the classification of bacteria very difficult. A statement which Hertwig makes concerning the idea of classification in zoology may be directly applied to bacteriology: up to the present time, neither by physiological nor by morphological evidence has there been found a criterion which can guide the systematist in deciding whether certain series of forms are to be regarded as good species or as varieties of a species.

Conn[1] in his classificatiom of dairy bacteria states that the "forms which we recognize in the following pages 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."

Chester[2] states "The question of what is a species in bacteriology, I shall not undertake to settle. Bacteria are so subject to morphologic, cultural and pathologic variations that one form appears to merge into another, making distinctions often difficult; and yet a typical form - an ideal species - may exist."

Winslow[3] has summed the present conception of this question up when he states that "for practical purposes, however, we must recognize certain types as "species" or "varieties" even though they may sometimes intergrade."

The Society Chart

Bacteriologists for some time have been working out a systemfor classifying bacteria. The chart of the Society of American Bacteriologists represents the present stage in the development of this effort. A history of the development of the card and the numerical system of recording characters of bacteria has been prepared by Doctor H. A. Harding. Since this may not be available to some of the readers, a brief outline of the chartʼs development will be given here.

Doctor Wyatt Johnson[5] first called attention to the

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1. Classification of Dairy Bacteria. Conn. Eston and Stocking. Am. Rept.Storrs. Ag.Exp.Sta. for 1906 p 107.
2. A Manual of Determinative Bacteriology. New York 1901 p.51
3. Winslow:- The Characterization and Classification of Bacterial Types. Science N.S. 39 (1914) 77-90

(4 and 5, see following page)

possibility of using some such system as the Dewey numerical system in the classification of bacteria. A committee was appointed which in its report (see page 23) suggested the beginnings of such a system.

Conn adopted their suggestions in the classification of dairy bacteria (see page 22 foot note 1.).

Rickards[1] used a decimal system for some time.

The real attempt to use such a group number as our present one was made by Kendall at the Lawrence Experiment Station. This work is reported by Gage[2] and Phelps. The classification of bacteria proposed by Migula was used as the basis for their chart. Doctor Harding has given the complete history of our present chart with specimens of the early ones and a complete discussion may be secured from that source.

The present chart has a group number depending on the determination of ten characteristics. It has been recently described by Doctors Rahn and Harding.[3]

This system of recording the characteristics of bacteria has not met with entire approval on the part of some bacteri-

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4. The constancy of Certain Physiological Characters in the Classification of Bacteria. Tech. Bul. No. 13 - 1910. N.Y. (Geneva) Ag. Exp. Station.

5. On the Grouping of Water Bacteria. Amer. Pub. Health Assoc. Proc. 20 (1895) 445-449.

1. A System of Recording Cultures of Bacteria generogically for Laboratory Purposes. Bost. Health Dept. Am. Rept. 30 (1901) 75-79.

2. On the Classification and Identification of Bacteria with a Description of the Card System in Use at the Lawrence Experiment Sta. for Records of Species. Amer.Pub.Health Assoc. Proc. 28 (1903) 494-505.

3. Die Bemuhungen zur einheitlichen Beschreibung der Bakterien in Amerika. Cent. f. Bakt. Abt. II 42 385-393.

ologists. One of those who was very instrumental in introducing this numerical system of recording bacterial characters has said that it was a thing he regretted. Whether it is or will be of any value may only be told after it has been used to study certain groups. This has been done by a number of workers and their conclusions follow.

Harding, 1910, in the paper mentioned above reports the study of the Pseudomonas campestris group of bacteria. For each strain the same group number was obtained which would indicate that the group number was well fitted to the study. This author believes that the group number may not "carry the separation to a group synonymous with the ordinary conception of species". This statement seems to have been borne out by later studies.

Harding and Prucha[1] used the Society Chart in the study of the flora in cheddar cheese. In their conclusions they state that this method of recording the reaction of cultures is a marked advance in technique. It seems to be their opinion that the shifts in the cheese flora may be traced more accurately. The sum and substance of their opinion of the chart in its application to the study of cheese bacteria is that the chart is a valuable means to an end.

Harding, Morse and Jones[2] in their study of soft rot

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1. Harding and Prucha - The Bacterial Flora of Cheddar Cheese. Tech. Bul. 8 - 1908. New York Agricultural Experiment Station, Geneva.
2. Harding, Morse and Jones - The Bacterial Soft Rot of Certain Vegetables. Tech. Bul. 11 - 1909. New York (Geneva) Agricultural Experiment Station.

organisms use the group number in studying their cultures.. By studying their data it is apparent that the group number is very valuable in studying soft rot organisms. This was a conclusion to that drawn by Harding in 1910 when the same group was studied.

Conn, in his classification of Dairy Bacteria used a group number but does not draw any definite conclusions with regard to its value. He still retained names for his organisms studied.

More recently, H. J. Conn[1] has studied 130 cultures of B. subtilis by means of the society chart. In selecting these cultures one-half of the determinations represented in the group number were excluded because they were implied in the definition of B. subtilis. His conclusions are that different group numbers do not always represent different speices, and that better methods for making these ten determinations represented in the group number should be devised.

Edson and Carpenter used the group number in studying bacteria in maple sap. No statements seem to have been made with regard to the value of the Society Chart in the study of the sap bacteria.

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1. Conn, H. J. A Study of B. subtilis by means of the Classification Card. Science N S. 41 (1915) 618.

A NUMERICAL SYSTEM OF RECORDING THE SALIENT CHARACTERS OF AN ORGANISM (GROUP NUMBER).

100.	Endospores produced
200.	Endospores not produced
10.	Aerobic (strict)
20.	Facultative anaerobic
30.	Anaerobic (strict)
1.	Gelatin liquefied
2.	Gelatin not liquefied
0.1	Acid and gas from dextrose
0.2	Acid without gas from dextrose
0.3	No acid from dextrose
0.4	No growth with dextrose
.01	Acid and gas from lactose
.02	Acid without gas from lactose
.03	No acid from lactose
.04	No growth with lactose
.001	Acid and gas from saccharose
.002	Acid without gas from saccharose
.003	No acid from saccharose
.004	No growth with saccharose
.0001	Nitrates reduced with evolution of gas
.0002	Nitrates not reduced
.0003	Nitrates reduced without gas formation
.00001	Fluorescent
.00002	Violet chromogen
.00003	Blue "
.00004	Green "
.00005	Yellow "
.00006	Orange "
.00007	Red "
.00008	Brown "
.00009	Pink "
.00000	Non-chromogenic
.000001	Diastasic action on potato starch, strong
.000002	Diastasic action on potato starch, feeble
.000003	Diastasic action on potato starch, absent
.0000001	Acid and gas from glycerol
.0000002	Acid without gas from glycerol
.0000003	No acid from glycerol
.0000004	No growth with glycerol

The genus according to the system of Migula is given its proper symbol which precedes the number thus:

Bacillus coli (Esch.)Mig.	becomes	B 222.111102
Bacillus alkaligenes Petr.	"	B 212.333102
Pseudomonas campestris	"	Ps 211.333151
Bacterium suicida (Mig)	"	Bact 222.232203

Bacteriological Groups.

The arrangement of bacteria into "physiological groups has been done for convenience. Quite often the limitations of some of these groups depend on one characteristic. The first five of the groups mentioned in Fuller and Johnson's classification of water bacteria are made on the basis of chromogenesis. The fluorescent group is the first group which they mention. Jordan, in the paper, previously mentioned found it convenient to group bacteria which produced the same color of pigment.

Rahn[1] states "this is convenient and helpful in describing certain characters though the classification and nomenclature has been accomplished as far as possible with strictly morphological characteristics as is the custom in all classification of plants and animals."

Ellis[2] states, "for descriptive purposes such a classification is extremely useful." He gives a list of twelve groups such as: pathogenic, saprophytic chromogenic, zymogenic, photogenic, bacteria, etc.

This group arrangement furnishes a very satisfactory method for handling the many forms of known bacteria. As has been mentioned above, many of these groups are abitrary and their limitations often depend too much upon characteristics which are not fixed.

Many of these groups have been subjected to an intensive

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1. Marshall. Microbiology. 1911. P. Blakiston's Sons & Co., Philadelphia.
2. Ellis. Outlines of Bacteriology 1909. Longman's Green & Co., New York.

study. These will tell whether the groups approach natural units and if they do, the relation and characters of the members. The groups of pathogenic bacteria and those which were of sanitary significance were the first to be studied. There are numerous studies of the Bacillus colon group and numerous studies of the Colon - Typhoid group. More recently, attention has been given to some of the groups which have no sanitary significance but which may be important from the changes which they produce in organic matter.

Members of the fluorescent group are very abundant in water and a study of these bacteria which constitute a "group" is reported in the following pages:

The Fluorescent Group.

This is probably a heterogenous group of bacteria having one property in common - that of excreting a green diffusible pigment capable of causing the medium to fluoresce. Kruse[1] believes that this group has little phylogenetic relationship but rather fall into widely related groups. These special bacteria are probably more conspicuous than the non-fluorescent bacteria and so have been put together into a group. By some bacteriologists, this group has been called a species.

Ruzicka[2] believes that in the fluorescent group we have a closely related group of bacteria with the semi pathogenic organism, B. pyocyaneus on the one end and B. fluorescens liquefaciens on the other. Some of his experiments seem to point to this conclusion.

Jordan[3] attempted to study the conditions under which the diffusible fluorescent pigment was produced. He uses synthetic media and concludes that the presence of phosphorus and sulfur is essential to vigorous fluorescing properties. He states that the fluorescent property is of no benefit to the cell.

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1. The Fluorescent Group of Bacilli. Die Mikroorganismen Flügge. 3rd Edition Vol. II p. 289. 1896

2. Bacillus pyocyaneus and Bac. fluorescens liquefaciens Arch. f. Hyg. 34 ( ) 140; 37 ( ) 1. Experimentelle Studien über die variabilitat wichtigen charaktere des B. pyocyaneus und B. fluorescens liquefaciens. Cent. f. Bakt. 24(1898) 11.

3. The Production of Fluorescent Pigment by Bacteria. Botan Gazette. 27 (1899) 19-36.

In his paper on "The Kinds of Bacteria In River Water" he discusses this group. He studied 58 strains of these bacteria, thirty-three of which liquefied gelatin. The power of liquefying gelatin was found to be closely associated with the ability to coagulate milk. In other characters, the two kinds were much alike. All of the strains studied by Jordan were short motile rods. Attention is called to the apparent identity of many of the various forms described in the literature. The first article by this author mentioned above includes the names of about fifty fluorescent bacteria described in the literature.

A number of questions may be asked with regard to this group. Have we any good reason other that the formation of this green pigment for setting aside these forms into a group? Is this character of sufficient importance for such a purpose? Such questions may be answered only after an analytical study of the group.

Niederkorn[1] studied fifteen strains of fluorescent bacteria in order to find, if possible, better methods for differentiating the various forms. He thinks that there are only two constant forms, Bacillus pyocyaneus (Gessard) and Bacillus fluorescens liquefaciens (Flügge) and that the others are varieties of both of these.

The work of Eisenberg[2] shows that this group of bacteria

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1. Niederkorn: Vergleichende Untersuchung über die verschiedenen Varietäten des Bacillus pyocyaneus und des Bacillus fluorescens liquefaciens. Cent. f. Bakt. Abt. I 27 (1900) 749-50.

2. Untersuchungen über die Variabilitat der Bakterien. Cent. f. Bakt. I 73 (1914) 466-70.

are closely related. He reports some work which he did on seven strains and finds intergrading characters which make it difficult to separate into species and varieties.

Significance of the Fluorescent Group.

Bacteria having such a strong aromatic odor may easily have some significance in the industries. Conn[1] ascribes some cases of rancidity in butter to the presence of fluorescent bacteria. The possibility of this is apparent to anyone who has extracted the pigment with any of the usual reagents. Even in small amounts the odor remains for a long time in the room in which such extractions have been made.

Griffon[2] calls attention to the wide distribution of the fluorescent bacteria in nature and that they are present in many plant diseases, and caused rot in vegetables.

Edson and Carpenter in their work, mentioned elsewhere prove that fluorescent bacteria play an important part in decompositions of maple sap.

Thöni[3] found fluorescent forms in a bacterial study of lemonade.

As has been mentioned before, bacteria are abundant in water supplies. The strains which were studied in this paper

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1. Conn. Practical Dairy Bacteriology 1910 p 217. Orange Judd & Co.

2. Griffon: Sur le role des bacilles fluorescent de Flügge en pathologie vegetale. Cormpt. Rend. Acad. Sci. 149 (1909) 51-53.

3. Thöni - Biologische Studien über Limonaden Cent. f. Bakt. 29 Abt. II (1911) 616-643.

were all taken from water supplies in Illinois. Schmelck[1] found fluorescent bacteria in cold glacial waters. Harrison[2] found these forms in hail. Belli[3] also found them in hail.

Routine analyses of samples of water sent to the Illinois State Water Survey indicate that fluorescent bacteria are rather common in waters from the Illinois and Mississippi rivers. They are especially abundant in the samples from the Illinois river which is rather heavily laden with organic matter. They seem to be abundant where organic matter is undergoing decomposition.

The fluorescent bacteria are resistant to quite varied conditions. It has been noticed that media which has spoiled after supposed sterilization in an autoclav often developed fluorescent bacteria. Some strains have great ability to resist hypochlorite. These forms are often abundant on plates made from water which has been sterilized by this reagent.

Previous Studies on this Group.

The only other study of this group, as such, which has been made was carried out at the Vermont Agricultural Experiment Station by Edson and Carpenter.[4] Since this is the most complete

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1. Schmelck - Steigerung des Bakterien gehalts im Wasser während des Schneeschmelzens. Cent. f. Bakt. 4 (1898) 11-17.

2. Harrison - Bacterial Content of Hailstones. Bot. Gaz. 26 (1898) 211-214.

3. Belli - Chemische, Mikroscopische und bakteriologische Unlersuchingen über den Hagel. Cent. f. Bakt. 8 Abt. 2 (1902) p. 445-446.

4. C. The green Fluorescent Bacteria Occurring in Maple Sap. Bul. 167-1912 Vermont Ag.Exp.Sta. Page 521-599.

study which has been made on this Group and since the Society Chart has been used, it will be rather fully abstracted here. These investigators used forty-two strains of fluorescent bacteria coming from maple sap. Their strains were collected from different orchards in Vermont. This paper contains a complete description of the strains with the following group numbers:

12	strains	pseudomonas	221.2332132
12	"	"	221.2332133
7	"	"	221.2322132
4	"	"	221.2332133
3	"	"	221.2333133
1	"	"	221.2323132
2	"	"	221.2222132
1	"	"	221.2232133
1	"	Bacillus	221.2222232

From these group numbers may be inferred the close relationship of the strains.

All of their strains liqefied gelatin but some of their gelatin tubes had to be kept for a much longer period than that of six weeks recommended on the society chart. Seven strains began to liquefy after three to five months. The time for complete liquefaction was not determined but at the end of eight and one-half months the tubes were melted and poured into plates. These showed growth in pure culture. Their group number is made up on the fact that all strains liquefied gelatin.

They found no spore formers in any of the strains which they studied.

Fluorescent Bacteria in the Literature.

Buchanan[1] has very aptly expressed the condition of the nomenclature of this group. "The naming of bacterial species, genera, and higher groups, indeed the whole subject of bacterial nomenclature, is in a condition which can best be described as chaotic." The members of the fluorescent group have been studied by many bacteriologists and many of them have received different names because they differed in one or few characters. Differences, though slight, were regarded as permanent and a new name was introduced for those forms which did not agree identically with any form described in the literature. Consequently names have little value when used in connection with any member of this group.

The following list of bacteria has been taken from the literature. These bacteria have been described by some bacteriologists as being fluorescent. Some of the references have not been seen in the original. In some cases the name of the investigator who first isolated them may not have been given, but the name which appears with the reference will furnish sufficient data for future investigation should it be desired.

