

MOORE (V.A.) Compliments

THE  
CHARACTER OF THE FLAGELLA

ON THE

BACILLUS CHOLERÆ SUIS (SALMON AND SMITH)  
BACILLUS COLI COMMUNIS (ESCHERICH)

AND THE

BACILLUS TYPHI ABDOMINALIS (EBERTH)

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*presented by the author*

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THE CHARACTER OF THE FLAGELLA ON THE BACILLUS CHOLERÆ SUI (SALMON AND SMITH), BACILLUS COLI COMMUNIS (ESCHERICH), AND THE BACILLUS TYPHI ABDOMINALIS (EBERTH).

By VERANUS ALVA MOORE.

Recent study of the morphology of bacteria has demonstrated the fact which Ehrenberg had foretold, that the motile forms are possessed of flagella. The further prophecy that in these minute hair-like appendages would be found vested the power of locomotion, was partially fulfilled as early as 1875 by Dallinger and Drysdale, who saw these filaments constantly lashing on a living, moving germ (*Bacterium termo*). More recently Straus has made similar observations on several species of bacteria. Cohn, in 1872, and Koch, in 1877, stained the flagella on a few of the larger saprophytic bacteria, but the methods which they employed were so defective that for more than a decade no further knowledge was gained concerning the character or existence of these minute filaments. The recent development of staining methods by which the flagella can be demonstrated on all the motile bacteria, is therefore of considerable importance, in opening before us a hitherto unexplored field in the study of the morphology of an exceedingly large and prominent class of the Schizomycetes.

Although considerable attention has been given to the character of the flagella, the greater part of the work which has been done on this subject has been directed to the development of methods for their demonstration rather than to the filaments themselves. As a natural result of this, our present knowledge of the flagella is exceedingly fragmentary, and the few statements concerning them are, in some instances, especially with the typhoid and coli bacteria, contradictory. The



intimate relation that exists between methods and results in the study of the morphology of bacteria will undoubtedly explain many of these discrepancies. In the endeavor to bring out more fully the character of the flagella of the three species of bacteria in question, it is desirable, on account of their intimate association with the development of our knowledge of flagella, to consider first, in a general manner, the methods that have been proposed for their demonstration and to give a brief résumé of our knowledge of these filaments and their accepted significance. I furthermore wish to acknowledge my indebtedness to the various writers on this subject for many valuable suggestions.

A SUMMARY OF OUR RECORDED KNOWLEDGE OF THE FLAGELLA OF MOTILE BACTERIA. (1) METHODS FOR THEIR DEMONSTRATION. (2) THEIR CHARACTER AND SIGNIFICANCE.

Cohn<sup>1</sup> and Koch<sup>2</sup> appear to be the first who stained the flagella. The methods by which they demonstrated the existence of these filaments have not been successfully applied to the smaller and especially the parasitic organisms. Although our instruments and methods have been much improved since Dallinger<sup>3</sup> and Drysdale made their observations, the detection of the flagella on living, moving bacteria is a result which has rarely if ever been attained with the small and pathogenic bacteria. From 1877 to 1889 no further knowledge of these filaments appears to have been acquired.

In 1889 Loeffler<sup>4</sup> succeeded in formulating a method by which he could stain the flagella on a considerable number of bacteria. The process, however, was not satisfactory, and a year later he published a second method<sup>5</sup> which has since borne his name, and which he believed would, if properly carried out, be applicable in staining the flagella on all the motile bacteria.

A few other methods\* have been proposed, but Loeffler's

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\* In an article<sup>10</sup> published in the *American Monthly Microscopical Journal*, (January, 1891), I reviewed the various methods that had been used in demonstrating the flagella on motile bacteria up to that time. See Literature, No. 5, 7, 10, and 11, at the close of this article.

has appeared to be the most trustworthy, although its application to the different species of bacteria has been attended with much difficulty. It has frequently happened, even when the method was apparently strictly adhered to, that the outcome has been absolutely negative, while in other instances the result would be entirely satisfactory.

Since the description of the original methods the literature on this subject has been exceedingly meager. In a communication to the American Society of Microscopists<sup>13</sup>, in 1891, I pointed out several of the difficulties usually experienced in the application of Loeffler's method, and suggested certain modifications, more especially in the technique, which I had found would to a great extent overcome these objections. In the same paper it was also stated that the principle laid down by Loeffler "that an acid\* producing germ required an alkaline mordant, and an alkali producing organism an acid mordant," need not be taken in a strict sense, as the flagella on certain of the acid and of the alkali producing bacteria could be stained by the use of either a neutral, acid, or alkaline mordant.

Straus<sup>17</sup> has recently reported a very simple method† by which he could stain the flagella on certain living bacteria. The process has given in my hands universally negative results.

Luksch<sup>15</sup> has modified Loeffler's method principally by

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\* In 1890, Dr. Theobald Smith (*Centralblatt f. Bakteriologie u. Parasitenkunde* VIII, (1890), p. 389) pointed out the fact that liquid cultures of certain bacteria would be acid or alkaline, according as glucose or other sugars were present or absent. These undergo fermentation with the formation of acids. In liquids free from sugars the reaction remains alkaline. (See his paper in this volume.) Petruschky's (*Ibid*, VI, 1889) classification of bacteria as acid or alkaline producing is thus shown to depend largely on the presence or absence of carbohydrates in the culture medium.

† The method is as follows: To a hanging drop preparation of the culture a drop of staining fluid consisting of carbol fuchsin one part, and distilled water three parts, is added and the preparation examined at once. He states that the moving flagella can be observed for about 15 minutes.

using a stronger solution of tannic acid in the preparation of the mordant and by the use of acetic instead of sulphuric acid in acidifying it. A very few other minor modifications of Loeffler's and other processes have been suggested, but they have in no way brought out results which entitle them to a consideration. Brown's<sup>19</sup> method is practically a modification of Trenkmann's process.