1. Buchanan. Nomenclature of the Coccaceae. Jour. Inf. Dis. 17 (1915) 528-41.

Name of Organism	Authority	Reference
1. Micrococcus chlorinus	Cohn	Beitrage z. Biol. d. Pflanzen 1872
2. Micrococcus diffluens	Schroetter	Kryptogamenflora der Schlesien Pilze 1886
3. Micrococcus fluorescens	Maggiora	Gioc. Soc. Ital. d'Lugen 16
4. Micrococcus lactis erythogenes	Conn	Classification of Dairy Bacteria. Ann. Rep. Conn. Ag. Exp. Sta. 1906.
5. Micrococcus lactis fluorescens	Conn	" " " " " " " "
6. Micrococcus versicolor	Flügge	Die Mikroorganismen 1886
7. Diplococcus fluo. foetidus	Klamann	Allgem. Med. Centralzeitung 1887.

11.	Pseudomonas aeruginosa	Schröter	The Classification and Distribution of the Intestinal Flora in Man. Studies from the Royal Victoria Hospital. Vol. I, No.5 p.67
12.	Ps. alba	Zimmermann	Die Bakterien unserer Trink und Nutz wasser I Reihe '1890) p. 18.
13.	Ps. aurea	Zimmermann	Loc. cit.
14.	Ps. capsulata	Pottien	Zeit. f. Hyg. 22 (1896) 146.
15.	Ps. chlorophaena	Migula	Compendium der Bakteriologischen Wasser-untersuchung (1901) 347.
16.	Ps. colloides	Tartaroff	Die Dorpater Wasserbaklerien Inang. Dissert. (1891) p. 40.
17.	Ps. convexa	Wright	Mem. Nat. Acad. Sci. 7 (1895) 438
18.	Ps. eisenbergii	Eisenberg	Bakt. Diagnostik III Auf. p. 145
19.	Ps. erythrospora	Cohn	Loc. cit.
20.	Ps. fluorescens	Zimmermann	Loc.cit.
21.	Ps. foliacea	Wright	Loc.cit.
22.	Ps. gracilis	Migula	Connpendium, etc.
23.	Ps. incognita	Wright	Loc.cit.
24.	Ps. lactis eurotas	Conn	Loc.cit.
26.	Ps. lactis nigra	Conn	Loc.cit.

27. Ps. lactis viridis	Conn	Loc. cit.
28. Ps. longus	Zimmermann	Loc. cit.
29. Ps. macroselmis	Tartaroff	Loc. cit.
30. Ps. mashekii	Mashek	Uniersuchung der Leitz Meritzer Trinkwasser
31. Ps. mesenterica	Tartaroff	Loc. cit.
32. Ps. ovalis	Ravenel	Mem. Nat. Acad. Sci. 8 (1896)
33. Ps. pseudo typhosa	Lustig	Diagnostik der Bakterium des Wassers. (1893) p. 16.
34. Ps. putrida	Flügge	Loc. cit.
35. Ps. pyocyanea	Gessard	System der Mikroorganismen II (1900)
36. Ps. rugosa	Wright	Loc. cit.
37. Ps. scissa	Frankland	Zeit. f. Hyg. 6 (1889) 399.
38. Ps. striata	Ravenel	Mem. Nat. Acad. Sci. 8 (1896)
39. Ps. syncyanea	Ehrenberg	Gurlt. Hertwig's Mag. f. ges Theirheilk I (1841)
40. Ps. tennis	Zimmermann	Loc. cit.
41. Ps. viridans	Symmers.	Brit. Med. Jour. 1991
42. Ps. viridescens	Ravenel	Loc. cit.
43. Ps. viscosa	Frankland	Loc. cit.
50. Bact. aeruginosum	Schröter	Conn's. Beit. zur Biol. Bd. I 126.

51.	Bact. commune	Miguel	Ann. d. Montsouris 1879
52.	Bact. fluor. alba	Zimmermann	Loc. cit.
53.	Bact. fluor. liq. minutissimus	Unna	Monatsch f. prakt. Dermatologie IX, 57.
54.	Bact. fluor. puditus colloides	Tartaroff	Loc. cit.
55.	Blaugrün fluor. Bakterium	Adametz	43 Mittheil. d. Versuchstation f. brauer u. Mälz Vienne 1888
56.	Bact. immobile	Kruse	Flügge Die Mikroorganismen 1896 p. 294.
57.	Bact. iris	Frick	Vischows Archives 96 (1899) 290
58.	Bact. lactis erythogenes	Conn	Loc. cit.
59.	Bact. lactis fluorescens	Conn	Loc.cit.
60.	Bact. osteophilum	Billet	Bull. Scient. de la France et de la Belgique 1890.
61.	Bact. puditum	Sternberg	Manual of Bact. 1892. 733
62.	Bact. smaragdinus foetidus	Reimann	Würzbourg (1887) 270.
63.	Bact. termo	Vigual	Arch. de Physiol. 1886 p. 71.
70.	B. aquatilis fluor.	Lustig	Arch. f. Hyg. 6 (1889) 391.
71.	B. butyri fluor.	Lafar	Arch. f. Hyg. 13 (1891) 1.
72.	B. caulivaris	Prillieux & Delarois	Compt. Reud. III (1890) 208.

73.	B. chronno aromaticus	Galtier	Compt. Reud. 106
74.	B. cloacae fluor.	Harrocks.	Bact. Exam. Water 1901. p. 142.
75.	B. cyanofluorescens	Zaugmeister	Cent. f. Bakt. 18 (1895) 34.
76.	B. cyanogenus	Ehrenberg	Hueppe - Mitt. a.d. Kaiserl Geo. 2
77.	B. dentalis viridans	Miller	Die Mikroorganismen der Mundhohle 316 (2nd Ed.)
78.	B. fluor. capsulatus	Pottien	Loc. cit.
79.	B. fluor. crassus	Flügge	Loc. cit.
80.	B. fluor. foliaceus	Wright	Mem. Nat. Acad. Sci. 7 (1895)
81.	B. fluor. immobilis	Flügge	Loc. cit.
82.	B. fluor. liquefaciens	Flügge	Loc. cit.
83.	B. fluor. mutabilis	Wright	Loc. cit.
84.	B. fluor. oralis	Ravenel	Loc. cit.
85.	B. fluor. ovalis	Ravenel	Loc. cit.
86.	B. fluor. Schuylkilli-ensis	Wright	Loc. cit.
87.	B. fluor. stercoralis	Horrocks	Loc. cit.
88.	B. fluor. nivalis	Schmolck	Cent. f. Bakt. 4 (1888) 545
89.	B. fluor. minutissimus	Unna	Loc. cit.
90.	B. graveoleus	Bordoni	Fortschr. d. Med. 1886
91.	B. lactis amberis	Conn	Loc. cit.

92.	B. lactis erythrogenes	Harrocks	Loc. cit.
93.	B. lactis fluor.	Conn	Loc. cit.
94.	B. lactis nebulus	Conn	Loc. cit.
95.	B. Lesagei	Conn	Loc. cit.
96.	B. leucaemiae canis	Conn	Baumgartens Jahrb. (1891) 319.
97.	B. lupuliperda	Behreus	Lafar. Tech. Mikologie I 166.
98.	B. melochloros	Winkler	Centr. f. Bakt. 9 (1891) 700.
99.	B. oogenes fluor.	Zärkendorfer	Arch. f. Hyg. 16 (1893) 300.
100.	B. parallelus	Edson & Carpenter	Micro-organisms in Maple Sap. Verm. Ag. Exp. Sta. 1912. 594.
101.	B. protens fluor.	Jager	Zeit. f. Hyg. 12 (1892) 525.
102.	B. pyocyanea	Gessard	Thisi de Paris 1882.
103.	B. smaragdinus	Reimann	Phil. Diss. Wurzburg 1887
104.	B. urinae Jager	Jager	Loc. cit.
105.	B. virescens	Frick	Virchow Archiv. 116 (1889) 292.
106.	B. virescens non liq.	Ravenel	Loc. cit.
107.	B. viridis	Lesage	Arch. de. Physiol 20 (1888) 212
108.	B. viridis pallescens	Frick	Loc. cit.
109.	Bacille de la diarrhee verte	Lesage	Loc. cit.
110.	B. fluor.	Zimmermann	Loc. cit.

Methods of Study.

The Descriptive chart of the Society of American Bacteriologists was used in the study of this group. The group number for each culture was determined under as uniform conditions as possible. A defect with this method is that some of the determinations which are called for are not sufficiently delicate. Individual workers have used their own methods which may cause some difficulty when group numbers determined in different laboratories are compared. It is understood that a code of standard methods is being prepared.

Media and Technique.

The media and technique used in this study conformed to Standard Methods for the Examination of Water and Sewage of the American Public Health Association, 1912, with one exception. Liebig's meat extract was substituted for the meat infusion in the preparation of the media. It is believed that more uniform media is thus secured. In those instances where special media have been prepared, the composition will be mentioned under the special heading.

Sources of Cultures.

All the cultures of fluorescent bacteria used in this study were isolated from water. The samples of water from which they came were collected at different places in the state of Illinois. It was decided to limit this study to those forms from water and at a later date, if possible to study another series from a different source. The investigation by Edson and Carpenter which has been previously mentioned, was made with

cultures many of which came from the same sap orchard. In this way their strains may have had some close relationship. The cultures which form the basis of the present study are probably in no way related. The following table presents a complete list of all the cultures with their sources and dates of isolation:

TABLE NO. 2.

Culture Number.	Place	Source	Date
1	Marseilles	Illinois River	June 19, 1914
2	Marseilles	" "	" " "
3	Casner	30' Dug Well	June 24, 1914
4	Marseilles	Illinois River	" 17, "
5	Chillicothe	" "	" 20, "
6	Chicago	Ill. Cent. Coach	" 6, "
7	Benton	16' Dug Well	" 11, "
8	De Kalb	Stream	" 23, "
9	De Kalb	"	" " "
10	Eureka	23' Dug Well	October 1, 1914.
11	Rock Island	Filtered Water	June 31, 1914.
12	Centralia	16' Dug Well	July 29, 1914.
13	Centralia	14' Dug Well	" " "
14	Havana	Illinois River	" " "
15	Chillicothe	" "	August 12, 1914
16	East St.Louis	Raw Water	September 9, 1914.
17	Loda	20' Dug Well	" 15, "
18	Lithia	Lithia Springs	" 19, "
19	"	" "	" " "

20	Danville	26' Dug Well	September 19, 1914
21	"	" " "	" " "
22	Pontiac	Raw River Water	" " "
23	Jacksonville	34' Dug Well	May 9, 1915
24	Broadlands	30' Dug Well	September 18, 1914
25	El Paso	35' Dug Well	" " "
26	" "	" " "	" " "
27	Elkhart	Spring	September 25, 1914
28	"	"	" " "
29	El Paso	35' Dug Well	" 22 "
30	Beardstown	Illinois River	" " "
31	"	" "	" " "
32	Decatur	15' Bored Well	" " "
33	"	" " "	" " "
34	"	" " "	" " "
35	Billwood	1535' Drilled Well	July 11, 1914
36	Chillicothe	Illinois River	" 6, "
37	Mowsaqua	Cistern	October 14, 1914
38	Jacksonville	Dug Well	July 25, 1914
39	Unknown	Water	" 20, "
40	Mattoon	60' Bored Well	" 11, "
41	Mattoon	52' Bored Well	" 29, "
42	White Hall	23' Dug Well	" " "
43	Mattoon	60' Bored Well	" " "
44	Canton	1800' Drilled Well	June 19, 1914.
45	Centralia	14' Dug Well	July 29, 1914.
46	Quincy	Cistern	October 15, 1914.

47	Marseilles	Illinois River	July 29, 1914
48	"	" "	" " "
49	Anna	Ice	October 23, 1914.
50	Joy	Cistern	November 5, 1914.
51	Pontiac	Vermillion River	July 29, 1914.
52	Evanston	Treated Water	October 15, 1914.
53	Champaign	Sewage	" 28 "
54	Marshall	22' Dug Well	November 2, 1914
55	"	" " "	" " "
56	Havana	Illinois River	December 3, 1914
57	Jacksonville	Well	November 5, 1914
58	San Jose	Well	" 6, "
59	Casey	18' Drilled Well	August 5, 1914
60	Blue Island	274' Drilled Well	" " "
61	Neoga	17' Dug Well	" " "
62	Virginia	51' " "	November 5, 1914
63	Abingdon	25' " "	August 6, 1914
64	St. Peter	14' " "	" " "
65	" "	" " "	" " "
66	Spring Valley	Illinois River	" 13, "
67	Henry	Illinois River	" " "
68	Roadhouse	29' Dug Well	November 6, 1914
69	Pearl	Illinois River	August 13, 1914
70	Peoria	30' Dug Well	February 16, 1915
71	Decatur	Swimming Pool	September 15, 1914
72	Charleston	Raw Water	
73	Breckenridge	23' Dug Well	November 6, 1914

74	Pekin	Illinois River	September 14, 1914
75	Sullivan	44' Dug Well	November 19, 1914
76	Roodhouse	15' Dug Well	November 6, 1914
77	Rock Island	Raw water	
78	Sullivan	44' Dug Well	November 20, 1914
79	Marseilles	Illinois River	November 20, 1914
80	Danville	Raw Water	" 25, "
81	Witt	18' Dug Well	" " "
82	"	" " "	" " "
83	Freeburg	30' " "	" 26, "
84	Pekin	Illinois River	September 24, 1914
85	Quincy	Filtered Water	November 26, 1914.
86	Granite City	18' Dug Well	" " "
87	Quincy	Filtered Water	" " "
88	Galesburg	City Water	" " "
89	Area	22' Dug Well	" 28, "
90	Quincy	Raw River Water	" " "
91	Pontiac	River Water	" " "
92	Area	22' Dug Well	" " "
93	Danville	Raw Water	" " "
94	Havana	Illinois River	December 3, 1914
95	Junction	22' Dug Well	November 28, 1914
96	Odin	16' " "	October 1, 1914
97	Carlyle	22' Bored Well	" 1, 1914
98	Carmi	22' Driven Well	" " "
99	Murphysboro	24' Bored Well	" " "
100	Neoga	25' Dug Well	" " "

Surface Waters	35
Deep Wells (Over 100' in depth)	3
Shallow Wells (Under 100' in depth)	47
Springs	4
Cistern	3
Swimming Pool	1
Sewage	1
Unknown	1
Interstate Carrier	1
Ice	1

The colonies were picked from agar and gelatin plates which had been made from water samples received at the laboratories of the Illinois State Water Survey. All of these samples were collected according to instructions which were furnished with the sterile container. They were shipped to the laboratory packed in ice where the examination was immediately started. After the plates had been counted for the routine analysis, they were examined for fluorescent bacteria. If none were evident, it was found that they very often appeared after the plates had been held at room temperature over night.

As is very important in a study of this kind every effort was made to conduct the study under as uniform conditions as possible. This is necessary because bacteria are capable of reacting easily to their environment.

Young cultures were used in each case. All inoculations into the various media were made either from a twenty four broth culture or from a twenty four hour agar slant culture.

Culturing in liquid media has been done in Erlenmeyer flasks containing at least 100 c. c. of the culture media. Acidity was determined by titrating aliquot portions with phenolphthalein as the indicator.

Description of Cultures.

Since the Society Chart was used in this study, the various cultural and morphological characteristics will be mentioned in somewhat the same order in which they appear on the chart. The following table presents the characteristics of the cultures used in this study. This obviates any necessity of extended discussions under the various headings to follow.

Morphology.

All the cultures studied were motile rods and usually grew in chains. The cells used in studying the morphology were young cells and were subcultured on plain agar slants before being stained. The vegetative cells were both long and short rods some of them being distinguished from coccis forms only with great difficulty. The agar slants were incubated at 37°C for 24 hours before staining. The study of morphology was made from smears stained with carbol fuchsin. The ends were rounded.

The cells were measured by means of a Leitz filar micrometer which had been standardized from a stage micrometer. This is not especially significant with these bacteria since there was such a large variation in size.

Only four of the cultures showed spore formation. These were Nos. 45, 47, 61, and 70. All of the strains possess a

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SHEET NO. 1

Culture Number	Date of Isolation	Group Number	Morphology: Diam. over 1 μ	Motility	Spores	Chaines	Flagella	Capsules	Gram stain	Broth: Turbidity	Sediment	Alkaline	Acid	Agar: Dull	Wrinkled
1	6-19-'14	222.2332/133	−	+	−	+	+	−	−	+	+			+	−
2	6-19-'14	221.2223/133	−	+	−	+	+	+	−	+	+			−	−
3	6-24-'14	221.2223/133	−	+	−	+	+	+	−	+	+			−	−
4	6-17-'14	222.2332/133	−	+	−	+	+	−	−	+	+			−	−
5	6-20-'14	222.2322/132	−	+	−	+	+	−	−	+	+			−	−
6	6- 6-'14	221.2232/133	−	+	−	+	+	+	−	+	+			−	−
7	6-11-'14	222.2333/133	−	+	−	+	+	−	−	+	+			−	−
8	6-23-'14	222.2333/133	−	+	−	+	+	−	−	+	+			−	−
9	6-23-14	222.2333/133	−	+	−	+	+	−	−	+	+			−	−
10	10- 1-'14	221.2332/133	−	+	−	+	+	−	−	−	−			−	−
11	6-31-'14	222.2332/133	−	+	−	+	+	−	−	+	+			−	−
12	7-29-'14	221.2333/133	−	+	−	+	+	−	−	+	+			−	−
13	7-29-'14	221.2222/133	−	+	−	+	+	−	−	+	+			−	−
14	7-29-'14	221.2332/133	−	+	−	+	+	+	−	+	+			−	−
15	8-12-'14	221.2222/132	−	+	−	+	+	−	−	+	+			−	−
16	9- 9-'14	221.2332/133	−	+	−	+	+	−	−	+	+			−	−
17	9-15-'14	221.2223/133	−	+	−	+	+	−	−	+	+			−	−
18	9-19-'14	222.2332/133	−	+	−	+	+	−	−	+	+			−	−
19	9-19-'14	222.2222/133	−	+	−	+	+	−	−	+	+			+	−
20	9-19-'14	221.2223/132	−	+	−	+	+	−	−	+	+			+	−
21	9-19-'14	221.2332/133	−	+	−	+	+	−	−	−	+			−	−
22	9-19-'14	221.2222/132	−	+	−	+	+	+	−	+	+			−	−
23	3- 9-'15	221.2223/132	−	+	−	+	+	−	−	+	+			−	−
24	9-18-'14	221.2223/133	−	+	−	+	+	−	−	+	+			−	−
25	9-18-'14	221.2233/133	−	+	−	+	+	−	−	+	+			−	−
26	9-18-'14	221.2233/133	−	+	−	+	+	+	−	+	+			−	−
27	9-25-'14	221.2322/132	−	+	−	+	+	−	−	+	+			−	−
28	9-25-'14	221.2332/133	−	+	−	+	+	−	−	+	+			−	−
29	9-22-'14	221.2233/133	−	+	−	+	+	−	−	+	+			−	−
30	9-22-'14	221.2323/132	−	+	−	+	+	−	−	+	+			−	−
31	9-22-'14	221.2223/133	−	+	−	+	+	−	−	+	+			−	−
32	9-22-'14	221.2333/133	−	+	−	+	+	−	−	+	+			−	−
33	9-22-'14	222.2223/133	−	+	−	+	+	−	−	+	+			−	−

Culture Number	Potato: Abundant	Moderate	Discolored	Chromogenic	Gel. stab: Surface growth	Needle growth	Grows at 37°C	Grows at 20°C	Cohn's solution	Uschinsky's solution
1	+	−	+	−	+	+	+	+		+
2	+	−	+	−	+	+	+	+		+
3	+	−	+	−	+	−	+	+		+
4	−	+	+	−	+	−	+	+		+
5	+	−	+	−	+	+	+	+		+
6	+	−	+	−	+	−	+	+		+
7	−	+	+	−	+	−	+	+		+
8	−	+	+	−	+	−	+	+		+
9	−	+	+	−	+	−	+	+		+
10	−	−	−	−	+	−	+	+		−
11	+	−	+	−	+	−	+	+		+
12	−	−	+	−	+	−	+	+		+
13	−	−	+	−	+	−	+	+		+
14	−	+	+	−	+	−	+	+		+
15	−	+	+	−	+	−	+	+		+
16	−	−	+	−	+	−	+	+		+
17	+	−	+	−	+	−	+	+		+
18	+	−	+	−	+	−	+	+		+
19	−	−	+	−	+	−	+	+		+
20	−	+	+	−	+	−	+	+		+
21	−	+	+	−	+	−	+	+		+
22	+	−	+	−	+	−	+	+		+
23	+	−	+	−	+	−	+	+		+
24	−	−	+	−	+	−	+	+		+
25	+	−	+	−	+	−	+	+		+
26	−	+	+	−	+	−	+	+		+
27	−	+	+	−	+	−	+	+		+
28	−	−	+	−	+	−	+	+		+
29	+	−	+	−	+	−	+	+		+
30	−	−	+	−	+	−	+	+		+
31	+	−	+	−	+	−	+	+		+
32	−	−	+	−	+	−	+	+		+
33	−	+	+	−	+	−	+	+		+

Culture Number	Liquefaction: Gelatin	Blood serum	Casein	Agar	Milk: Acid	Alkaline	Curd	Dextrose: Acid	Alkaline	Closed arm	Gas	Lactose: Acid	Alkaline	Closed arm	Gas
1	−		−	−	−	+	+	+	−	−	−	−	+	−	−
2	+		+	−	+	−	+	+	−	+	−	+	−	+	−
3	+		+	−	+	−	−	+	−	+	−	+	−	−	−
4	−		−	−	−	+	−	+	−	−	−	−	+	−	−
5	−		−	−	+	−	+	+	−	+	−	−	+	+	−
6	+		+	−	−	+	−	+	−	−	−	+	−	−	−
7	−		−	−	−	+	−	+	−	+	−	−	+	−	−
8	−		−	−	−	+	−	+	−	+	−	−	+	−	−
9	−		−	−	−	+	−	+	−	−	−	−	+	−	−
10	+		−	−	−	+	−	+	−	−	−	−	+	−	−
11	−		−	−	−	+	−	+	−	−	−	−	+	−	−
12	+		+	−	+	−	+	+	−	+	−	−	+	+	−
13	+		+	−	−	+	−	+	−	+	−	+	−	+	−
14	+		+	−	−	+	−	+	−	−	−	−	+	+	−
15	+		−	−	−	+	−	+	−	−	−	+	−	+	−
16	+		+	−	−	+	−	+	−	−	−	−	+	+	−
17	+		+	−	−	+	+	+	−	−	−	+	−	+	−
18	−		−	−	−	+	−	+	−	−	−	−	+	+	−
19	−		−	−	+	−	−	+	−	−	−	+	−	−	−
20	+		+	−	−	+	−	+	−	−	−	+	−	−	−
21	+		+	−	−	+	−	+	−	−	−	−	+	−	−
22	+		+	−	−	+	−	+	−	+	−	+	−	−	−
23	+		+	−	+	−		+	−	−	−	+		−	−
24	+		−	−	+	−	+	+	−	+	−	+	−	−	−
25	+		+	−	−	+	−	+	−	−	−	+	−	−	−
26	+		+	−	−	+	−	+	−	−	−	+	−	−	−
27	+		−	−	−	+	−	+	−	−	−	−	+	−	−
28	+		−	−	−	+	−	+	−	−	−	−	+	−	−
29	+		+	−	−	+	−	+	−	−	−	+	−	−	−
30	+		+	−	+	−	−	+	−	−	−	−	+	+	−
31	+		+	−	−	+	+	+	−	−	−	+	−	−	−
32	+		+	−	−	+	−	+	−	−	−	−	+	−	−
33	−		+	−	−	+	−	+	−	+	−	+	−	−	−

Culture Number	Saccharose: Acid	Alkaline	Closed arm	Gas	Glycerol: Acid	Alkaline	Closed arm	Gas	Indol	Hydrogen sulfid	Nitrates reduced	Ammonia	Flourescence	Remarks
1	−	+	−	−	−	+	−	−	−	−	−	−	+	
2	+	−	−	−	−	+	−	−	−	+	+	+	+	
3	+	−	−	−	−	+	−	−	+	−	+	+	+	
4	−	+	−	−	−	+	−	−	−	−	−	−	+	
5	+	−	−	−	+	−	+	−	−	−	−	−	+	
6	−	+	−	−	−	+	−	−	−	−	−	−	+	
7	−	+	−	−	−	+	+	−	−	−	−	−	+	
8	−	+	+	−	−	+	+	−	−	−	−	−	+	
9	−	+	−	−	−	+	+	−	−	−	−	−	+	
10	−	+	−	−	−	+	−	−	−	−	−	−	+	
11	−	+	−	−	−	+	+	−	−	−	−	−	+	
12	−	+	+	−	−	+	−	−	−	+	−	−	+	
13	+	−	−	−	−	+	−	−	−	+	−	−	+	
14	−	+	+	−	−	+	−	−	−	+	−	−	+	
15	+	−	+	−	+	−	−	−	−	+	−	−	+	
16	−	+	−	−	−	+	−	−	−	−	−	−	+	
17	+	−	+	−	−	+	−	−	−	+	+	+	+	
18	−	+	−	−	−	+	−	−	−	−	−	−	+	
19	+	−	−	−	−	+	−	−	−	+	−	−	+	
20	+	−	−	−	+	−	−	−	−	+	−	−	+	
21	−	+	−	−	−	+	−	−	−	−	−	−	+	
22	+	−	−	−	+	−	−	−	−	+	−	+	+	
23	+	−	−	−	+	−	−	−	−	+	−	−	+	
24	+	−	+	−	−	+	+	−	−	+	+	+	+	
25	−	+	−	−	−	+	−	−	−	+	+	+	+	
26	−	+	−	−	−	+	−	−	−	−	+	+	+	
27	+	−	−	−	+	−	−	−	−	−	−	−	+	
28	−	+	−	−	−	+	−	−	−	−	−	−	+	
29	−	+	+	−	−	+	−	−	−	+	+	+	+	
30	+	−	−	−	+	−	+	−	−	+	+	+	+	
31	+	−	−	−	−	+	−	−	−	+	+	+	+	
32			−	−	−	+	−	−	−	+	+	+	+	
33	+	−	+	−	−	+	−	−	−	+	+	+	+	

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SHEET NO. 2

Culture Number	Date of Isolation	Group Number	Morphology: Diam. over 1 µ	Motility	Spores	Chaines	Flagella	Capsules	Gram Stain	Broth: Turbidity	Sediment	Alkaline	Acid	Agar: Dull	Wrinkled
34	9-22-'14	221.2232/133	−	+	−	+	+	−	−	+	+			−	−
35	7-11-'14	221.2333/133	−	+	−	+	+	−	−	+	+			−	−
36	7-6-'14	222.2332/133	−	+	−	+	+	−	−	+	+			+	−
37	10-14-'14	221.2333/133	−	+	−	+	+	+	−	+	+			+	−
38	6-25-'14	222.2332/133	−	+	−	+	+	−	−	+	+			+	−
39	6-20-'14	222.2333/132	−	+	−	+	+	−	−	+	+			+	−
40	6-11-'14	222.2332/133	−	+	−	+	+	−	−	+	+			−	−
41	6-29-'14	222.2332/133	−	+	−	+	+	−	−	+	+			+	−
42	6-29-'14	222.2332/133	−	+	−	+	+	−	−	+	+			−	−
43	6-29-'14	222.2333/133	−	+	−	+	+	−	−	+	+			−	−
44	6-29-'14	222.2332/133	−	+	−	+	+	−	−	+	+			−	−
45	6-29-'14	121.2332/133	−	+	+	+	+	−	−	+	+			−	−
46	10-15-'14	221.2332/133	−	+	−	+	+	+	−	+	+			−	−
47	6-29-'14	121.2333/133	−	+	+	+	+	−	−	+	+			−	−
48	6-29-'14	221.2323/133	−	+	−	+	+	−	−	+	+			+	−
49	10-23-'14	222.2332/133	−	+	−	+	+	−	−	+	+			−	−
50	11-5-'14	221.2223/133	−	+	−	+	+	+	−	+	+			−	−
51	6-29-'14	222.2332/133	−	+	−	+	+	−	−	+	+			−	−
52	10-15-'14	222.2233/133	−	+	−	+	+	−	−	+	+			−	−
53	10-28-'14	222.2333/133	−	+	−	+	+	−	−	+	+			−	−
54	11-2-'14	222.2333/133	−	+	−	+	+	−	−	+	+			−	−
55	11-2-'14	222.2333/132	−	+	−	+	+	−	−	+	+			−	−
56	12-3-'14	222.2333/133	−	+	−	+	+	−	−	+	+			−	−
57	11-5-'14	222.2333/132	−	+	−	+	+	−	−	+	+			−	−
58	11-6-'14	222.2332/133	−	+	−	+	+	−	−	+	+			−	−
59	7-5-'14	221.2332/132	−	+	−	+	+	−	−	+	+			−	−
60	7-5-'14	222.2223/132	−	+	−	+	+	+	−	+	+			−	−
61	7-5-'14	121.2332/133	−	+	+	+	+	−	−	+	+			−	−
62	11-6-'14	221.2332/132	−	+	−	+	+	−	−	+	+			+	−
63	7-6-'14	221.2333/133	−	+	−	+	+	−	−	+	+			+	−
64	7-6-'14	221.2333/133	−	+	−	+	+	−	−	+	+			−	−
65	7-6-'14	221.2333/133	−	+	−	+	+	−	−	+	+			−	−
66	7-12-'14	221.2333/132	−	+	−	+	+	−	−	+	+			−	−

Culture Number	Gel. Col.: Round	Proteus like	Curled	Filamentious	Potato: Abundant	Moderate	Discolored	Chromogenic	Gel. Stab: Surface growth	Needle growth	Grows at 37°C	Grows at 20°C	Cohn's solution	Uschinsky's solution
34					−	−	+	−	+	+	+	+		+
35					−	+	+	−	+	−	+	+		+
36					−	−	+	−	+	−	+	+		+
37					+	−	+	−	+	−	+	+		+
38					−	+	+	−	+	−	+	+		+
39					+	+	+	−	+	−	+	+		+
40					−	+	+	−	+	−	+	+		+
41					−	+	+	−	+	−	+	+		+
42					−	+	+	+	+	−	+	+		+
43					+	−	+	−	+	−	+	+		+
44					−	+	+	−	+	−	+	+		+
45					−	+	+	−	+	−	+	+		+
46					+	−	+	−	+		+	+		+
47					−	−	+	−	+	−	+	+		+
48					−	+	+	−	+	−	+	+		+
49					−	+	+	−	+	+	+	+		+
50					+	−	−	−	+	−	+	+		+
51					−	+	+	−	+	−	+	+		+
52					−	+	+	−	+	−	+	+		+
53					+	−	+	−	+	+	+	+		+
54					+	−	+	−	+	−	+	+		+
55					+	−	−	−	+	−	+	+		+
56					−	−	+	−	+	+	+	+		+
57					−	−	+	−	+	+	+	+		+
58					−	+	+	−	+	+	+	+		+
59					+	−	+	−	+	−	+	+		+
60					+	−	+	−	+	−	+	+		+
61					+	−	+	−	+	−	+	+		+
62					+	−	+	−	+	−	+	+		+
63					+	−	+	−	+	−	+	+		+
64					+	−	+	−	+	−	+	+		+
65					+	−	+	−	+	−	+	+		+
66					+	−	+	−	+	−	+	+		+

Culture Number	Liquefaction: Gelatin	Blood serum	Casein	Agar	Milk: Acid	Alkaline	Curd	Dextrose: Acid	Alkaline	Closed arm	Gas
34	+		−	−	−	+	−	+	−	−	−
35	+		+	−	−	+	−	+	−	−	−
36	−		−	−	−	+	−	+	−	−	−
37	+		+	−	−	+	+	+	−	−	−
38	−		−	−	+	−	−	+	−	−	−
39	−		−	−	+	+	+	+	−	−	−
40	−		−	−	−	+	−	+	−	−	−
41	−		−	−	−	+	−	+	−	−	−
42	−		−	−	−	+	−	+	−	−	−
43	−		−	−	−	+	−	+	−	−	−
44	−		−	−	−	+	−	+	−	−	−
45	+		−	−	−	+	−	+	−	−	−
46	−		+	−	−	+	−	+	−	−	−
47	+		−	−	+	−	−	+	−	−	−
48	+		−	−	−	+	−	+	−	−	−
49	−		−	−	−	+	−	+	−	−	−
50	+		+	−	−	+	−	+	−	−	−
51	−		−	−	−	+	−	+	−	−	−
52	+		+	−	−	+	−	+	−	−	−
53	+		+	−	−	+	+	+	−	−	−
54	+		+	−	−	+	−	+	−	−	−
55	+		+	−	−	+	−	+	−	−	−
56	+		−	−	−	+	−	+	−	−	−
57	+		+	−	−	+	−	+	−	−	−
58	+		−	−	−	+	−	+	−	−	−
59	−		−	−	−	+	−	+	−	−	−
60	+		+	−	−	+	−	+	−	+	−
61	−		−	−	−	+	−	+	−	−	−
62	−		−	−	−	+	−	+	−	−	−
63	−		−	−	−	+	−	+	−	−	−
64	−		−	−	−	+	−	+	−	−	−
65	−		−	−	−	+	−	+	−	−	−
66	−		−	−	−	+	−	+	−	−	−