During the brief period of time that the existence of flagella on the smaller and especially the pathogenic bacteria has been known, two valuable applications of this knowledge have been made :

(1) *Their use as a basis for a general classification of the Schizomycetes.* Soon after the appearance of Loeffler's first method (1889) Messea<sup>8</sup>, an Italian investigator, proposed a new systematic classification\* of bacteria based upon the number and arrangement of the flagella. His classification is as follows :

- I. GYMNOBACTERIA (*non-motile*).
  - II. TRICHOBACTERIA (*motile*).
- |                        |                        |
|------------------------|------------------------|
| 1. <i>Monotricha.</i>  | 3. <i>Amphitricha.</i> |
| 2. <i>Lophotricha.</i> | 4. <i>Peritricha.</i>  |

The *Monotricha* have one flagellum at one pole of the bacillus (*Bacillus pyocyaneus*). The *Lophotricha* have a tuft or bunch of flagella at one pole of the bacillus (*Bacillus of blue milk*). The *Amphitricha* have a flagellum at each pole (*Spirillum volutans*). The *Peritricha* are provided with rows of flagella (*Bacillus typhosus*).

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\* Kruse (*Centralblatt f. Bakteriologie u. Parasitenkunde*, IX, (1891), p. 107) in reviewing Messea's classification, considers it of only secondary importance. His reason for this is, that it is unnatural in that it places bacilli, spirilla, and a micrococcus together in one group (the *Monotrichia*). It does not seem necessary to consider a pioneer classification secondary merely because of an apparent inconsistency, especially in a subject about which so little is known. Undoubtedly Messea's classification was based on too limited a number of examinations, and more extended investigations will probably cause many changes to be made. At present, however, it is the only classification which incorporates the complete morphology, as known at the present time, of motile bacteria.

(2) *Their assistance in differentiating closely allied species.* Luksch<sup>15</sup> in differentiating *Bacillus typhi abdominalis* and *Bacillus coli communis* made use of their flagella. He found from one to three flagella on the colon bacteria, but the typhoid bacilli were endowed with from 8 to 12 filaments, a difference sufficiently great to be of differential value. He experienced much more difficulty in staining the flagella on the colon germ than on any of the other motile bacteria.

Tavel<sup>18</sup> has recently made the statement that *Bacillus coli communis* has no flagella\* but that the typhoid bacillus is provided with them. This statement is qualified by a preceding one, that *Bacillus coli communis* is a non-motile organism, which would indicate that he was working with a different species.

In the articles, already mentioned, on the demonstration of the flagella a brief description is given of the motile appendages on the typhoid and a few other bacteria. Dallinger<sup>4</sup> and Zettnow<sup>14</sup> have discussed the flagella on a few species, more especially the saprophytic spirilla. In addition to these, there are brief mentions of the filaments on the typhoid and other species of bacteria scattered throughout the literature of the past three years, but so far as I am aware, they are too incomplete to be considered of any specific value.

THE COMPARATIVE DIFFERENTIAL VALUE OF THE FLAGELLA AND  
THE BIOLOGICAL PROPERTIES OF THE BACILLUS CHOLERÆ SUIS,  
B. COLI COMMUNIS, AND B. TYPHI ABDOMINALIS.

Bacteriology recognizes more fully, perhaps, than any other branch of biological science, functional properties in the formation of species and varieties. There are writers on this subject who consider every variation in the characters or properties of bacteria of specific or varietal significance, while there are others who hold more rigidly to the morphology of these organisms in determining their specific differences. The question, therefore, are specific differences in bacteria determined by their morphological characters or by their biological

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\* In 1891 I published a brief description of the flagella on the colon bacteria (Proceedings of the Am. Soc. of Microscopists, 1891).

(including etiological) properties, is one which, as yet, has no clearly defined and uniformly accepted answer. Messea's classification of motile bacteria is based on morphological characters only, and it recognizes genera, but not species. Luksch goes still further and points out a specific difference in the number of flagella of the typhoid and colon bacilli, two well recognized species of one genus. On the other hand, the more extended investigations of the biological properties of bacteria are continually bringing forth new facts, and revealing variations in the properties of these organisms which are not explained by corresponding differences in their morphology. There are bacteria which possess marked difference in their biological manifestations which resemble each other so closely in their structure that, with our present knowledge, their differentiation by purely morphological characters, is practically impossible. In the group of bacteria which includes *Bacillus cholerae suis*, *Bacillus coli communis*, and *Bacillus typhi abdominalis* we have an excellent illustration of this fact. These species resemble each other very closely morphologically but they are readily differentiated by means of their biological and etiological properties. In this group, therefore, is found a most rigid test for the differential value of the flagella.

It may be asserted that these bacteria do not belong to the same group, and while in a narrow sense (considering their more characteristic physiological manifestations only) it may be true, yet morphologically they are small, motile bacteria belonging to the *Peritricha* (Messea) and, in certain other respects, they are closely enough related to one another to be enrolled in the same genus, while their physiological and etiological properties mark their specific differences. In order that the differential importance of the biological and etiological properties of these bacteria may not be overlooked in the subsequent consideration of the specific character of their flagella, a brief summary of the more important of these properties for each of the germs in question, is appended :

(a). *Bacillus cholerae suis* is a small actively motile germ which is found in the organs of swine suffering from hog cholera. It

has not been found outside of the diseased animal body (or its immediate surroundings). It is *fatal* to experimental animals when injected beneath the skin in small numbers. In larger doses it will produce the disease in swine. *It ferments glucose with the formation of gas. It does not coagulate the casein in milk.*

(b). *Bacillus typhi abdominalis* is slightly larger than the bacillus of hog cholera and not so uniformly actively motile. It is found in the intestines and organs of people suffering from typhoid fever and is generally accepted to be its cause. Like the hog cholera bacillus, its natural habitat outside of the diseased body is not known. It is *not fatal* to experimental animals when they are inoculated with moderate doses. *It does not ferment glucose with the formation of gas\*. It does not coagulate the casein in milk.*