Culture Number	Lactose: Acid	Alkaline	Closed arm	Gas	Saccharose: Acid	Alkaline	Closed arm	Gas	Glycerol: Acid	Alkaline	Closed arm	Gas	Indol	Hydrogen sulfid	Nitrates reduced	Ammonia	Flourescence	Remarks
34	+	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	+	
35	−	+	−	−	−	+	−	−	−	+	−	−	−	+	+	+	+	
36	−	+	−	−	−	+	−	−	−	+	−	−	−	+	−	−	+	
37	−	+	−	−	−	+	−	−	−	+	−	−	+	−	+	+	+	
38	−	+	−	−	−	+	−	−	−	+	−	−	−	−	−	−	+	
39	−	+	−	−	−	+	−	−	+	−	−	−	−	−	−	−	+	
40	−	+	−	−	−	+	−	−	−	+	−	−	−	−	−	−	+	
41	−	+	−	−	−	+	−	−	−	+	−	−	−	−	−	−	+	
42	−	+	−	−	−	+	−	−	−	+	−	−	−	−	−	−	+	
43	+	−	−	−	−	+	−	−	−	+	−	−	−	+	+	+	+	
44	−	+	−	−	−	+	−	−	−	+	−	−	−	+	−	−	+	
45	+	−	−	−	−	+	−	−	−	+	−	−	−	+	−	−	+	
46	−	+	−	−	−	+	−	−	−	+	−	−	−	+	−	−	+	
47	+	−	−	−	−	+	−	−	−	+	−	−	−	+	+	+	+	
48	−	+	−	−	+	−	−	−	−	+	−	−	−	+	+	+	+	
49	−	+	−	−	−	+	−	−	−	+	−	−	−	−	−	−	+	
50	+	−	−	−	+	−	−	−	−	+	−	−	−	−	+	+	+	
51	−	+	−	−	−	+	−	−	−	+	−	−	−	−	−	−	+	
52	+	−	−	−	−	+	−	−	−	+	−	−	−	+	+	+	+	
53	−	+	−	−	−	+	−	−	−	+	−	−	−	+	+	+	+	
54	−	+	−	−	−	+	+	−	−	+	−	−	−	−	+	+	+	
55	−	+	−	−	−	+	−	−	+	−	−	−	−	+	+	+	+	
56	+	−	−	−	−	+	−	−	−	+	−	−	−	−	+	+	+	
57	−	+	−	−	−	+	−	−	+	−	−	−	−	−	+	+	+	
58	+	−	−	−	−	+	−	−	−	+	−	−	−	+	−	−	+	
59	+	−	−	−	−	+	−	−	+	−	−	−	−	+	−	−	+	
60	+	−	+	−	+	−	+	−	+	−	−	−	−	+	+	+	+	
61	+	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	+	
62	−	+	−	−	−	+	−	−	+	−	−	−	−	−	−	−	+	
63	+	−	−	−	−	+	−	−	−	+	−	−	−	−	+	+	+	
64	+	−	−	−	−	+	+	−	−	+	−	−	−	−	+	+	+	
65	+	−	−	−	−	+	−	−	−	+	−	−	−	−	+	+	+	
66	+	−	−	−	−	+	−	−	+	−	−	−	−	−	+	+	+	

SUBJECT
DATA
DATE

SHEET NO. 3

Culture Number	Date of Isolation	Group Number	Morphology: Diam. over 1 μ	Motility	Spores	Chaines	Flagella	Capsules	Gram stain	Broth: Turbidity	Sediment	Alkaline	Acid	Agar: Dull	Wrinkled	Gel. col.: Round	Proteus like	Curled	Filamentious	Potato: Abundant	Moderate	Discolored	Chromogenic	Gel. stab: Surface growth	Needle growth	Grows at 37°C	Grows at 20°C	Cohn's solution	Uschinsky's solution
67	7-12-'14	221.2333133	−	+	−	+	+	−	−	+	+			−	−					−	+	+	−	+	−	+	+		+
68	11-6-'14	222.2233133	−	+	−	+	+	+	−	+	+			+	−					+	−	+	−	+	−	+	+		+
69	7-13-'14	221.2333132	−	+	−	+	+	−	−	+	+			−	−					+	−	+	−	+	−	+	+		+
70	2-16-'15	122.2333133	−	+	+	+	+	−	−	+	+			−	−					+	−	+	−	+	−	+	+		+
71	9-15-'14	221.2233133	−	+	−	+	+	−	−	+	+			+	−					+	−	+	−	+	−	+	+		+
72		221.2333133	−	+	−	+	+	−	−	+	+			+	−					+	−	+	−	+	+	+	+		+
73	11-6-'14	221.2332133	−	+	−	+	+	−	−	+	+			−	−					+	−	+	−	+	−	+	+		+
74	9-14-'14	221.2332133	−	+	−	+	+	−	−	+	+			+	−					+	−	+	−	+	−	+	+		+
75	11-19-'14	221.2333133	−	+	−	+	+	+	−	+	+			−	−					+	−	+	−	+	+	+	+		+
76	11-6-'14	222.2333133	−	+	−	+	+	−	−	+	−			−	−					−	+	+	−	+	−	+	+		+
77		222.2333133	−	+	−	+	+	−	−	+	−			−	−					+	−	+	−	+	−	+	+		+
78	11-20-'14	222.2223133	−	+	−	+	+	−	−	+	+			−	−					+	−	+	−	+	+	+	+		+
79	11-20-'14	222.2223133	−	+	−	+	+	+	−	+	+			−	−					+	−	+	−	+	+	+	+		+
80	11-25-'14	222.2223133	−	+	−	+	+	−	−	+	+			−	−					−	+	+	−	+	−	+	+		+
81	11-25-'14	222.2233133	−	+	−	+	+	−	−	+	+			−	−					+	−	+	−	+	−	+	+		+
82	11-25-'14	222.2233132	−	+	−	+	+	−	−	+	+			+	−					+	−	+	−	+	+	+	+		+
83	11-26-'14	222.2223133	−	+	−	+	+	−	−	+	+			+	−					+	−	+	−	+	−	+	+		+
84	11-24-'14	221.2332133	−	+	−	+	+	−	−	+	+			−	−					+	−	+	−	+	−	+	+		+
85	11-26-'14	221.2332133	−	+	−	+	+	−	−	+	+			−	−					+	−	+	−	+	−	+	+		+
86	11-26-'14	221.2232133	−	+	−	+	+	−	−	+	+			−	−					+	−	+	−	+	−	+	+		+
87	11-26-'14	221.2332133	−	+	−	+	+	−	−	+	+			−	−					+	−	+	−	+	−	+	+		+
88	11-26-'14	221.2232133	−	+	−	+	+	−	−	+	+			−	−					+	−	+	−	+	−	+	+		+
89	11-28-'14	222.2233133	−	+	−	+	+	−	−	+	−			−	−					+	−	+	−	+	−	+	+		+
90	11-28-'14	221.2333133	−	+	−	+	+	−	−	+	+			−	−					+	−	+	−	+	−	+	+		+
91	11-28-'14	221.2332133	−	+	−	+	+	−	−	+	+			−	−					+	−	+	−	+	−	+	+		+
92	11-28-'14	222.2233133	−	+	−	+	+	−	−	+	−			−	−					+	−	+	−	+	−	+	+		+
93	11-28-'14	222.2223133	−	+	−	+	+	−	−	+	−									+	−	+	−	+	−	+	+		+
94	12-3-'14	221.2332133	−	+	−	+	+	−	−	+	+			−	−					+	−	+	−	+	−	+	+		+
95	11-28-'14	222.2232133	−	+	−	+	+	−	−	−	−			+	−					+	−	+	−	+	−	+	+		+
96	10-31-'14	222.2233133	−	+	−	+	+	−	−	+	+			−	−					+	−	+	−	+	+	+	+		+
97	10-1-'14	222.2333133	−	+	−	+	+	−	−	+	+			−	−					+	−	+	−	+	−	+	+		+
98	10-1-'14	221.2333133	−	+	−	+	+	−	−	+	−			−	−					+	−	+	−	+	−	+	+		+
99	10-1-'14	221.2332133	−	+	−	+	+	−	−	+	+			−	−					+	−	+	−	+	−	+	+		+
100	10-1-'14	222.2323133	−	+	−	+	+	−	−	+	−			−	−					+	−	+	−	+	−	+	+		+

Culture Number	Liquefaction: Gelatin	Blood serum	Casein	Agar	Milk: Acid	Alkaline	Curd	Dextrose: Acid	Alkaline	Closed arm	Gas	Lactose: Acid	Alkaline	Closed arm	Gas	Saccharose: Acid	Alkaline	Closed arm	Gas	Glycerol: Acid	Alkaline	Closed arm	Gas	Indol	Hydrogen sulfid	Nitrates reduced	Ammonia	Flourescence	Remarks
67	−		−	−	−	+	−	+	−	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−	−	+	+	+	
68	+		+	−	−	+	−	+	−	−	−	+	−	−	−	−	+	−	−	−	+	−	−	−	+	+	+	+	
69	−		−	−	−	+	−	+	−	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−	−	+	+	+	
70	+		+	−	−	+	−	+	−	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−	+	+	+	+	
71	+		+	−	+	−	−	+	−	+	−	+	−	−	−	−	+	−	−	−	+	−	−	−	+	+	+	+	
72	+		+	−	−	+	−	+	−	+	−	−	+	+	−	−	+	+	−	−	+	−	−	−	+	+	+	+	
73	−		−	−	−	+	−	+	−	+	−	−	+	−	−	−	+	−	−	−	+	−	−	−	−	−	−	+	
74	−		−	−	−	+	−	+	−	+	−	−	+	−	−	−	+	−	−	−	+	−	−	−	−	−	−	+	
75	+		−	−	−	+	−	+	−	+	−	−	+	+	−	−	+	−	−	−	+	−	−	−	−	+	+	+	
76	+		−	−	−	+	−	+	−	+	−	−	+	−	−	−	+	−	−	−	+	−	−	−	+	+	+	+	
77	+		+	−	−	+	−	+	−	+	−	−	+	−	−	−	+	−	−	+	−	−	−	−	+	+	+	+	
78	+		−	−	−	+	−	+	−	+	−	+	−	−	−	+	−	−	−	−	+	−	−	−	+	+	+	+	
79	+		+	−	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	−	+	−	−	−	+	+	+	+	
80	+		−	−	−	+	−	+	−	−	−	+	−	−	−	+	−	−	−	−	+	−	−	−	−	+	+	+	
81	+		+	−	−	+	−	+	−	−	−	+	−	−	−	−	+	−	−	−	+	−	−	−	+	+	+	+	
82	+		+	−	+	−	−	+	−	+	−	+	−	−	−	−	+	+	−	+	−	−	−	−	−	+	+	+	
83	+		−	−	−	+	−	+	−	+	−	+	−	−	−	+	−	−	−	−	+	−	−	−	−	+	+	+	
84	−		−	−	−	+	−	+	−	−	−	−	+	−	−	−	+	−	−	+	−	−	−	−	+	−	−	+	
85	−		−	−	−	+	−	+	−	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−	−	−	−	+	
86	−		−	−	−	+	−	+	−	−	−	+	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	+	
87	−		−	−	−	+	−	+	−	−	−	+	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	+	
88	−		−	−	−	+	−	+	−	−	−	+	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	+	
89	+		+	−	−	+	−	+	−	−	−	+	−	−	−	−	+	−	−	−	+	−	−	−	+	+	+	+	
90	−		−	−	−	+	−	+	−	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−	−	+	+	+	
91	−		−	−	−	+	−	+	−	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−	−	−	−	+	
92	+		−	−	−	+	−	+	−	−	−	+	−	−	−	−	+	−	−	−	+	−	−	−	−	+	+	+	
93	+		+	−	−	+	−	+	−	−	−	+	−	−	−	+	−	−	−	−	+	−	−	−	−	+	+	+	
94	+		−	−	−	+	+	+	−	−	−	+	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	+	
95	+		−	−	−	+	−	+	−	−	−	+	−	−	−	−	+	−	−	−	+	−	−	−	+	−	−	+	
96	+		+	−	−	+	+	+	−	−	−	+	−	−	−	−	+	−	−	−	+	−	−	−	−	+	+	+	
97	+		+	−	+	−	−	+	−	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−	−	+	+	+	
98	−		−	−	−	+	−	+	−	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−	+	+	+	+	
99	−		−	−	−	+	−	+	−	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−	−	−	−	+	
100	+		+	−	+	−	+	+	−	−	−	−	+	−	−	+	−	−	−	−	+	−	−	−	−	+	+	+	

great similarity as is shown by the following group numbers.

45 and 61	121.2332133
47	121.2333133
70	122.2333133

Culture No. 70 differs from the other three spore formers in not liquefying gelatin. Nitrate reduction is absent in strains Nos. 45, and 61. Three of them are from shallow wells while No. 47 comes from the Illinois river at Marseilles.

All of the cultures were pseudomonas forms. Loeffler's method was used to stain the flagella. Most of the cultures possessed a tuft of flagella on one end while those with but one flagellum were very rare. The length of the flagella was not measured.

Motility was observed with each strain. A few strains failed to show motility on the first observation. Subsequent observations however, give ample proof of motility.

The staining properties were not especially characteristic. All were gram negative.

Capsules were demonstrated on the following cultures by means of Welch's[1] method.

2, 3	Ps.	221.2223133
6	"	221.2232133
14	"	221.2332133
22	"	221.2222132
26, 75	"	221.2233133

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1. Muir and Richie. Manual of Bacteriology. 1913. Page 110.

37	Ps.	221.2333133
46	"	221.2332133
50	"	221.2223133
60	"	222.2223133
68	"	222.2333133
79	"	222.2223133

The broth cultures of these cultures were invariably viscid. The group numbers are quite similar but differ slightly in a few characteristics. All but three strains liquefy gelatin.

Agar Stroke.

All cultures were grown on this medium and produced a distinct fluorescence. Abundant growth was secured at 37° C. In a few cases the fluorescent property was not evident until after two or three days but was intensified by holding at room temperature or in an ice box.

Potato Slants.

The potato slants were made in the usual way. A little distilled water was placed into the bottom of the test tube and the potato slant was supported above this by means of a small piece of glass tubing. Sterilization was conducted in the autoclav since it was found to be very difficult to sterilize this medium in the Arnold.

The growth on potato was not significant. In almost every case the growth was spreading and was moderate to abundant. Upto about two days incubation the growth was limited to the line of inoculation but spread rapidly after slightly longer incubation. The potato was turned to a dark brown or green.

Observations were made at the end of ten days after which time the potato and the water in the bottom of the tubes was poured into a mortar and tested for starch destruction.

Starch.

No official method for determining starch reduction exists. Each worker uses his own method, but in this way it is questionable whether the work of one worker may be closely compared with that of another. The method used by Edson and Carpenter was the addition of 2% thymol starch paste to a 10 day old broth culture. After incubation for 8 hours the culture was filtered and tested for reducing sugar by means of Fehling's solution.

Smith's method was used in this work. Cultures of the bacteria were grown on potato slants for ten days at 37° C. At the end of this time these slants were crushed in a mortar together with the water in the bottom of the culture tubes. This mixture was diluted with distilled water and tested with a weak solution of iodine in potassium iodide for the split products of starch. If the starchs mixture is not sufficiently diluted the blue color which this substance gives with iodine will entirely mask the color given by some of the decomposition products. The presence of erythrodextrin is shown by the presence of a red color. Good results may be obtained with this method after one has become accustomed to the color changes with are involved. This method has been fully described by Harding.[1]

1. Harding and Morse. The bacterial rot of Certain Vegetables. Technical Bulletin No. 11, 1909. New Agricultural Experiment Station, Geneva, N.Y. Page 268.

A few of the cultures were examined according to the method of Edson and Carpenter except that Kahlbaums phenol was used in place of the thymol. No reduction of Fehling's solution could be demonstrated even when large amounts of the sample were added.

None of the cultures studied were able to decompose starch. This is not to be expected when one recalls the strong proteolytic action of this group. Over forty-one per cent of these cultures were able to break up gelatin while none were able to split starch. This, however is in accord with the work of other investigators.

Agar Colonies.

Colony growth on agar was always abundant. After the colonies were well developed a large amount of soluble pigment was formed. The outline was always irregular and never entire. These colonies were not characteristic.

Gelatin Colonies.

All cultures gave abundant growth on this medium. The non-liquefiers grew on the surface and often spread over a large area. Fluorescence was very evident on this medium. The liquefying cultures gave large saucer-shaped colonies which were filled with a flocculent growth.

Gelatin Stab.

The medium used in this test was made from Gold Label French gelatin according to the Standard Methods for Water Analysis of the American Public Health Association, 1912. Twelve per cent of gelatin was added to plain peptone bouillon.

The medium was then put into test tubes to the depth of about seven centimeters. Inoculations were then made into these tubes by means of a platinum needle, stabbing to the bottom of the test tube. These cultures were then incubated for thirty days at 20° C. which is the incubation period suggested on the Descriptive Chart of the Society of American Bacteriologists which was used in this study.

At the end of thirty days fifty-nine of the cultures had liquefied the gelatin. The gelatin cultures of the other forty-one were left in the incubator for over eight months. During that time strains 7, 19, 67, and 83 liquefied the medium. With numbers 7 and 19, this liquefaction took place at the end of three months. The others required about six months for this change. The tubes were fitted with rubber caps to prevent evaporation. In determining the group number, however, only those strains were considered as liquefiers which produced liquefaction in thirty days. In all cases the growth seemed to be best at the surface, although it often extended down into the medium along the line of inoculation. At the end of six or seven days, fluorescence was noticeable. This soon extended throughout the tube with the greatest amount at the surface.

This characteristic is recognized as an important one in separating bacteria. The possession of a proteolytic enzyme very sharply sets a culture off from those which do not possess this enzyme. The method for determining this characteristic must be improved in order that comparable results may be secured by workers from the different laboratories.

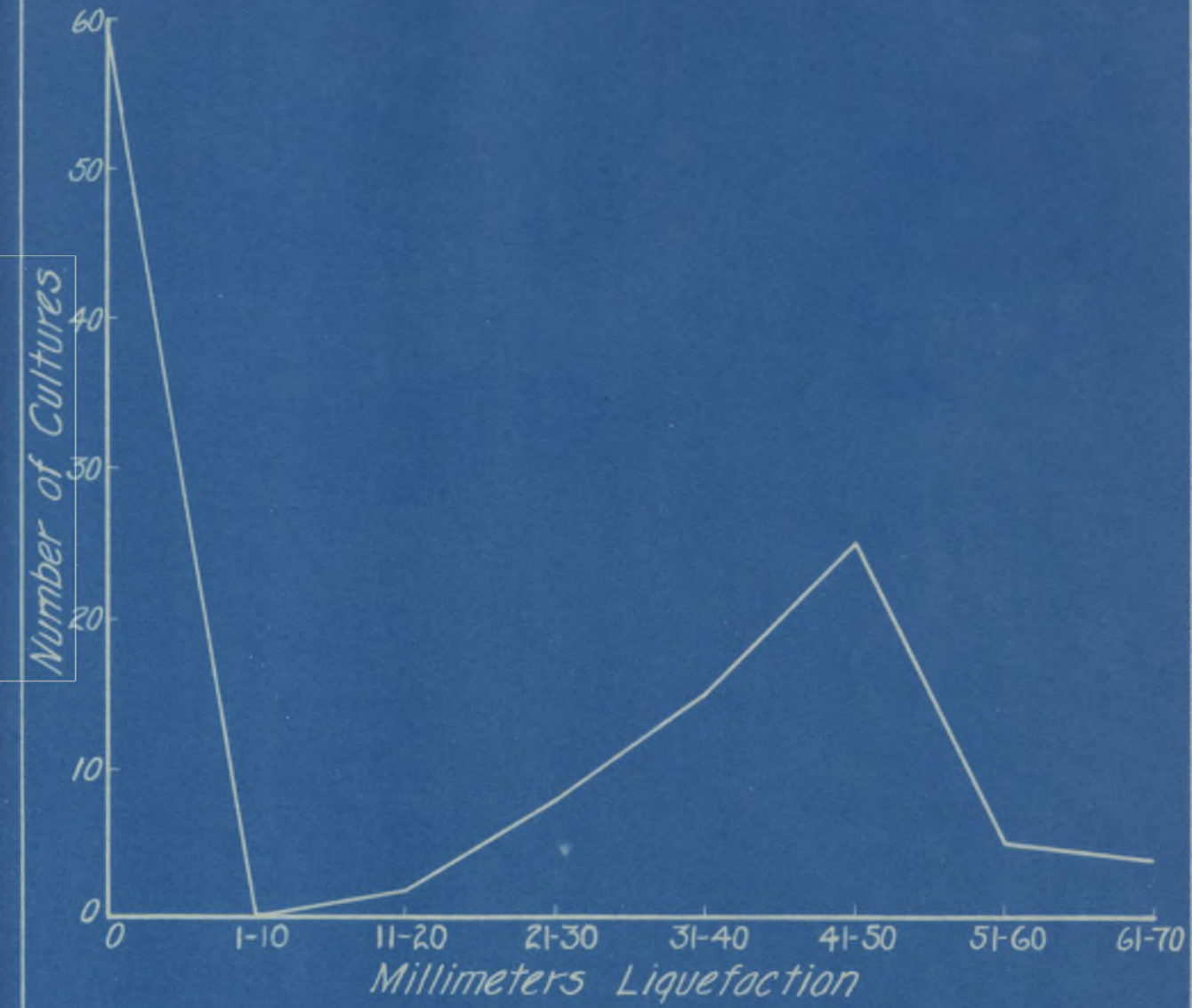
TABLE INDICATING AMOUNT OF GELATIN LIQUEFACTION.

Culture Number	Millimeters Liquefaction	Culture Number	Millimeters Liquefaction
1	0	27	30
2	30	28	30
3	45	29	39
4	0	30	28
5	0	31	35
6*	40	32	35
7	0*	33	0
8	0	34	39
9	0	35	20
10	35	36	0
11	0	37	56
12	42	38	0
13	35	39	0
14	25	40	0
15	45	41	0
16	40	42	0
17	65	43	0
18	0	44	0
19	0	45	50
20	65	46	42
21	40	47	40
22	47	48	20
23	60	49	0
24	70	50	29
25	40	51	0
26	50	52	60

Culture Number	Millimeters Liquefaction	Culture Number	Millimeters Liquefaction
53	40	79	50
54	47	80	45
55	44	81	46
56	50	82	42
57	27	83	0
58	47	84	0
59	0	85	0
60	65	86	0
61	0	87	0
62	0	88	0
63	0	89	50
64	0	90	0
65	0	91	0
66	0	92	44
67	0	93	45
68	40	94	24
69	0	95	35
70	50	96	44
71	43	97	60
72	40	98	0
73	0	99	0
74	0	100	42
75	50		
76	47		
77	53		
78	45		

*Liquefied in three months.

Mm. liquefaction	0	1-10	11-20	21-30	31-40	41-50	51-60	61-70
No. of cultures	59	0	2	8	15	25	5	4



INCIDENCE OF GELATIN LIQUEFACTION

Nutrient Broth.

The medium used in this study was made up according to Standard Methods of Water Analysis, 1912. Liebig's meat extract was used and the reaction was adjusted to neutrality with phenolphthalein.

Good growth was secured with all strains when grown in nutrient broth at 37° C. The medium was practically always cloudy with a pellicle and sediment. Varying degrees of fluorescence were manifested by the cultures. A few of the cultures were viscid and would string out when a platinum needle was used. This has been mentioned above under head of Staining Properties. The growth in nutrient broth was not characteristic.

Indol.

A one per cent solution of Witte's peptone was used in this test. None of the cultures formed indol from this medium. The tubes were inoculated from a twenty-four-hour broth culture and were incubated at 37° C. for ten days. At the end of this incubation period, 1 c.c. of paradimethylamidobenzaldehyde was added to the culture tube. A red color indicated the presence of indol. With those strains which produced a large amount of fluorescent pigment a red color was very often secured but this was not due to indol since the culture tubes did not have the characteristic odor of putrefaction.

Hydrogen Sulfid Formation.

To determin this characteristic, a special

medium prepared by Redfield[1] was used. This was prepared by adding 300 grams of Witte's peptone and 75 grams of potassium chloride to 700 c.c. of boiling tap water. This mixture is heated to dissolve as much peptone as possible. After this, the solution is cooled and diluted to one liter. This is again boiled, plugged with cotton and allowed to stand in an ice box for at least twenty-four hours. At the end of this time the medium is filtered and transferred to special flasks which are used in the routine analysis of water.

The method here used was a modification of Redfield's in that the flasks used by him in routine water analysis could not readily be adapted to the study of pure cultures. In his method 10 c.c. of his special medium is diluted to 100 c.c. in a special flask with 90 c.c. of the sample under investigation. This flask was fitted with a ground glass stopper which held the lead acetate paper. In order to adapt this medium to the study of pure cultures, it was made up one-tenth as strong as given above and put into a test tube fitted with a short piece of glass tubing in a one-hole glass stopper. A strip of lead acetate paper was held in this tube by means of cotton. Tin foil was twisted about the end of the glass tube. After inoculation from a twenty-four culture the tubes were incubated at 37° C. for thirty days. At the end of this period darkening of the paper indicated hydrogen sulfide formation.

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1. Redfield. A study of Hydrogen Sulfid Production by Bacteria and Its Significance in the Sanitary Examination of Water. Thesis, 1912. Cornell University.

Some of the strains studied in this series produced prodigious amounts of hydrogen sulfid. Cultures Nos. 45, 47, 48, 53, 12, 19, 23, 30, and 43 produced such an amount of hydrogen sulfid at 37° C. in thirty days that 2 cm. of a strip of paper 25 x 2 mm. was darkened. Forty-six of these cultures produced hydrogen sulfid from Redfield's medium.

Redfield has collected the names of those bacteria which have been reported to produce hydrogen sulfid. The following fluorescent bacteria are included.

In two days.

Bacillus fluorescens non-liquefaciens.

In three days.

Bacillus pyocyaneus.

In ten days.

Bacillus fluorescens liquefaciens.

In thirty days.

Bacillus cyanogenus. (Fluorescent?)

In varying lengths of time.

Bacterium immobile.

Pseudomonas fluorescens (Flügge)

Pseudomonas pilocyanea.

Edson and Carpenter secured hydrogen sulfidformation in twenty of the forty-two cultures which were isolated from maple sap. This gives a percentage of approximately 48 per cent while that obtained with cultures from water was 46 per cent. The method Edson and Carpenter consisted in suspending astrip of lead acetate paper above an ordinary broth culture. From

these two investigations it would seem that plain broth was about as efficient as Redfield's medium for the formation of hydrogen sulfid by bacteria. In a paper by Chamot and Redfield[1] on the same subject the statement is made that hydrogen sulfid is more rapidly produced in a culture in which several organisms are growing to-gether than by pure cultures. More work on this subject is promised. The peptone must be split down to abiuret compounds among which are cysteine and cystine. From these it is easy to imagine how H_2S could be split.

Sugar Broths.

No gas was formed by any of the cultures. This indicates that they are of no sanitary significance if gas formation is taken as an indication of this. The reaction in sugar broths was determined after two days incubation at 37° C. Five c.c. of the medium was diluted with 50 c.c. of distilled water and titrated after boiling with N/20 NaOH using phenol phthalein as the indicator. The table which follows presents the results which were secured. Rogers and Davis[2] comment on the value of these data for classification work. "Mention has already been made of the objections to the use of fermentation of sugars and similar substances. The question of the constancy of of these reactions has been the subject of investigation, and

1. Chamot and Redfield. Studies on the Culture Media Employed for the Bacteriological Examination of Water. Jour. Amer. Chem. Soc. 37 (1915) 1601-30.

2. Rogers and Davis. Methods of Classifying the Lactic Acid Bacteria. U.S. Dept. Agric., Bureau of Animal Industry. Bul. 154-1912.

while there is some disagreement of opinion of those who have studied the question most carefully seems to be that they are at least as constant as any of the characters ordinarily used in classification."

The rectangular polygons for the reactions in dextrose, lactose sucrose and glycerol follow the table which indicates the amounts of acid and alkali formed in these media.

With dextrose, all cultures formed acid. The polygons for the acidities divide the bacteria into three groups. A fourth group contains but one culture.

Thirty-nine cultures form acid in lactose broth. The acid formers are grouped closely about the neutral point and seem to grade into those which form alkali in this medium. No other distinct groups are made.

In sucrose broth, twenty-three form acid. Among the acid formers, five distinct groups are secured. Two of them, however, are made up of but one culture each. Among the alkali formers, there are two groups which are separated from each other by o.10 of alkali. Action on sucrose indicated that the fluorescent bacteria are not well differentiated.

With glycerol, seventeen cultures produced acid while eighty-three cultures produced alkali. The rectangular polygons are not very characteristic.

FERMENTATION OF CARBOHYDRATES

Per Cent of Acid or Alkali Formed in Carbohydrate Broths.