(c). The *Bacillus coli communis* is a very feeble or more actively motile bacillus varying somewhat in size but usually about as large as the typhoid bacillus. It is found in the healthy intestines of both man and the lower animals. It does not appear to live in nature outside of the animal body. It is *fatal* to rabbits when they are inoculated with large doses of the pure culture. *It ferments glucose with the formation of gas and coagulates the casein in milk.*

Another very important feature in the study of the relation, from a differential standpoint, that exists between the biological properties and the character of the flagella of these bacteria, is the fact, which is verified by many observations, that these organisms exist in nature (*i. e.*, the coli in the healthy intestines, and the hog cholera and typhoid bacteria in the organs of the victims of their respective diseases), in variously modified forms in which the differences (biological and pathogenic) which separate the more typical species are very much diminished. This variation, especially in the pathogenic properties of the typhoid and colon bacteria, has given rise to a theory, advanced by Rodet and Roûx<sup>16</sup>, that the typhoid germ is a modified form of the colon bacillus. In the investigation of animal diseases, hog cholera and colon bacteria have been found which varied in size and in their biological and pathogenic properties to a marked degree from

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\* The fact that the typhoid bacillus would not ferment glucose with the formation of gas, was first pointed out by Dr. Theobald Smith in 1890. (*Centralblatt f. Bakteriologie u. Parasitenkunde* VII, 1890. p. 502).

the more typical forms of these species.\* This would indicate that the evolutionary theory of Rodet and Roux is as applicable to the hog cholera germ as it is to the typhoid bacillus. Although the development of these specific pathogenic bacteria from a common intestinal germ is improbable, yet the possibility can not be gainsaid and the importance which this theory has in its bearing upon public health, as well as in its relation to pure bacteriology, renders its demonstration of much interest. This, and many other interesting problems connected with the specific limitations of these bacteria, emphasize the importance of determining as far as possible the extent to which their flagella may be deemed of differential value. In my efforts to do this, I have considered the flagella not only of the more typical species but also of a considerable number of modified forms (excepting the typhoid bacillus) of these bacteria. From these investigations, I have found their flagella to be much more constant than their biological properties, but contrary to Luksch, I have not found them to be of specific differential importance. The evidence to support this conclusion is found in the subsequent description of the character of the flagella.

I have limited myself to a consideration of a very few of the many interesting questions which have presented themselves in the study of the flagella of these bacteria. A large amount of experimental work was necessary before a satisfactory method for their demonstration could be formulated. This being accomplished the specific objects which I endeavored to attain are :

1. To complete as far as possible our knowledge of the morphology of each of these organisms.

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\*In a communication to the Biological section of the American Association for the advancement of Science, in 1890 (*New York Med. Journal* LII (1890) p. 485). Dr. Theobald Smith described a hog cholera bacillus which was, "in every way nearer the saprophytic forms (coli) than the germ usually found in epizootics of that disease." I have found similar variations in the colon bacteria isolated from variously diseased organs of different animals. In an outbreak of swine disease in 1892, I isolated several colon bacteria which approached in their biological characters very closely to the hog cholera organism.

2. To determine whether or not there is a difference in the number or character of the flagella of the modified forms of these bacteria corresponding with the variations that are found to exist in their physiological and etiological manifestations.

3. To determine the significance of the flagella in classifying motile bacteria, as illustrated by a comparative study of these filaments on three typical species, representing morphologically three closely allied groups\*, and biologically three distinct groups, of bacteria.

It was my opinion in 1891 that there was a well marked specific difference in the flagella of these species, but more extended investigations have caused me to recede somewhat from that opinion and to call attention at this time, in accordance with the results of my observations, to the similarity which exists between them.

#### METHOD FOR STAINING THE FLAGELLA.

The difficulties which have been experienced in the demonstration of the flagella both by myself and others, necessitate a somewhat careful consideration of the method which has been followed in obtaining the results herein recorded. As I have been unable to detect the flagella on living bacteria, my descriptions apply to stained preparations only. With the exception of certain modifications, Loeffler's method has been followed. The advantages that are claimed for the modified method over the original process are: (1) the preparations are more uniformly free from a deeply stained background which conceals entirely or renders indistinct the individual filaments; (2) there is a better distribution of bacteria on the cover-glass; (3) the application is more simple and the results more trustworthy. The majority of the changes which I have made were fully described in a previous article<sup>13</sup>. The others are incorporated here. The method as I have used it, is as follows: the principle that is involved in staining the flagella is simply the one employed in the use of a mordant or fixative. The methods which have been at all successful (beyond the experience of the originator) in staining the flagella require a

\* Group is here taken in a narrow sense, signifying a species and its varieties only.

mordant which contains tannic acid. The other elements have varied. The technique of the method therefore consists in treating the cover-glass preparations of the bacteria with a mordant prior to, or together\* with, the application of the staining fluid.

*The mordant* : †

A 20 per cent. solution of tannic acid . . . . . 10 cc.  
 A cold saturated solution of sulphate of iron . . . . . 5 cc.  
 A saturated alcoholic solution of fuchsin . . . . . 1 cc.

If possible chemically pure tannic acid should be used. This mordant can be used in staining the flagella of these three species of bacteria, but slightly better results appear to be obtained with the typhoid and colon bacteria when a few drops of a 10 per cent. solution of sulphuric or acetic acid are added. With the hog cholera bacteria a mordant containing a 10 per cent. solution of tannic acid gave equally good results.

*The staining fluid* : ‡ For this I have used carbol fuchsin prepared after Ziehl's formula.

\* I have found, since the greater part of the work on this article was completed, that certain staining fluids (alcoholic solutions) could be mixed with the mordant thus eliminating their subsequent application. I have not perfected the formula for this combination but have obtained excellent results with a fluid composed of a 20 per cent. solution of tannic acid 2 cc., saturated solution of sulphate of iron 1 cc., and a saturated alcoholic solution of fuchsin 1 cc., filter and apply in the same manner as the mordant, but for a much longer time.