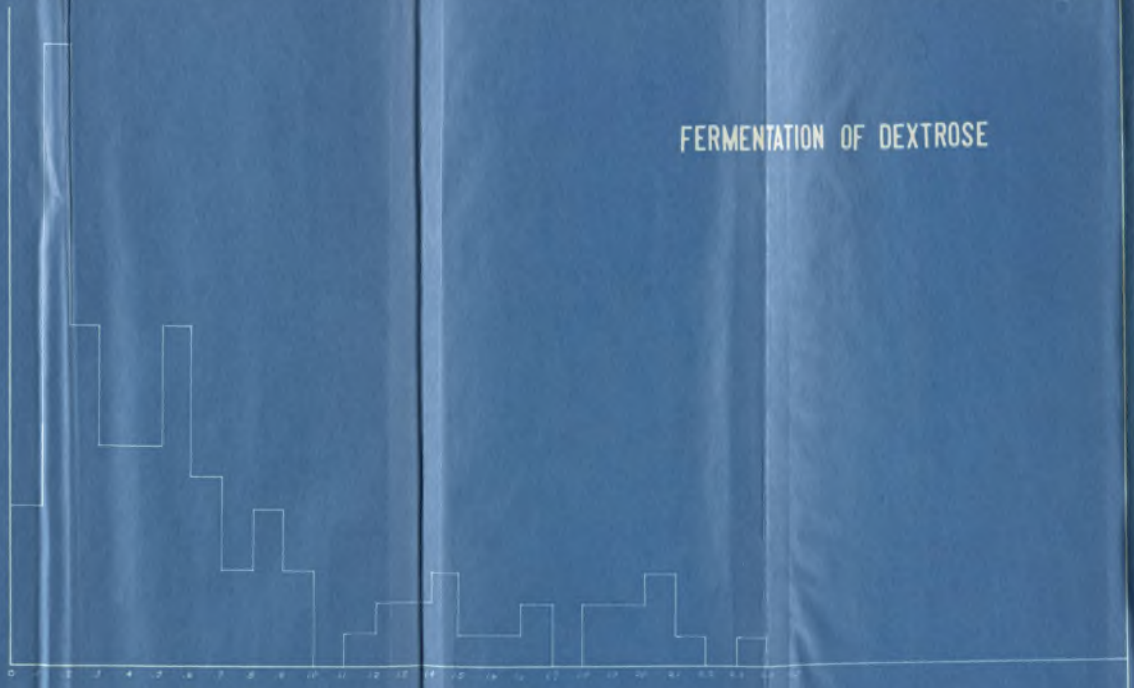
A = Alkaline

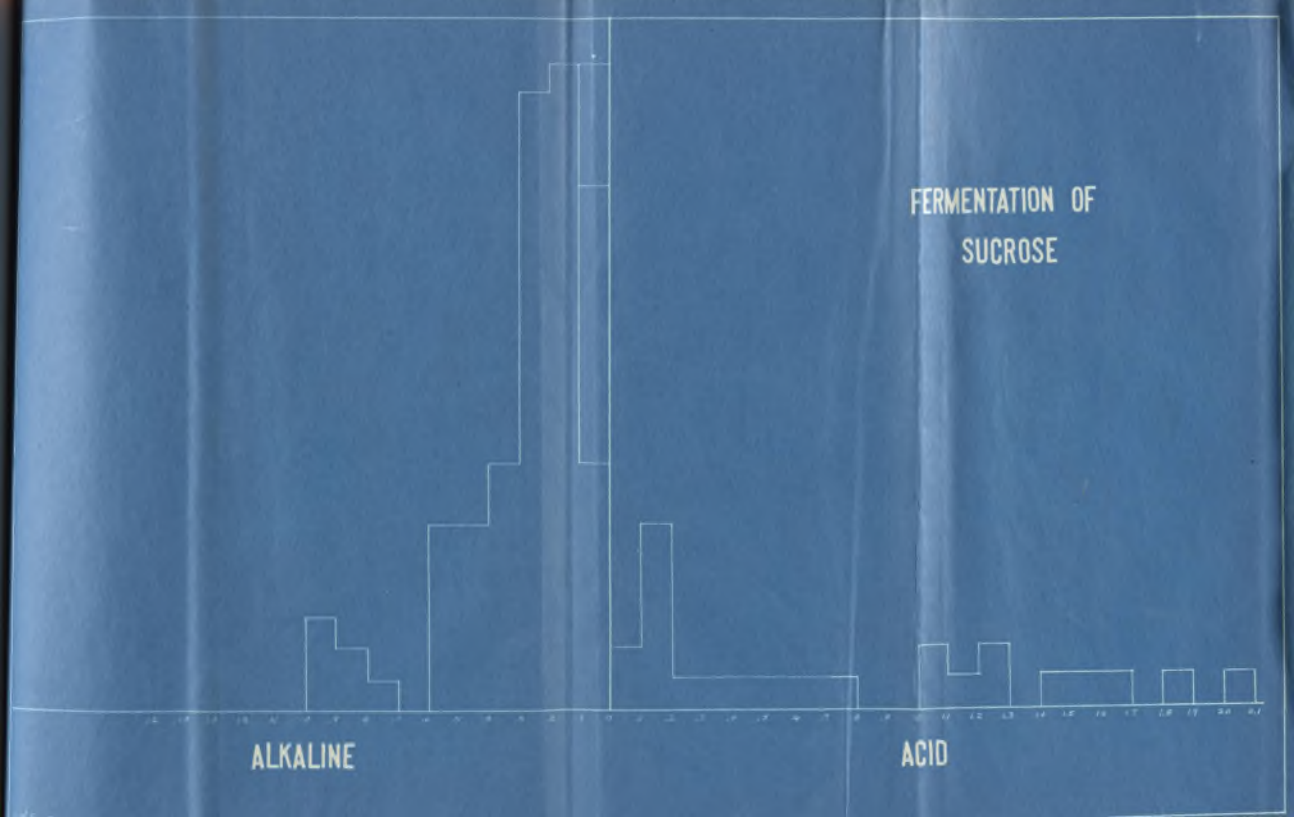
Culture Number	Dextrose Broth	Lactose Broth	Sucrose Broth	Glycerol Broth
1.	1.41	A 0.24	A 0.54	A 0.53
2.	1.55	1.34	1.63	A.0.51
3.	0.64	.32	.12	A 0.59
4.	0.86	A 0.22	A 0.40	A 0.49
5.	1.97	A 0.15	1.24	.39
6.	0.51	.11	A 0.28	A 0.46
7.	0.41	A 0.16	A 0.33	A 0.80
8.	1.26	A 0.20	A 0.24	A 0.64
9.	0.69	A 0.21	A 0.31	A 0.85
10.	0.75	A 0.19	A 0.23	A 0.63
11.	1.70	A 0.21	A 0.28	A 0.19
12.	0.61	A 0.19	A 0.23	A 0.26
13.	0.95	0.34	0.16	A 1.80
14.	0.55	A .34	A 0.10	A 0.60
15.	0.64	0.25	0.02	0.31
16.	2.45	A 0.34	A 0.58	A 0.73
17.	0.24	0.75	1.39	A 0.75
18.	2.16	A 0.36	A 0.54	A 0.69
19.	1.60	0.04	0.14	A 0.73
20.	0.31	0.03	1.04	0.36
21.	0.57	A 0.04	A 0.24	A 0.70
22.	1.21	0.52	0.02	0.41
23.	1.67	0.85	1.04	0.06

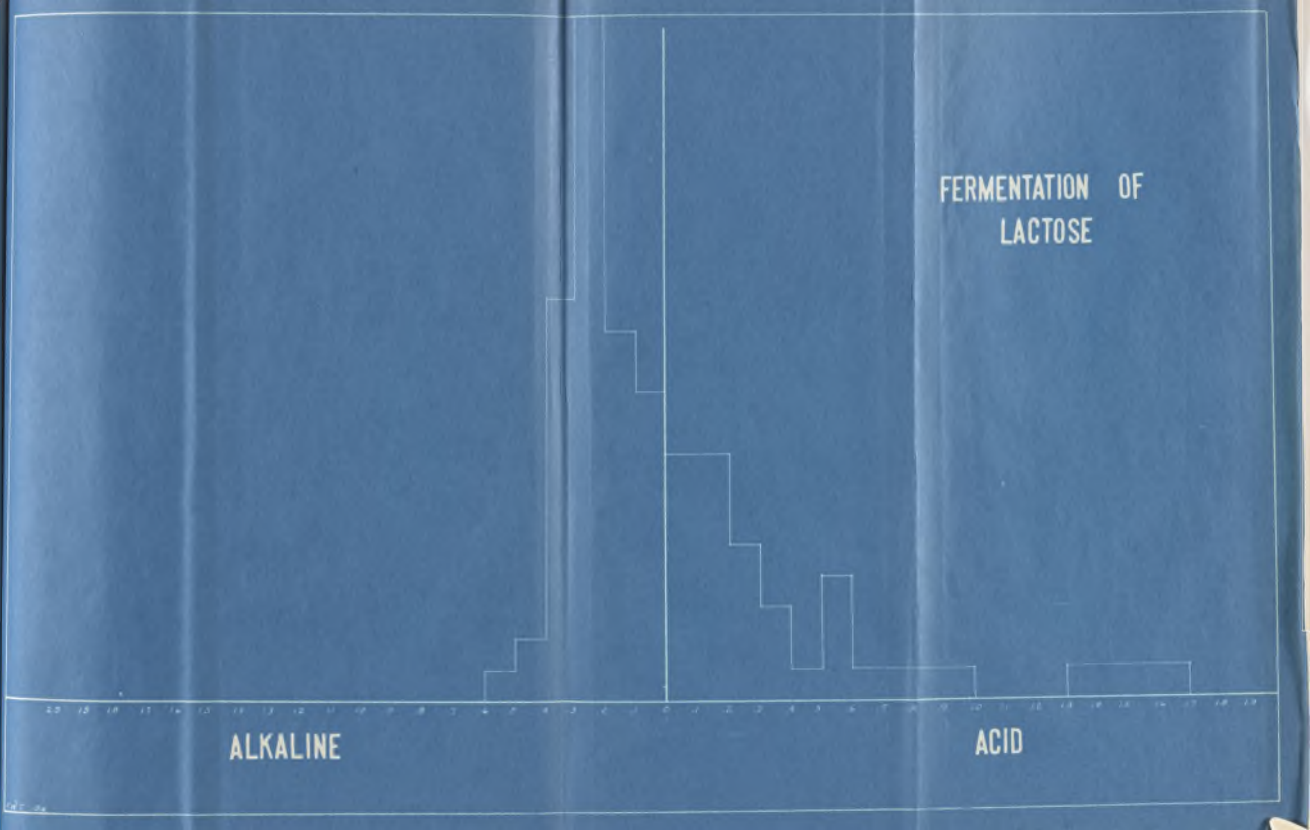
Culture Number	Dextrose Broth	Lactose Broth	Sucrose Broth	Glycerol Broth
52.	0.56	0.04	A 0.18	A 0.14
53.	0.29	A 1.86	A 0.13	A 0.65
54.	0.25	0.20	A 0.18	A 0.12
55.	0.17	A 0.28	A 0.07	0.05
56.	0.15	A 0.28	A 0.20	A 0.07
57.	0.15	A 0.28	A 0.15	0.49
58.	0.10	A 0.28	A 0.24	A 0.14
59.	0.40	A 0.18	A 0.33	0.06
60.	2.25	0.59	2.00	1.71
61.	0.00	A 0.31	A 0.27	A 0.21
62.	0.50	A 0.34	A 0.19	0.06
63.	0.85	A 0.33	A 0.28	A 1.07
64.	0.10	A 0.27	A 0.18	A 0.21
65.	0.04	A 0.32	A 0.30	A 0.20
66.	0.57	A 0.34	A 0.15	0.06
67	0.50	A 0.29	A 0.19	A 0.13
68.	0.75	0.05	A 0.12	A 0.34
69.	0.36	A 0.25	A 0.19	A 0.06
70.	0.35	A 0.20	A 0.40	A 0.28
71.	2.19	0.40	A 0.20	A 0.74
72.	1.49	A 0.10	A 0.10	A 0.28
73.	0.15	A 0.33	A 0.19	A 0.90
74.	0.11	A 0.36	A 0.16	A 0.09
75.	0.82	A 0.01	A 0.03	A 0.06
76.	0.11	A 0.08	A 0.18	A 0.31
77.	0.13	A 0.28	A 0.19	0.06
78.	2.00	1.51	0.59	A 0.61
79.	1.75	1.45	1.60	A 0.30

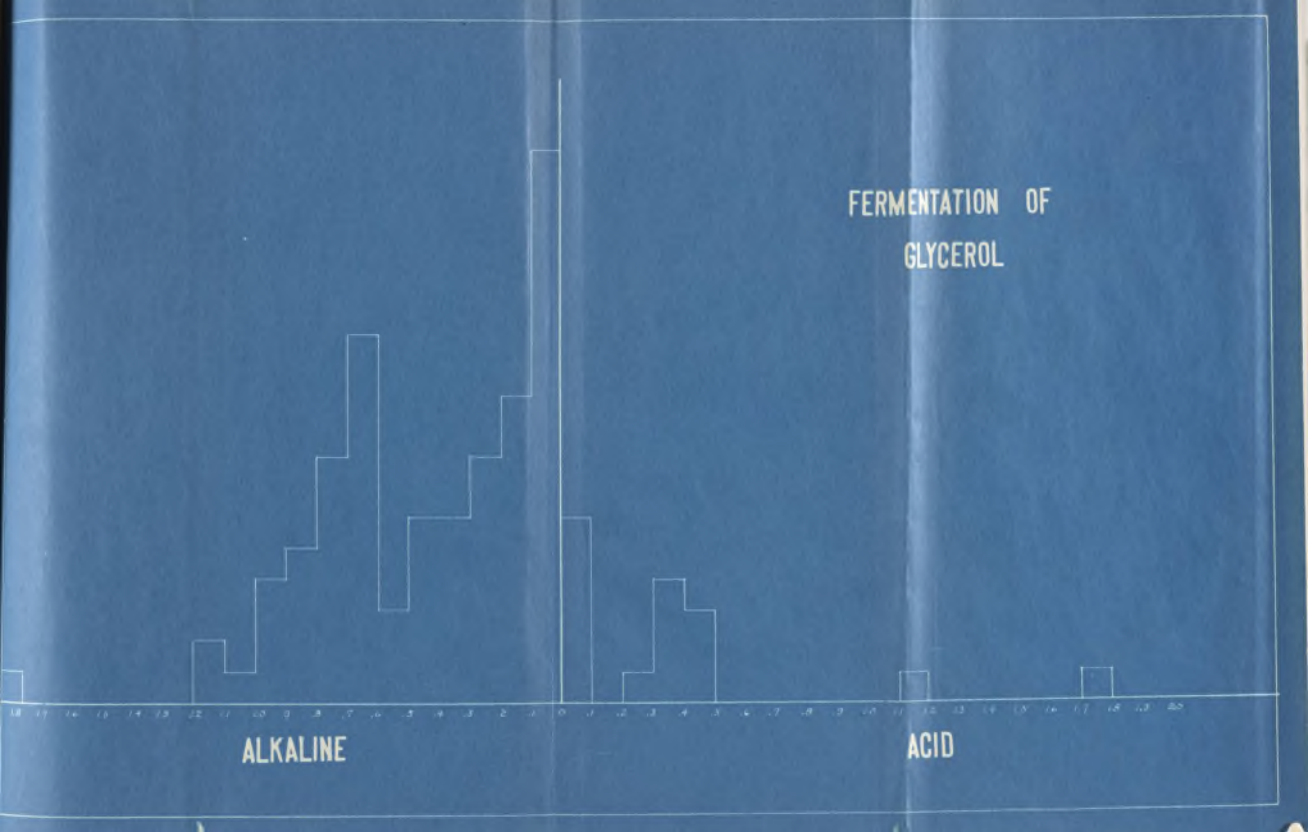
Culture Number	Dextrose Broth	Lactose Broth	Sucrose Broth	Glycerol Broth
24.	1.9	0.65	1.53	A 0.31
25.	0.71	0.10	A 0.05	A 0.09
26.	0.91	0.50	A 0.15	A 0.10
27.	0.80	A 0.19	0.28	0.49
28.	1.36	A 0.07	A 0.28	A 0.89
29.	1.13	0.11	A 0.32	A 1.13
30.	1.96	A 0.05	1.76	1.11
31.	2.14	0.12		A 0.83
32.	0.55	A 0.02		A 0.66
33.	1.37	1.60	1.92	A 1.15
34.	0.19	0.20	A 0.58	A 0.87
35.	0.39	A 0.23	A 0.49	A 0.90
36.	0.10	A 0.07	A 0.57	A 0.73
37.	0.50	A 0.05	A 0.38	A 0.39
38.	0.35	A 0.25	A 0.38	A 0.49
39.	0.67	A 0.25	A 0.18	0.36
40.	0.25	A 0.05	A 0.93	A 0.66
41.	0.40	A 0.05	A 0.92	A 0.49
42.	0.04	A 0.30	A 0.81	A 0.49
43.	0.21	0.95	A 0.92	A 0.62
44.	0.28	A 0.46	A 0.83	A 0.66
45.	0.17	0.10	A 0.49	A 0.49
46.	0.99	A 0.25	A 0.47	A 0.60
47.	1.41	0.09	A 0.58	A 0.99
48.	0.10	A 0.25	0.74	A 0.62
49.	0.40	A 0.58	A 0.58	A 0.70
50.	0.05	0.05	0.19	A 0.99
51.	0.20	A 0.48	A 0.24	A 0.79

Culture Number	Dextrose Broth	Lactose Broth	Sucrose Broth	Glycerol Broth
80.	0.45	0.54	0.30	A 0.20
81.	0.20	0.15	A 0.25	A 0.12
82.	0.12	0.11	A 0.05	0.28
83.	0.10	0.25	0.11	A 0.09
84.	0.66	A 0.28	A 0.06	0.00
85.	0.22	A 0.28	A 0.14	A 0.22
86.	0.06	0.15	A 0.15	A 0.09
87	0.52	A 0.30	A 0.10	A 0.09
88.	0.17	0.05	A 0.11	A 0.09
89.	0.16	0.20	A 0.07	A 0.03
90.	0.29	A 0.26	A 0.12	A 0.08
91.	0.30	A 0.12	A 0.21	A 0.09
92.	0.16	0.05	A 0.07	A 0.07
93.	0.86	0.30	0.49	A 0.06
94.	0.42	A 0.15	A 0.14	A 0.17
95.	0.16	0.16	A 0.08	A 0.09
96.	0.27	0.15	A 0.29	A 0.16
97.	0.18	A 0.32	A 0.75	A 0.04
98.	0.35	A 0.16	A 0.20	A 0.04
99.	0.41	A 0.20	A 0.24	A 0.14
100.	0.50	0.00	0.19	A 0.07


FERMENTATION OF DEXTROSE
ACID


FERMENTATION OF
SUCROSE
ALKALINE
ACID


FERMENTATION OF
LACTOSE
ALKALINE
ACID


FERMENTATION OF
GLYCEROL
ALKALINE
ACID

Uschinsky's Medium.