† This is Loeffler's standard or neutral mordant. It is successfully employed in staining the flagella on many motile bacteria. The addition of a few drops of a 10 per cent. solution of sulphuric acid or a similar quantity of a 1 per cent. solution of sodium hydrate appears to give better results, if indeed it is not absolutely necessary, with certain other species. My personal experience tends to prove that no sharp and fixed lines can be determined for the preparation of a universal mordant. I have been unable, however, to stain a single flagellum by the use of a mordant not containing tannic acid, although I have tried nearly all of the known "fixatives." As I have stated elsewhere<sup>13</sup> a weaker solution of tannic acid can sometimes be used with advantage.

‡ I have stained the flagella with nearly all of the basic aniline dyes ordinarily used in bacteriological work. The carbol fuchsin gives a deeper stain and consequently a clearer definition of the filaments is obtained.

Fuchsin . . . . .	1 gram
Absolute alcohol . . . . .	10 cc.
A 5 per cent. solution of carbolic acid . . . . .	100 cc.

The fuchsin is dissolved in the alcohol, after which the acid solution is added.

*The cover-glass preparations:* The cover-glasses must be perfectly clean. The desired number of cover-glasses are then arranged on a level tray. A large drop of warm water (distilled or hydrant) is placed upon each cover-glass by means of a sterile pipette. If the cover-glass is properly cleaned\* the drop of water will spread over its entire surface. The point of a cooled, flamed platinum wire is very gently touched to the surface growth of the germ on agar or gelatine and carefully immersed in the water near the center of the cover-glass. A sufficient number of bacteria will adhere to the wire to make from six to ten preparations. The tray with the cover-glasses is then placed in an incubator at a temperature of about 36° C. until the water is evaporated. Many of the bacteria on account of their power of locomotion, and by means of the currents produced in the liquid during its evaporation, will be found, in the dried preparations, to be isolated from the clumps of bacteria that were introduced with the wire and distributed very evenly over a large portion of the surface of the cover-glass. This natural distribution prevents, to a marked extent, the breaking off of the flagella which occurs when the distribution is made by mechanical means in a smaller quantity of the diluent. If the water to be used

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\* Weber (*Fortschritte der Medicin* Bd. XI. (1893), p. 49) has found that when the percentage of calcium is too small in proportion to the alkalies (sodium and potassium) in the composition of the glass, the atmosphere will produce a chemical change in the glass which gives its surface a peculiar moist condition. This may explain to some extent the cause of the deeply stained background where the proper precautions were taken to prepare the cover-glasses. I have frequently noticed that in the use of cover-glasses that had been cleaned and exposed to the atmosphere for a considerable time prior to their use, the water would "roll up" in drops, as though the surface was covered with a film of oil, and when stained they would invariably exhibit a deeply stained background.

on the cover-glasses is heated to a temperature of about 40-45° C. and the preparations placed at once in the incubator, the bacteria are more evenly distributed.

After the preparations have dried the bacteria are fixed to the cover-glass by heat. This can be accomplished either by passing them, film upward, *twice* through the flame of a spirit lamp or Bunsen burner, or by heating them from 120°-140° C. for from five to ten minutes in a hot air chamber. The latter is to be recommended when facilities will permit.

*The application of the mordant and staining fluid:* In applying the mordant the preparations are completely immersed in the fluid. In place of a watch glass as formerly recommended, I have used a large (one inch) test tube for this purpose. From three to four c. c. of the mordant is placed in the tube into which the cooled, heated cover-glass preparation is dropped. The tube is held over a flame until steam is given off when it is removed. The mordant should be frequently agitated by gently shaking the tube. After from five to ten minutes the cover-glass is removed by the use of a wire hook on the end of a glass rod and with a pair of fine forceps. The cover is thoroughly rinsed in clean water, or better, in a stream from a spigot or wash bottle. If there is a grayish film on the preparation it can usually be removed by rinsing it in strong alcohol and again in water.

The staining fluid is applied in a test tube in precisely the same manner as the mordant. It is allowed to act for from one to three minutes.

The mordant should be fresh and always *filtered* before it is used. The carbol fuchsin can be kept in stock solution for a considerable time. The success of the operation depends very largely upon the *care with which it is performed*. The method as described gives excellent results with the three species of bacteria under consideration, and it has been successfully applied to a few others.\*

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\*In the application of the method to other bacteria whose flagella will not stain by the use of a neutral solution, trials must be made with acid and alkaline mordants, and possibly with mordants containing different quantities of tannic acid, until a successful combination is found.

*Cultures used* : In this work, agar cultures have been employed. Unfortunately the staining method is not so applicable to preparations of bacteria from bouillon cultures on account of the presence of organic substances in the bouillon, which form a deeply stained back ground. I have, however, succeeded in making a few quite satisfactory preparations from these cultures. Gelatin and potato can be used, but with our present methods the surface of inclined agar appears to be the most satisfactory sub-stratum upon which to grow the bacteria for this purpose.

A DESCRIPTION OF THE COMMON AND MORE SPECIFIC CHARACTERS OF THE FLAGELLA ON THE BACILLUS CHOLERÆ SUI, B. COLI COMMUNIS, AND B. TYPHI ABDOMINALIS.

In stained preparations for the exhibition of the flagella on these bacteria, there are so many variations and exceptions to what might be termed a typical presentation of the body of the germ and its motile appendages, that at present, a formula for their description can not well be written. In order to avoid repetition I shall first describe in a general way, such characters of the flagella as are common to the three species.\*

The staining process necessary to bring out the flagella increases to a slight extent the size of the body of the germs. This is probably due either to the staining of a "capsular" substance which may surround the bacteria, and which is not brought out by the ordinary staining methods, or to the swelling of the cellular substance on account of the action of the mordant.†

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\* In this discussion the well-known morphological characters (size and form) of these bacteria are omitted. The rod-shaped forms will be spoken of as the body of the germ wherever it is necessary to distinguish between it and the flagella. The terms motile appendages, and filaments are used synonymously with flagella.