This medium was made up after the following formula and was sterilized in the Arnold on three successive days:

Distilled Water	1000 c.c.
Glycerol	35 gms.
Sodium chloride	6 "
Calcium chloride	0.1 gms.
Magnesium sulfate	0.35 "
Di potassium phosphate	2.00 "

Vigorous growth was secured in this medium in all strains except No. 10. In many cases the medium was strongly fluorescent. A pellicle was formed by many strains and many of them exhibited a very viscid growth.

Frankel's Medium.

This medium was prepared after the following formula:

Sodium chloride	5.0 gms.
Monocalcium phosphate	3.0 "
Ammonium lactate	6.0 "
Asparagin	4.0 "
Distilled water	1000.0 c.c.
N NaOH	20.0 c.c.

The medium was filtered and sterilized in the Arnold sterilizer on three successive days.

All strains grew abundantly in this medium.

Sullivan's Medium.

This medium[1] was found to support good pigment formation:

Asparagin	1.00%
Magnesium sulfate	0.20%
Dipotassium phosphate	0.10%

Growth in this medium was abundant but did not reach a maximum as quickly as with Frankel's medium. None of these strains could reduce the magnesium sulfate to hydrogen sulfid.

Oxygen Relations.

All cultures used in this study were found to be facultative anaerobes. The test was carried out in a vacuum dessicator. Into the bottom of the dessicator was placed about fifty grams of pyrogallol. The cultures were made on agar slants and placed in the dessicator; and by means of a vacuum pump the pressure was reduced as far as possible under these conditions. After this was done a strong solution of KOH was allowed to be sucked into the bottom of the dessicator by slightly opening the glass stop cock. By this technique it is believed that a minimum supply of oxygen was left in the dessicator.

The dessicator was incubated at room temperature which was about 25°C. for five days. At the end of that time there was visible growth. It was very restricted but was quite apparent. Some doubt was felt with regard to these results since these bacteria have always been described as strict aerobes; but

1. Sullivan: Loc. cit.

since growth was quite visible, the group number is determined on the ground that they are facultative anaerobic and are able to carry on a limited activity under reduced supplies of oxygen.

Plain Milk.

This medium was made by thoroughly mixing 100 grams of Merrill-Soule skim milk powder in 1000 c.c. of distilled water. This was then beat with an egg beater and filtered. The medium was sterilized in 75 c.c. quantities of Erlenmeyer flasks. Inoculations were made from twenty-four hour cultures and the flasks were incubated at 37° C.

The fluorescent bacteria seem to arrange themselves into four general groups with regard to their action on this medium.

One group is made up of those cultures which produce prompt coagulation in two days. Cultures No. 1, 5, 13, 16, 17, 18, 23, 24, 30, 31, 39, 57, and 60 coagulated the milk in this time. The curd formed was solid in consistency and in many cases settled to the bottom of the flask. If peptonization took place, this curd was decomposed until the whole flask had assumed a golden color.

Another group produced clearing without coagulation. The rapidity of this varied. With some of the cultures this was evident after twenty-four hours incubation at 37° C. This usually started at the surface - a dark transparent layer being formed which rapidly extended downward. With a few of the cultures, however, the entire contents of the flask seemed to change to a thinner consistency. The final decomposition product was a golden color. The green pigment which was often produced in

large amounts in this medium together with this golden color gave a solution which was highly colored.

The third type was made up of those cultures which seemed to have no effect on this medium in twenty days. These cultures were not held longer than this time but it is possible that some change might have been secured.

The fourth group consisted of those cultures which rendered this medium slimy. This was very apparent after twenty days. Those cultures which possessed capsules always produced a slimy growth in broth and milk.

The Chart of the Society of American Bacteriologists calls for the titration of the reduction at the end of one, two, four, ten, and twenty days. This was done with these cultures but erratic results were obtained. These were due to the fact that the medium was so highly colored with the pigment and decomposition products from the casein that it was impossible to determin the real end point with phenolphthalein. It was masked by these substances. The green pigment seemed to be the greatest factor. Another possible reason why varying results were obtained is that phenolphthalein is not sensitive in solutions which contain ammonia. Much ammonia is formed in the splitting of casein. Were the solution boiled to remove carbon dioxid, this might boil off some of the volatile compounds and cause a greater error than the carbon dioxid would cause.

For determining the group number, the reaction at the end of the tenth day was taken. By that time a constant reaction had been established and continued incubation simply increased it

in the same direction.

Litmus Milk.

This medium was prepared after the same method that was used in the preparation of plain milk. Kahlbaum's azolitmin was used as the indicator. The milk was inoculated from a twenty-four-hour broth culture by means of a platinum loop, and incubated at 37° C. for about twenty-five days. A detailed account of all the changes in this medium will not be included. In many cases the tubes turned alkaline at the surface and gradually extended downward until the whole tube was changed. When peptonization occurred, this continued until the whole tube had turned to an orange color.

Many of the strains possessed no proteolytic enzyme and thus produced no change in the milk. Some of the strains formed alkali which soon changed to a permanent acid. In many cases the curdling of the milk could not be entirely explained by the concentration of the acid. It was doubtless due to the presence of a rennin-like enzyme.

Temperature Relations.

The temperature relations of any group of bacteria are very important. It is believed by some that since an organism is found in nature, that it has its optimum temperature or grows best at temperatures near 20° C. and that growth is restricted at 37° C. The literature on fluorescent bacteria indicate that very few of these forms refuse to grow at 37° C. All the cultures used in this study were found to grow well at this temperature.

The amount of change brought about by bacteria may be said, generally speaking, to be a function of the incubation temperature and period of incubation. This again reverts to the laws which govern enzyme action and the relation of temperature thereto.

Fluorescence.

This characteristic was necessary in order for a culture to be included in this study. It is closely related to the pigment which these bacteria form. Some bacteriologists have attempted to separate Bacillus pyocyaneus and Bacillus fluorescens liquefaciens on the ground that B. pyocyaneus possessed no fluorescent pigment. This separation is probably more apparent than real. There were all gradations among the cultures of this series with regard to this characteristic. Those formes which liquefied gelatin and casein produced more fluorescent pigment than those which did not break up these two compounds. This might have some bearing to show that the pigment is an excretion - a substance of no value to the bacterium because the more food that was used up would cause an increase in catabolic products.

A little work has been done on this pigment which simply confirms some work which appears in the literature. This is a subject of sufficient magnitude for a separate research.

The Pigment.

This group has been selected for study solely on account of its ability to form a diffusible fluorescent pigment. It is easily recognizable that by selecting this group

in this way that members of other groups have been included.

Why these bacteria form these pigments is not known. It may be merely a waste product which happens to be colored. On the other hand, it may serve its purpose in protecting the bacterial cell form harmful influences in the same way that certain animals have of excreting a substance which is obnoxious to other animals. Much work has been done on colors in nature and also directly on the pigment produced by this group.

Color in Nature.

This subject has been studied by many investigators. The theories with regard to color or pigment formation are reviewed by Sullivan[1]. He divides pigments into two divisions - structural and pigmental. Pigments in nature are divided into:

A. Pigments of direct importance, as in respiration.

B. Derivatives of such pigments.

C. Waste products, or derivatives of such.

D. Introduced pigments.

E. Reserve pigments or pigments associated with reserves.

Sullivan also reports Beyerinks[2] division of chromogenic bacteria. This is as follows:

A. Chromophorous Bacteria - forms in which the pigment serves some purpose in the cell as the chlorophyll.

B. Chromoparous Bacteria - forms which excrete the pigment as a waste substance.

C. Parachrome Bacteria - forms which retain the pigment in their cells.

1. Sullivan - Synthetic Culture Media and the Biochemistry of Bacterial Pigments. Jour.Med.Res. 14 109-160 1905. 2.(next page)

The subject of bacterial pigments has been much discussed. Only a few of these publications will be mentioned here. Wasserzug[1] studied B. pyocyaneus or the organism of green pus and found that in the same cultures not all the cells produced the same pigment. To avoid this as far as possible, he worked with cultures which had been rejuvenated by successive transfers and inoculations into rabbits. Wasserzug states, "Il semble qu' on puisse distinguer deux periodes dans la vie de l' organisme colore: dans la premiere it prefere et accommode a ses besoins son milien de culture; dans la seconde, it produit et secrete, la matiere colorante. He tried the effects of antiseptics and found that the points where pigment formation and growth stopped, differed but slightly.

This would seem to indicate that the colored pigment was merely a waste product of the cells' metabolism.

Babes[3] studied the colored materials from B. pyocyaneus. To secure his pigments he inoculated neutral peptone gelatin with a strain coming from an abscess. The media was inoculated and left for six weeks at room temperature. The color varied and an odor of linden flower was noticeable. Babes used solvents and distillation methods to remove his pigment. Pyocyanin of Fordos was probably secured which was blue in alkaline and red in acid solution. It crystallizes in rhombic crystals. A red-brown

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2. Beyerink - Die Lebengeschichte einer Pigment bacterie Bot. Zeit. (1891)

1. Wasserzug - Sur la formation de la matiere colorante chez le Bacillus Pyocyaneus. Ann L'Inst Pasteur I (1887)581-591.

3. Babes - Note sur quelques matieres colorantes et aromatiques produites par le bacille pyocyanique Comp. Rend. Soc. Biol. (9th Series 1) 41 (1889) 438-9.

substance was also secured. This was greenish in refracted light, soluble in H_2O and insoluble in chloroform. In acid solution fluorescence is lost but is taken on again in alkaline solution.

By distillation, a colorless substance with a peculiar odor was obtained. This differed from the original and was supposed to be a decomposition product.

Thumm[2] found that all species produced the same pigment which differs with the reports of other investigators. He was unable to confirm the findings of others that several pigments were produced. He found that this group was made up of vigorous alkali formers and that dextrose was fermented to acids which were neutralized later by a formation of ammonia.

Boland[3] believes that two pigments are formed, a fluorescent one which is formed by many others and pyocyanin which goes to a red brown pigment by oxidation.

Krause[4] studied the symbiosis of B. pyocyaneus with pus formers and found that the aromatic odor was almost always

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Charrin & Nittis - Sur la production simultanee des pigments noir, bleu, vert, jaune par un bacille pyocyanique Compt. Rend 1 Series 10 Vol. 5 (1898) 721.

2. Thumm - Beitrage sur Biologie der fluorescirenden Bakterien. Arbeiten aus dem Bakt. Inst. der Technischen Hochschute zu Carlsruhe Band I (1895) 291-377. Reviewed by Smith - Bacteria in Relation to Plant Diseases p. 238.

3. Boland - Uber Pyocyanin den blanen Farbstoff des Bacillus Pyocyaneus. Cent. f. Bakt. 25 (1899) 897-902.

4. Krause - Beitrage zur Kentniss des Bacillus pyocyaneus Cent. f. Bakt. 27 (1900) 769-775.

present and that as long as B. pyocyaneus predominated over the pus formers, the green color did not appear. When B. pyocyaneus cells were removed from the culture, they again formed the green pigment. Certain gases were tried and with hydrogen, good growth was secured but no pigment. With CO_2 and a vacuum, no growth was secured. In his study of the pigments the following were secured:

1. Water extract	Greenish yellow fluorescence
2. 80% alcohol	Yellowish green "
3. Glycerol	Blue-green
4. Amyl alcohol	Grass green
5. Chloroform	Blue
6. Ether	Yellow blue

Nogier[1], Dufourt and Dujol studied a strain from a lesion and found a red pigment of the color of vinegar along with the fluorescent and red-brown pigment usually described. This red pigment was formed in glycerol broth on the fourth day. Acid and alkali seemed to suppress pigmentation as did pure oxygen.

Gessard[2] believed that two pigments were formed, a fluorescent green and pyocyanin.

Sullivan[3] comes to opposite conclusions and differs with Gessard. Sullivan finds that pyocyanin formation is independent of the presence of phosphate or sulfate. He did find, though,

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1. Nogier, Dufourt and Dujol - Contribution a l' etude des pigments du bacillus pyocyaneus - Jour. Physiol. path. gen. 15 (1913) 633-635.

2. Gessard - Des Races du Bacille Pyocyanique. Ann. l' Inst. Past. 5 (1890) 65.

3. Sullivan- Synthetic Culture Media and the Biochemistry of Bacterial Pigments. Jour. Med. Res. 14 (N.S. No.9) (1905) 109-160.

the phosphorus and sulfur were essential. He recognizes a fluorescent pigment and pyocyanin and states that the same variety of B. pyocyaneus may be made to take up either of these functions or both. Sullivan believes that "the production of pigment is not an essential vital act. As it is of no discoverable advantage to the organism possessing the power of producing it, its production is purely accidental."

A little work has been done on this pigment. A four liter flask of plain broth was inoculated with strain No. 37 and left for eight weeks at a temperature of about 25° C. at the end of that time the medium had assumed a dirty green color with a heavy precipitate in the bottom of the flask. When the flask of broth was tested for growth, it was found that there was a large number of bacteria.

This culture was filtered through paper into a large bottle from which different portions were taken for study.

About one and one-half liters were precipitated with lead acetate and allowed to stand over night. In the morning this was filtered and divided into three portions. Each of these was extracted with ether, chloroform and ligroin. The chloroform was the only solvent which removed any of the pigment. It removed the blue pigment. Subsequent shakings in a separatory funnel continued to remove small amounts of the blue pigment. In four or five days, this chloroform solution would change to a red if left in bright light. No reagents seemed to be able to change it back to the green color. In the dark, the blue chloroform solution would change from a deep blue color to

a dark green which was permanent.

Some of this chloroform solution was allowed to evaporate in a crystallizing dish. A black residue was left which had a very strong odor similar to some of the aromatic ammonium bases. This substance was soluble in alcohol and water, insoluble in ether and red in acid solution. Neutralization restored the green color again.

The work here reported agrees with that reported by the early authors. Ledderhose[1] believes that this pigment which has been called pyocyanin belongs to a group of aromatic substances closely related to the anthracene group.

It was thought that this pigment might be related to anthocyanin which is supposed to have some relation to the flavone or anthone groups. This may be in the form of a colorless glucoside in the plant and capable of oxidation only after it has been liberated. Repeated tests by Molisch's reaction failed to demonstrate its glucosidal character either before or after hydrolysis.

Reduction of Nitrates.

This test was made on media of the following composition:

Distilled Water	1000 c.c.
Witte's peptone	1 gm.
Potassium nitrate	3 gm.

The medium was sterilized in small Erlenmeyer flasks

1. Ledderhose. Ueber den blanen Eiter. Dentsch. Zeitschr. f. Chirurgie. 28 (1888) 201. Not.Seen. Cent. f. Bakt. 4 (1888) 432-4.

holding about 30 c.c. These were inoculated from a twenty-four hour broth culture and incubated for five days at 37° C. At the end of this period, 1 c.c. of the nitrate broth culture was removed by a sterile pipetted and diluted to 50 c.c. in a nessler tube with N free water.

The method of testing for NO_2 was that usually used in a water laboratory and it is believed that it is not too delicate for bacterial work if blank determinations are made. To each of the nessler tubes prepared above was added 1 c.c. of an acid solution of naphthylamine hydrochloride and 1 c.c. of a saturated solution of sulfanilic acid. The tubes were allowed to stand for thirty minutes in the colorimeter before being examined for presence of NO_2. Controls were always examined at the same time in order to check the reagents and apparatus.

Fifty-one of the cultures studied reduced the nitrate broth given above when incubated for five days at 37° C.

Ammonia Production.

This was examined for in the same cultures in nitrate broth in which nitrate reduction was detected. At the end of ten days 2 c.c. of this broth culture was withdrawn and diluted to 50 c.c. with N free water. Nessler's reagent was added to the tubes and control tubes and comparison made in a colorimeter used in determining N in water analysis.

Fifty-two of the 100 cultures produced ammonia in ten days at 37° C. Probably, if plain nutrient broth had been used to test for ammonia production all strains would have been found to produce ammonia. The plain broth cultures were usually alkaline to phenolphthalein.

Peptonization of Casein.