† Zettnow<sup>14</sup> holds with Klebs and Bütschli, that the part of the germ which is easily brought out by the ordinary staining methods is the nucleus only, and that the additional part which is demonstrated by the use of Loeffler's method is a plasma which surrounds the nucleus. Wahrlich (Article reviewed in *Centralblatt f. Bakteriologie u. Parasitenkunde* XI, (1892) p. 49) found two substances in bacteria cells, (1) the basis, a substance which gave the micro-chemical reaction of linen, and (2) a *chromatine* substance or nucleus which is contained within the meshes of the basis.

In the microscopical examination of well-executed preparations for exhibiting the flagella three conditions have been universally observed: (1) In certain fields there were a greater or less number of bacteria which exhibited no flagella; (2) there were a considerable number of detached or free flagella lying between the bacteria; and (3) the numbers of flagella on the different bacilli were not constant. On the other hand, however, fields could be selected in which there were no detached filaments and where every germ was provided with motile appendages.

The flagella appear as hair-like appendages or filaments, which radiate from the bacteria. They are given off from the cell wall of the germs of which they appear to be continuations or projections. This can be clearly shown by their reaction to the following staining fluid. If to 3 c. c. of the mordant 1 c. c. of a saturated alcoholic solution of fuchsin is added and the preparation treated in the usual way with this solution for about five minutes, the flagella and periphery of the bacteria will be stained with equal intensity, while the central portion of the cells will remain unstained. By allowing the reagent to act for a much longer time or by applying the usual stain, the entire organism will become deeply tinted. This would indicate that the cell wall and flagella were alike in their composition, or at least in their reaction to a certain staining fluid, and that the contents or nucleus was different. The sharp outlines of the flagella as observed in stained preparations, would indicate that they are organized elements. I have been unable to make out any differentiation of their structure.

The appearance of shorter and longer free flagella and the difference in the length of those still attached to the body of the germs is due apparently to their detachment and breaking during the separation of the individual bacteria from the closely packed masses in which they grow on solid media. In these cultures the long filaments on the different individuals appear to become entangled with each other, causing the separation of the bacteria to be accomplished with difficulty and presumably with more or less injury to their organs of

locomotion. This is suggested by watching the clumps of bacteria in a hanging-drop preparation. The germs at the edge of these masses, when about to separate themselves from the others, exhibit first a trembling motion, then a jerking, reeling and pitching movement, until finally they are free\* and move across the field.

Furthermore, in the examination of a hanging drop preparation made from a bouillon culture the bacteria are observed to move much closer to each other than the length of their flagella, and it seems highly probable that detachment or breaking of the appendages is produced during these voluntary movements, by their contact and possible entanglement with each other. Free flagella have been found to be numerous in stained preparations made from liquid cultures. For the ultimate settlement of these questions a method must be devised by which the motile appendages can be observed on the living and moving germs. At present we are forced to be content with the study of the appearances that are presented in stained specimens, presuming that as the conditions of preparation are the same the characters that are revealed will be correspondingly similar in the different species.

The length of the flagella as seen in stained preparations varies to a marked degree. The longest I have measured was  $18 \mu$  or about nine times the length of the body of the bacillus (hog cholera).

The diameter of the flagella varies in different preparations and frequently in the same specimens, to a marked degree. In a deeply stained preparation they are occasionally  $0.4 \mu$  in diameter, or about one-third of the diameter of the body of the germ. More commonly they are about  $0.2 \mu$  in diameter, or about one-sixth the thickness of the organism. Again they may appear as extremely delicate lines, so fine that it is with difficulty that they can be seen at all. Usually, however, they are about  $0.2 \mu$  in diameter. The unexpected appearance of these variations has thus far baffled an explanation,

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\* It is an observable fact that the character of the movement of the individual germs is somewhat varied. This may be due to the loss of certain of their motile appendages.

although many series of preparations have been carefully made to discover the cause. I believe, however, that the failure is due to the technique rather than to a variation in the filaments. The diameter of the flagella is appreciably the same at the distal end as at the union with the body of the germ. Occasionally, however, the distal portion appears to be very slightly tapering.

Among the free flagella is sometimes observed what appears to be a strand or bunch of twisted filaments, varying from one to two  $\mu$  in width, which presents a uniformly, deeply stained appearance. At one or both ends the filaments are separated from each other, giving the appearance of the frayed end of a cord. In preparations from old cultures several flagella which radiate from the same point are frequently observed. They present the appearance of the filaments of a single germ. These are occasionally observed in preparations from young cultures.

Frequently a flagellum will present throughout the greater part of its length a very close, wavy condition. There are in addition to these many other anomalous appearances which have as yet no clearly defined significance.

In the study and comparison of the flagella, I have employed cultures varying in age from sixteen hours to three days. There appeared to be no difference in the character of the flagella on the bacteria in cultures of these ages, but in preparations made from those of a longer growth there was usually a much larger number of broken and detached filaments.

The exact arrangement of the flagella on the body of the germ is hard to determine. In stained preparations the organisms are dried to the cover-glass with the filaments in such positions that they seem to radiate from the outer edges of the germs as they appear in the stained specimen. Frequently they all appear to come from a very small arc on the circumference. This is undoubtedly due to our inability to detect the filaments as they cross the body of the germ. The flagella are given off from both extremities, and at variable points along the intervening portion of the body of the germ,

although a single bacillus which exhibits this uniform radiation of motile appendages is rarely observed in stained preparations. The flagella are usually more or less wavy, and it is the rule, though it has many exceptions, that the waves in a single flagellum are uniform.

I have studied very carefully the flagella on several hog cholera bacteria. These were obtained from different sources (outbreaks of hog cholera) and a few of them exhibited slight variations in their biological characters and more marked difference in their virulence. So great have been these variations in a few cases that the bacteria have been deemed modified forms. I have also studied a larger number (about 20) of colon bacteria isolated from variously diseased organs of different animals and from the human intestine. These have also shown a marked difference in their properties. The two cultures more specifically described represent (1) the more typical form (2) a somewhat modified form. Of the typhoid bacteria, only two cultures have been at my disposal. All of these bacteria have been carefully studied and their identification clearly established. In these examinations I have been unable to detect any constant, specific difference in the character of the flagella on the germs from the different cultures of the same species. On this account bacteria from only two cultures of each species will be considered in the more specific description of the flagella.

*The flagella on Bacillus cholerae suis.* (Plate 1, Fig. 1.)

(1) A culture of hog cholera bacteria which was obtained from a pig that died in an outbreak of hog cholera in the State of Illinois in the fall of 1891. An examination of the bacteria in a hanging drop preparation showed them to be universally actively motile. They were virulent.

The number of flagella on the different germs, as observed in the stained preparations, was variable. The most usual number was from two to five. A few germs have been found upon which nine filaments could be counted, but it is the exception to find more than eight. Frequently the filaments are bent upon themselves in such a way that it is very difficult to determine the exact number, especially when it is large. In

a few instances I have thought it possible for as many as twelve filaments to be present. In order to estimate the most usual number an actual count of the flagella on a large number of germs was made. These were taken from somewhat ideal fields on a considerable number of preparations. In these fields there were from 2 to 10 bacteria which were well separated\* from each other and on each of which all of the flagella could be counted; that is, there were no clumps of bacteria present. Care was always taken to avoid extreme conditions.

Of these the number of flagella on two hundred individual germs was as follows, 12 had no flagella, 23 had one, 30 had two, 47 had three, 39 had four, 22 had five, 12 had six, 8 had seven, 5 had eight and 2 had nine. In many of the fields there were no free filaments, while in others there was a variable number.

The longest flagellum that I measured was  $18\mu$ .† The usual length was from 7 to  $12\mu$ . Shorter ones were quite common. Occasionally the ends of the filaments were curved into nearly or quite perfect circles or rings with a diameter of about  $1.5\mu$ . These were not uniformly present but in occasional preparations they were quite conspicuous.

(2) A culture obtained from an outbreak of hog cholera in the State of Maryland. A microscopical examination of a hanging drop preparation showed the bacteria to be actively motile. They were less virulent than those in the previous culture. Cover-glass preparations treated and stained as in the preceding case revealed no appreciable difference in their staining properties, the number, arrangement, and character

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\*This is very important, for the tendency of the bacteria to be united in twos or small clumps which might easily be mistaken for a single germ is very marked, and the flagella which belong to two germs could be readily considered as those of a single individual. This is especially true where the bacilli are united end to end.

† In measuring the length of the flagella the distance from the body of the germ to the distal end of the filament along its general course was taken, without allowance for the minute curves or waves which would in some instances, if considered, add an appreciable amount to the recorded length.

of their flagella. To complete the comparison the number of flagella on 200 germs is recorded. Of these, 10 had no flagella, 33 had one, 33 had two, 45 had three, 38 had four, 19 had five, 6 had six, 8 had seven, 6 had eight; and 2 had nine. The longest flagellum observed measured 11  $\mu$ . The usual (about 75 per cent.) length was from 6 to 8  $\mu$ . The small circles at the distal ends were also present but in small numbers.

*Flagella on Bacillus coli communis.* (Plate 1, Fig. 2). A culture of the bacillus coli communis, obtained by Dr. Theobald Smith from the human intestine. A microscopical examination of a hanging drop preparation from an agar culture of this species showed comparatively few of the germs to be in motion. Upon watching it carefully for several minutes, many of the individual germs which were first at rest exhibited an active motility.

The diameter and arrangement of the flagella on this species do not differ to any appreciable degree from those on the hog cholera bacteria. The number of filaments on the individual germs varied considerably. Seven was the maximum number found on a single organism. As before, an actual count of the flagella on 200 individual germs selected from representative fields was made. Of these, 9 exhibited no motile appendages, 33 had one, 58 had two, 44 had three, 34 had four, 15 had five, 4 had six and 3 had seven. The number of the free flagella in many of the fields averaged about one to each germ. This is unimportant as in other parts of the preparations the number was greater and in still others less. The length of the filaments varied from 2 to 12  $\mu$ . The greatest number (66 per cent. of a large number measured) were from 5 to 7  $\mu$ . It is of interest to note that the number of individual bacteria which exhibited no flagella was no larger in this species than in the preparations of the hog cholera bacteria where apparently every germ was actively motile. A very few of the flagella formed nearly or quite perfect circles or rings at their distal ends. Their existence, even in small numbers eliminates their specific value when compared with hog cholera and typhoid bacteria.

(2). A culture *Bacillus coli communis* obtained from the pectoral muscle of a pig which died in an outbreak of swine disease in 1892. The motility of the bacteria in this culture was less marked than in those from the previous culture. The flagella extended out from the body of the bacteria in delicate, more or less wavy filaments. In diameter, course, and general appearance they differed in no perceptible manner from the flagella on the previously described germ. The maximum number of filaments that was found on an individual was one less than in the previous case, and the number which possessed but one flagellum was proportionally larger. The number of detached filaments was not in excess of those in the preparations of the colon bacteria from the human intestine. In order to arrive at a more accurate comparison, the flagella on 200 individual bacteria from selected fields were counted. Of these, 11 showed no flagella, 83 had one, 55 had two, 29 had three, 13 had four, 6 had five, and 3 had six. The maximum length of the filaments measured was 15  $\mu$ . The length of the greatest number varied from 5 to 9  $\mu$ .

*Flagella on Bacillus typhi abdominalis.*—(Plate I, Fig. 3). For my cultures of typhoid bacilli I am indebted to Dr. Theobald Smith. These cultures were from two to four years old. Unfortunately I have no knowledge of the history of these cultures prior to their arrival in our laboratory. (1) A culture obtained from the Johns Hopkins Hospital, Baltimore, Md. When examined in a fresh condition (hanging drop) the majority of the bacilli were observed to be in active motion.