With most of the cultures, casein peptonization was apparent in the flasks used in the titration of the acidity in milk, but with the others it was impossible to tell whether this medium was attacked. In order to determin this character Hastings'[1] milk agar was used. This was prepared by cooling a tube of melted agar and adding to it 1 c.c. of sterile skimmed milk. This was then poured into a sterile Petri dish and thoroughly stirred. When cool, it was inoculated by making streaks of the cultures on the surface, after which the plates were incubated for five days at 37° C. A clear zone appeared around the line of inoculation on those plates which were inoculated with these cultures which peptonized the casein. To verify this dilute acetic acid was added to the plate and if the zone remained clear, the culture was recorded as one which would peptonize casein.

Forty-two cultures of the series were able to decompose casein. This of course was more marked with a few of the forms than with the majority.

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1. Hastings. The Action of various classes of Bacteria on Casein as Shown By Milk Agar Plates. Cent. f. Bakt. II 12 (1904) 590-93.

Vitality of Fluorescent Bacteria.

The members of this group seem to be able to resist very unfavorable conditions. No special experiments have been made but much evidence is available from a number of sources.

Broth suspensions of Bacillus pyocyaneus were suspended in the Urbana septic tank and daily counts made. These increased regularly until the end of the period which indicates that this bacterium is able to live in such an environment.

It has been noticed that tubes of culture media which spoiled in the ice chest were very often infected with these bacteria. Thus it is apparent that these bacteria are able to resist high temperatures for short periods. It is well known among physicians that once a hospital is infected with Bacillus pyocyaneus, it is disinfected with great difficulty.

Cultures of these bacteria live for a long time on laboratory media. Agar slants which have been allowed to dry for six months at room temperature were found to support living fluorescent bacteria. Cultures of strains Nos. 22 and 37 were left for a year and a half with very infrequent transfers and each case good growth was secured from these old cultures. These cultures did lose some of their pigment producing property.

Rettger and Sherrick[1] report a type of resistance by a member of this group. They state that an old culture of Bacillus pyocyaneus began to lose its property of producing the green pigment. An agar slant of this bacterium was placed on the top

1. Rettger and Sherrick. Studies on Bacterial Variation. Jour. Med. Research N.S. 19 (1911) 265-84.

of an incubator in April and left until October. When examined at this time the agar was dried to a hard mass and it was thought unlikely that any living bacteria were present. Transfer to fresh media, however, give good growth and much pigment was finally produced.

Birge and Neill[1] in a paper on the comparative resistance of fluorescent and non-fluorescent bacteria to ultra-violet light conclude that fluorescent bacteria are better able to resist ultra-violet light than those which do not produce this pigment. Their cultures of fluorescent bacteria were secured from those which form the basis of this study. The following table indicates the results which they secured with the fluorescent bacteria. The numbers in parentheses are those which the cultures bear in this paper; the other numbers are those which Burge and Neill assigned to the cultures.

	Numbers of Bacteria per ccm. Fluorescent Bacteria						
Time of Exposure	1 (2)	2 (3)	3 (13)	4 (15)	5 (22)	6 (25)	7 (37)
0 Sec.	72 mill	80 mill	28 mill	70 mill	75 mill	33mill	25 mill
20							
40							
60	750	813	55	210	460	12	88
80	459	623	29	79	211	10	20
100	223	221	25	56	113	7	14
120	128	102	20	29	103	5	12
140	97	88	15	18	56	4	8
160	81	73	12	13	38	3	2
180	43	29	10	6	25	3	1
2000	31	15	3	3	11	2	1

1. See following page.

This table indicates that these bacteria have a greater resistance to unfavorable conditions than do many other common forms. In order to secure some basis for comparing this character, these authors subjected such common forms as Bacillus colon communis, Pseudomonas violacea, Sarcina lutea and Bacillus proteus vulgaris which are not fluorescent to the effect of the ultra-violet light. In no case were any of these bacteria able to resist the effects of the ultra-violet light for more than 200 seconds. Most of them could not survive an exposure of 160 seconds. With the fluorescent bacteria, however, the results are quite different. In each case a few cells were found to be alive at the end of the 200 seconds. Curves accompany the paper by these investigators which indicate the death rate is according to the monomolecular law. Under these conditions the fluorescent property enables the bacteria to withstand unfavorable conditions. Burge and Neill explain this resistance of fluorescent bacteria to ultra-violet light by assuming that "the fluorescent bacteria protect themselves from the coagulating effect of the ultra-violet light by converting the short wave lengths to longer waves and hence dispose of energy of the absorbed short waves." Non fluorescent bacteria were unable to do this.

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1. Burge and Neill. The Comparative Rate at which Fluorescent and Non-fluorescent Bacteria are killed by Exposure to Ultra-violet Light. American Journal of Physiology 38 (1915) 399-403.

Division of Strains into Groups.

The group number allows the following separation of strains:

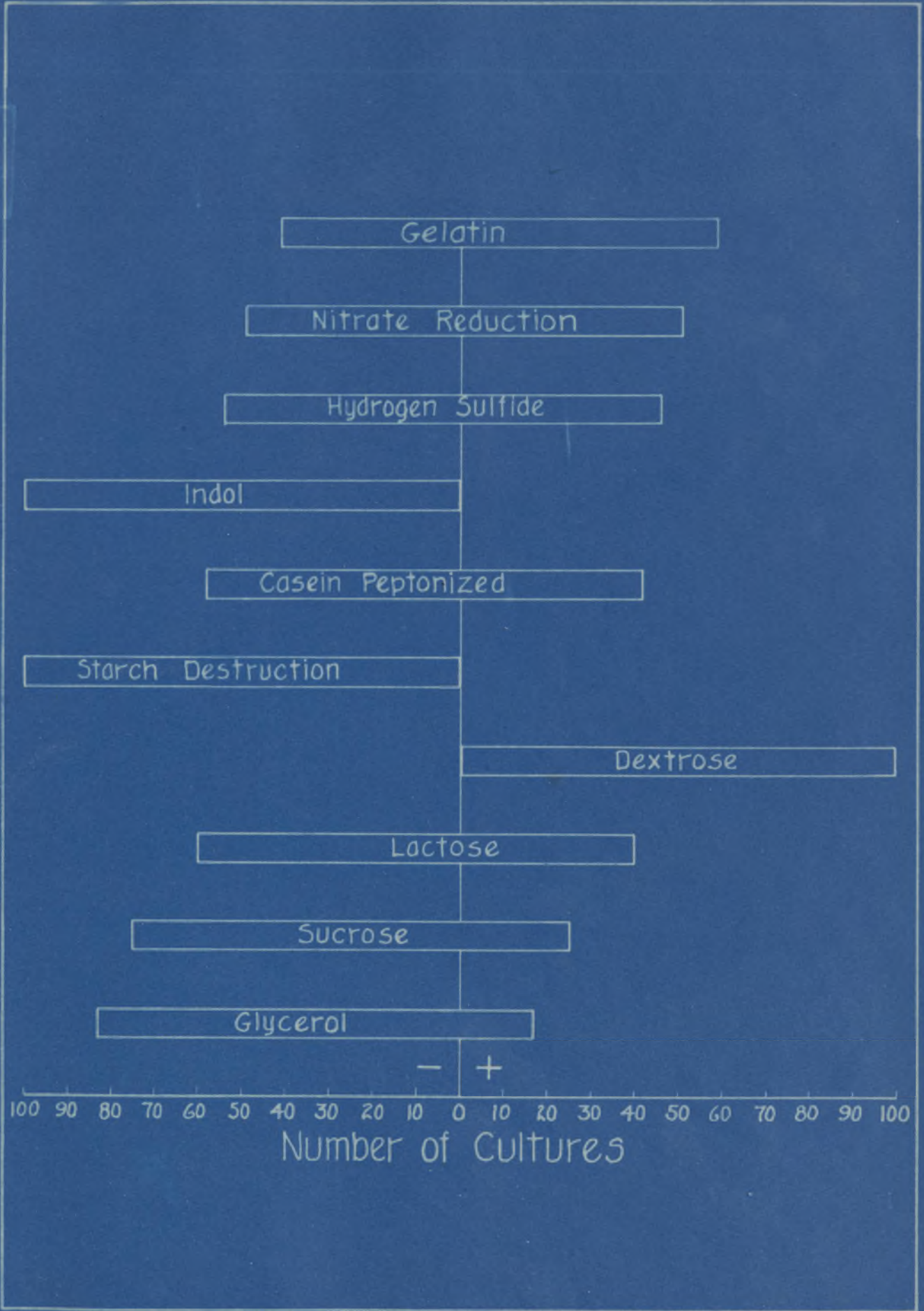
121.2332133	61, 45
121.2333133	47
122.2333133	70
221.2222132	22, 15
221.2222133	13
221.2223132	23, 20
221.2223133	24, 17, 2, 50, 31, 3
221.2232133	34, 6, 88, 87, 86
221.2233133	71, 29, 26, 25
221.2322132	27
221.2323132	30
221.2323133	48
221.2332132	62, 59
221.2332133	99, 91, 94, 85, 84, 73, 46,28,21 16, 14, 10, 74
221.2333132	69, 66
221.2333133	98, 90, 75, 72, 67, 64, 63, 37 35, 32, 12
222.2222133	19
222.2223132	60
222.2223133	93, 83, 80, 79, 78, 33
222.2232133	95
222.2233132	82

222.2233133	92, 68, 81, 52, 96, 89
222.2322132	5
222.2323133	100
222.2333132	39, 57, 55
222.2333133	97, 77, 76, 56, 54, 53, 43, 9 8, 7
222.2332133	58, 51, 49, 44, 42, 41, 40, 38 36, 18, 11, 4, 1

This method of separating these strains makes twenty-seven groups. Many differ by but one characteristic from those closely related to it. With the group number as it now stands it is possible to obtain 276,480 different bacteria. This large number of possibilities allows ample opportunity in applying the group number to include the known forms.

The following table presents a brief characterization of the action of these cultures on some of the more important test substances. Such a table has been used by Rogers and Davis.[1]

1. Rogers and Davis. Loc. cit.


Gelatin
Nitrate Reduction
Hydrogen Sulfide
Indol
Casein Peptonized
Starch Destruction
Dextrose
Lactose
Sucrose
Glycerol
−
+
100 90 80 70 60 50 40 30 20 10 0 10 20 30 40 50 60 70 80 90 100
Number of Cultures

Discussion of Results

A comparative study of one hundred cultures of fluorescent bacteria by means of the group number as expressed on the Descriptive Chart of the Society of American Bacteriologists places them in twenty seven groups. Twelve of these twenty seven groups are made up of but one culture. In several cases they are separated by simply one characteristic. For instance the greater number of these cultures form alkali in glycerol broth. The acid formers may differ from those which form alkali by this one determination which allows a different group number.

Four of the cultures form endospores. This character has been accepted, for some time by bacteriologists, as a reliable and important basis for separating bacteria. DeBary placed so much importance on this that he described minutely the formation and germination of spores. This characteristic is sufficient basis for believing that the fluorescent bacteria do not form a genetic group.

Gelatin liquefaction has been considered of sufficient importance in classification work. This, in the past, has been taken as the sole difference between certain members of this group. To liquefy gelatin, a bacterium must possess a proteolytic enzyme; this is of importance since it determins, to some extent the food for the bacterium. The one hundred cultures forming the basis of this study were about evenly divided with respect to this characteristic. Gelatin liquefaction paralleled casein digestion closely. Sixteen cultures

which liquefied gelatin failed to split casein. Of the cultures which liquefied gelatin, the greater number liquefied between forty and fifty millimeters.in a test tube. The others graded away from this group. From this work, one must infer that the test, as it is now made, is not sufficiently delicate for classification purposes. Those cultures which required four or five months to liquefy might be regarded as the links between the liquefyers and the non-liquefyers. Investigation with regard to the best technique for determining this character are urgently needed. The percentage of gelatin to be used must be determined, the composition of the medium and possibly some other method of determining liquefaction than the eye.

These cultures are reported as facultative anaerobes. This is another determination called for in the group number which has no satisfactory technique. Formerly, much importance, in this connection was attributed to growth along the line of inoculation in stab cultures. This is beset with too many objections for unlimited application. Media, itself may contain sufficient dissolved oxygen to support growth. Evidence of growth in the closed arm of the fermentation tube has also been used to determin anaerobiosis. This limits the determination to the particular substance from which the oxygen was taken. A standard technique for this determination would have much significance.

The fallacies in recording diastatic action on starch, as called for in the group number, are evident. Personal equation is given too much importance in determining whether growth is feeble or strong.

Despite these objections, the group number is a satisfactory method for handling a group of closely related bacteria. It obviates the necessity of bacterial names which, in the past, have been carried to an extreme. When the group number is used, the main characters, which maynnot be expressed in the name, are known. This is especially significant with the fluorescent bacteria.

All of these cultures correlate with respect to acid formation in dextrose broth, production of fluorescent pigment, no diastatic action upon potato starch and negative indol formation. Since these characters agree for so many cultures, they may be of some significance. The absence of diastatic action upon starch presupposes no amylase. Negative indol formation may indicate that peptone is not split to products including tryptophane which is the precursor of indol.

The explanation of the correlation between acid formation in dextrose broth and production of fluorescent pigment is probably bound up with the structure of pyocyanin and its formation by the bacterial cell. If the opinion of Ledderhose is accepted that this pigment is a derivative of the anthracene group, it is evident that no apparent relation exists.

The fluorescent bacteria produce a green diffusible pigment Along with this, is produced a large amount of ammonia. The pigment itself, is probably basic in character.

From this study, it is evident that these bacteria do not form a genetic group. The property of pigment formation has been used in the past, on account of its ease of detection, as a basis for forming a "group". Many of the bacteria which have

been included in this group might be classed in other groups provided other bases for classification were used.

Summary.

I The present status in the classification of bacteria is their their arrangement in to groups.

II These groups should be studied to determin

(a) Whether the group is a logical one, its members not possessing widely divergent characters.

(b) The relation of the various members within the group.

III Among water bacteria, the fluorescent group, characterized by a green diffusible pigment, is commonly recognized.

(a) The pigment is produced most profusely when the bacteria are grown in Frankel's solution.

(b) All cultures correlate with regard to production of fluorescent pigment, formation of acid in dextrose broth, no diastatic action upon potato starch and negative indol formation.

(c) These cultures when studied according to the group number as expressed on the Descriptive Chart of the Society of American Bacteriologists fall into twenty seven groups.

(d) The fluorescent bacteria are about evenly divided with regard to gelatin liquefaction. The test is not delicate when applied to this group and requires further study.

(e) Four of the one hundred cultures are spore formers. This characteristic is recognized as one which logically separates a bacterial species.

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The greater portion of the experimental work reported in this paper was done while the author held an assistantship with the Illinois State Water Survey. It was in these laboratories that the real investigation - isolation of the cultures, morphological and cultural studies, etc. - were made. The inception of the investigation was made possible through the interest and sympathy of Professor Edward Bartow Director.

The analytical discussion of the data, in part the study of the literature and the writing of the paper was done during the tenure of an assistantship in the Bacteriology Department of the University of Illinois. Under these conditions both departments contributed to the possiblities for this study, and it is to them that the author is greatly indebted.

The study was briefly outlined by Professor Otto Rahn in June 1914. On account of his absence from the University during the two succeeding years, Professor H. A. Harding became the principal advisor and it is a pleasant duty to acknowledge his advice and assistance through the work.

Thanks are also extended to those others, who by various means gave assistance in the completion of this work.

BIOGRAPHY

Fred Wilbur Tanner received his common school education in the public schools of Western New York. He graduated from Warsaw High School, Warsaw, New York in 1908, and entered Wesleyan University, Middletown, Connecticut in the fall of the same year. In June 1912, he received the degree of Bachelor of Science from this institution having taken the scientific course with chemistry as the major study.

He entered upon duties as assistant bacteriologist with the Illinois State Water Survey, University of Illinois Urbana, in July 1912. In August 1915, he resigned from the Illinois State Water Survey to accept an assistantship for the succeeding academic year, in the department of Bacteriology in the University of Illinois. During the tenure of these two assistantships, advanced work was taken in the Graduate School of the University of Illinois. The degree of Master of Science was received from this University in June 1914.

He is a member of the American Chemical Society, the American Water Works Association, The American Public Health Association and the Society of American Bacteriologists. He is also a member of Sigma Xi and an abstractor for Chemical Abstracts and the American Journal of Bacteriology.