The arrangement of their flagella was not perceptibly different from those on the hog cholera and colon bacteria. In the examination of different preparations of this bacillus a considerable variation was found to exist in the appearance of its flagella. A certain number of the bacilli were provided with long, nearly straight or wavy filaments which extended in various directions from the body of the germs, while the motile appendages on the greater number of the organisms were shorter, more curved, and intertwining, presenting a

somewhat bushy appearance about the body of the germs. In many specimens there was an exceedingly large number of flagella which were bent at their distal ends, if attached to the germ, into nearly or quite perfect circles or rings varying from 1.2 to 2  $\mu$  in diameter, while other filaments were curved to such an extent that the free ends passed either above or below the body of the germ. In many preparations I have found fields in which one or more of the filaments on nearly every germ exhibited these circles or rings. Their formation is somewhat curious and their significance is as yet speculative. Frequently many of the "rings" are broken from the flagella. The filaments were usually bent at right angles to their course at the point where the curve begins. This gave them the appearance of the ends of certain screw hooks. Less frequently the rings were formed by the distal part of the filaments bending to one side, forming perfect or nearly complete circles. In some preparations a considerable number of bacilli were observed around which were from one to four rings or inbending filaments with few, if any, extended flagella. The terminal rings and incurved flagella on the typhoid bacilli were very marked, but their differential value is destroyed by the fact that they occasionally appeared in preparations of the other bacteria.

The detached filaments vary in number, as in the case of the previously described organisms. The number of flagella on the individual germs also varied; ten was the maximum number that I was able positively to determine on a single germ; Luksch found twelve; and according to Sternberg<sup>20</sup>, Babes found as many as twenty on a single germ. In the photographs of typhoid bacillus published by Fraenkel and Pfeiffer<sup>21</sup>, and Migula<sup>22</sup>, showing their flagella, ten is the largest number positively represented as belonging to a single germ. In these photographs, clumps and pairs of bacilli show a much larger number. A count of the flagella on 200 germs in well selected fields showed 9 bacilli to be without motile appendages, 23 had one, 39 had two, 45 had three, 27 had four, 15 had five, 23 had six, 11 had seven, 3 had eight, 3 had nine, and 2 had ten. The length of the filaments

varied considerably. The maximum length of those measured was 11  $\mu$ ; 78 per cent. of a large number that were measured varied in length from 3 to 6  $\mu$ .

(2). A culture which was obtained from Koch's Laboratory (Germany). The bacteria were not quite so actively motile as the hog cholera germs. They stained readily and the flagella differed in no appreciable manner from those on the bacteria from culture (1). There were a large number of short flagella and rings. The number of flagella on the individual germs was estimated in the manner heretofore described. Seventeen of the 200 germs exhibited no flagella, 43 had one, 42 had two, 45 had three, 24 had four, 18 had five, 5 had six, 3 had seven, and 3 had eight. It is possible that a few bacteria had nine filaments each, but there was a doubt as to the exactness of the count. The longest filament measured was 13  $\mu$ . A large majority of those measured varied from 3 to 7  $\mu$  in length.

From the detailed descriptions of the flagella on these three species of bacteria a few comparisons may be made. These can be stated best in tabulated form :

A COMPARISON OF THE NUMBER OF FLAGELLA ON THE INDIVIDUAL GERMS.

BACILLUS.	Cul- ture	The Number of Flagella.										Total number of bacteria.	Average number of flagella on each germ
		0	1	2	3	4	5	6	7	8	9		
Cholerae suis .	(1)	12	23	30	47	39	22	12	8	5	2	200	3.3
"    "	(2)	10	33	33	45	38	19	6	8	6	2	200	3.1
Coli communis	(1)	9	33	58	44	34	15	4	3			200	2.6
"    "	(2)	11	83	55	29	13	6	3	(?)			200	1.8
Typhi abdomi- nalis . . . .	(1)	9	23	39	45	27	15	23	11	3	3	200	3.5
"    "	(2)	17	43	42	45	24	18	5	3	3	3	200	2.6

In comparing the figures in the tables the fact should be kept clearly in mind that they have only a relative significance. The large number of preparations examined and the number of counts and measurements made give them,

A COMPARISON OF THE LENGTH, DIAMETER AND CHARACTER OF THE FLAGELLA.

BACILLUS.	Culture	Length of longest flagellum.	Lgth. of 70 per ct or more of the flagella.	Usual diameter of flagella.	Appearance of flagella.
Cholerae suis	(1)	18 $\mu$	7-12 $\mu$	0.1-0.2 $\mu$	Usually extended, wavy, few terminal rings.
" "	(2)	11 $\mu$	6-8 $\mu$	0.1-0.2 $\mu$	" " "
Colicommunis	(1)	12 $\mu$	5-7 $\mu$	0.1-0.2 $\mu$	" " "
" "	(2)	15 $\mu$	5-9 $\mu$	0.1-0.2 $\mu$	" " "
Typhi-abdominalis . . .	(1)	11 $\mu$	3-6 $\mu$	0.1-0.2 $\mu$	Many incurved, wavy, large number of terminal rings.
" "	(2)	13 $\mu$	3-7 $\mu$	0.1-0.2 $\mu$	" " "

however, a good representative value. To illustrate this point and to show how easily different results could be obtained, especially in reference to the number of flagella, by considering a smaller or possibly larger number of bacteria, I have appended the results of the count of the flagella of 200 individual germs. Here also a further difference of opinion as to the fields to be selected might vary the final result. My counts in this case were made from eight preparations.

BACILLUS CHOLERAÆ SUIS.	Number of fields examined.	Number of Flagella.									Total number of bacteria.	Aver. number of bacteria in a field.	
		0	1	2	3	4	5	6	7	8			9
Preparation I.	5		3	6	2	8	7	1	2			32	6.4
" II.	4	2	1	5	7	5	2	4	1	2	1	30	7.5
" III.	6	1	1	2	10	6	1	3	1	3	1	29	4.8
" IV.	3	2	2	1	8	1						14	4.6
" V.	8	2	6	8	6	5	5	1				35	4.3
" VI.	7	2	3	2	6	4	1	1	1			20	2.8
" VII.	3	1	5	3	7	7	3		1			27	9
" VIII.	2	2	2	2	1	3	3	2	1			15	7.5
		12	23	30	47	39	22	12	8	5	2	200	

In comparing the specific characters of the flagella of the three species, it will be observed that while there are manifest

differences there are likewise striking resemblances. A few of the more important facts which have been brought out in this study to illustrate their differences and similarities are appended.

Their difference is shown from the observation, (1) that the length of the greater number of the flagella is greatest on the hog cholera and least on the typhoid bacilli, while those of the colon bacteria are of intermediate length; (2), that the average number of flagella on the colon bacteria is less than that on either of the other species; and (3), that the terminal and free rings are much more numerous in the preparations of the typhoid bacillus than in those of the other bacteria. This is also true of the incurving flagella.

Their similarity is illustrated by the fact (1), that the numbers of flagella on the individual bacteria vary in the different fields in the preparations from the same species as much as in those from different species, excepting in the maximum numbers; this is also true of the length of the flagella; (2), that the diameter of the flagella on the three species is identical; (3), that the position of the flagella on the body of the germ is the same; and (4), that fields could be selected in preparations from the three species in which no difference could be detected in the character of the flagella.

#### CONCLUSIONS.

The foregoing examinations and the results of a careful comparative study of the flagella of these three species of bacteria appear to sustain the following conclusions:

1. These three species of bacteria belong to the *Peritricha* (Messea).

2. There are apparently slight differences in their flagella, but the differences are not marked enough to be deemed of differential value. This is evidenced by the fact that the flagella in different preparations from the same species exhibit quite as marked variations.

3. There is no difference in the flagella of modified forms of the same species to correspond with the difference in their physiological and etiological manifestations.

4. Until further facts are determined, the character of the flagella will not furnish a means for specific differentiation. The species and varieties must be determined by their physiological and pathogenic properties while the genera may be fixed by the character of the flagella.

5. The proposition that the *Bacillus typhi abdominalis* is a modified form of *Bacillus coli communis* cannot be justly refuted on their morphological characters. The similarity in the structure (as it is now understood) of these bacteria increases the importance, from a differential standpoint, of the differences found to exist in their biological and etiological manifestations.

WASHINGTON, D. C.,

July 31, 1893.

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## DESCRIPTION OF PLATE.

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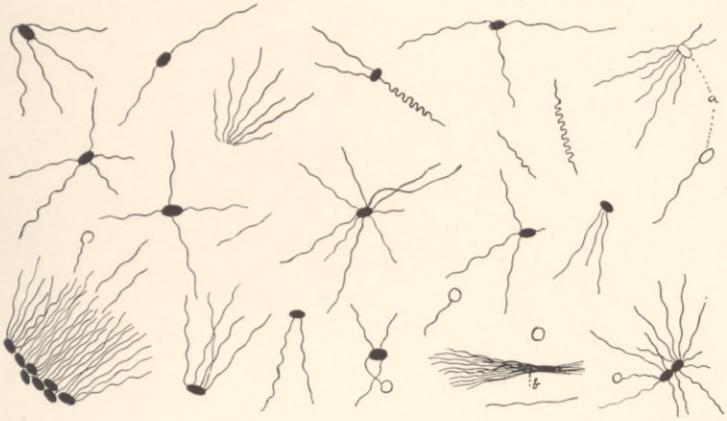
The figures in the plate are to illustrate the flagella on these three species of bacteria as they appeared in stained cover-glass preparations. The drawings were made by the aid of a Zeiss apochromatic objective, 2 mm., 1.30 n. a. and the measurements were made with the compensating micrometer ocular No. 6. Each germ and its flagella were carefully measured and in the drawings each micromillimetre is represented by a millimeter, thus giving a magnification of a thousand diameters. The curves in the flagella were carefully counted and reproduced as accurately as it was possible by freehand drawing. The position of the flagella was also carefully determined. In the preparation of the plate care has been taken to avoid extremes. Individual bacteria have been selected from different fields to represent the various number, lengths and position of the filaments on the body of the germs as they appeared in the preparations. A few free, or detached flagella are also indicated. The drawing of each germ is practically equivalent to a photograph. It is possible to find all of the structures represented in a few fields of the microscope in a well executed preparation. The germ in the center of each figure represents the maximum number of flagella on a single individual. In the left lower corner of each is a drawing of a clump of bacteria with their flagella. There are a few drawings of bacteria (a) with only their periphery and flagella stained.

Fig. 1. *Bacillus cholerae suis*. Drawings made from preparations of the culture of hog cholera bacteria obtained in the State of Illinois. (b) A bunch or strand of flagella.

Fig. 2. *Bacillus coli communis*. Drawings made from preparations from the culture obtained from the human intestine.

Fig. 3. *Bacillus typhi abdominalis*. Drawings made from preparations of the typhoid bacillus which was obtained from the Johns Hopkins Hospital. The upper right hand corner, enclosed in dotted lines, represents all of the bacteria and flagella from a single microscopic field.

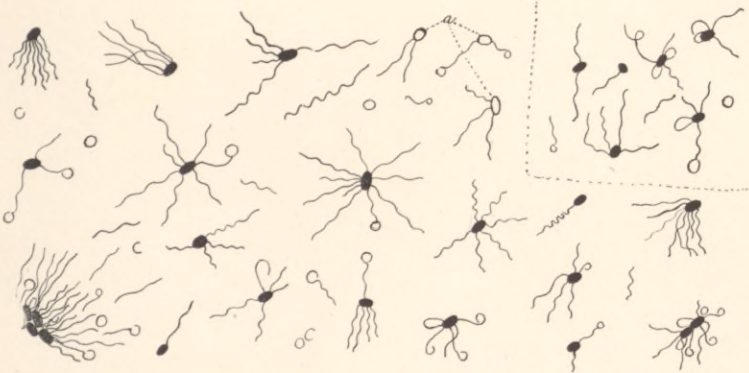
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FLAGELLA OF BACTERIA—MOORE.











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