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15

# A STUDY

OF SOME OF THE

# BACTERIA FOUND IN THE DEJECTA OF INFANTS AFFLICTED WITH SUMMER DIARRHŒA.

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346
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# A STUDY OF SOME OF THE BACTERIA FOUND IN THE DEJECTA OF INFANTS AFFLICTED WITH SUMMER DIARRHŒA.

## UNE ÉTUDE DES BACTÉRIES TROUVÉES DANS LES DÉJÉCTIONS D'ENFANTS AFFLIGÉS DE LA DIARRHÉE D'ÉTÉ.

## UNTERSUCHUNG EINIGER DER BAKTERIEN IN DEN ENTLEERUNGEN VON KINDERN, WELCHE AM BRECHDURCHFALL LEIDEN.

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Baltimore, Md.

346
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The term summer diarrhœa is used in this article to include conditions usually classified as functional or non-inflammatory diarrhœa, gastro-enteric catarrh, dysentery and cholera infantum. The transition from one of these conditions to the other is often so gradual and their symptoms are so often blended that it is not permissible, or even possible, to draw sharp lines of distinction between them.

The great increase of diarrhœal affections in children with the increased heat of summer was observed first by American physicians, and a thorough report upon the subject was made by Benjamin Rush in 1789.* For a long time this disease was supposed to be confined to America, but since the middle of the present century it has been recognized in England, Germany, France and other countries. During the last twenty-five years the European contributions to the literature of cholera infantum are not less than those from American sources.†

Of the causes which lead to the greater frequency and severity of these affections in summer, the effect of heat has been naturally considered to be the most important, but in what manner this influence is exerted is still a subject of discussion.

That microörganisms are in some way concerned in the causation of the group of affections embraced under the name summer complaint or summer diarrhœa of children, is a view which has often been suggested and which, of late years, has gained favor. The increasing popularity of this mycotic theory is based more upon the advance

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*B. Rush. "Medical Inquiries and Observations," Philadelphia, 1789. The article "Cholera Infantum" bears the date 1773.

† A full bibliography of cholera infantum, up to the year 1882, is to be found in the Index Catalogue of the Library of the Surgeon-general's Office, United States Army, Vol. III, p. 148, et seq.

in our knowledge in general concerning the relation between bacteria and infectious diseases, than upon any actual discoveries of specific pathogenic organisms in the diseases now under consideration.

In the absence of any such actual discoveries the following facts have been urged, with more or less plausibility, in favor of the mycotic theory. Under favoring external conditions the summer diarrhœa of children occurs as an epidemic. It is not possible to explain at least a large number of the cases by the anatomical lesions found after death, which are often entirely disproportionate to the severity of the symptoms during life, and when present may often be regarded as secondary. It is generally admitted that abnormal processes of fermentation and of decomposition in the gastric and intestinal contents play an important rôle in the etiology and symptomatology of these affections, and these processes outside of the body have been proven to depend upon the presence of various low vegetable organisms. The influence of heat is no less apparent in favoring the development of these processes than in favoring the development of the summer diarrhœa of children.

In view of these circumstances, and others of a similar purport might be adduced, it is not unnatural that many investigators have directed their attention to the microscopic study of the dejecta, in the hope of finding there microörganisms which might be regarded as the cause of the disease. Although these researches brought to light the fact that the diarrhœal stools are swarming with a large number of various forms of bacteria and of fungi, no positive conclusions could be drawn from this fact, inasmuch as the normal stools also contain countless bacteria. Indeed, many observers went further than this, and drew the unwarrantable inference that bacteria cannot be concerned in the causation of diarrhœal affections, because the normal stools as well as the diarrhœal stools contain an enormous number of various bacteria. This inference is unwarrantable, inasmuch as the mere microscopical examination of the stools affords only imperfect information as to the morphological characters, and no information at all as to the biological characters of the organisms present, and it may well be that in diarrhœal stools organisms with specific pathogenic properties are present which do not exist in healthy stools.

Evidently the only way in which to attack with any hope of success the problem as to the significance of the microörganisms in the diarrhœal stools of children is to isolate so far as possible the various species according to the modern bacteriological methods, which we owe to Koch. The organism thus isolated must be studied morphologically and biologically, more especially with reference to their fermentative and pathogenic properties. Furthermore, such organisms must be compared with those found in healthy stools, and the frequency of their presence in diarrhœal stools and their relation to different varieties of summer diarrhœa of children must, if possible, be established.

Before the work thus outlined can be satisfactorily prosecuted, it is necessary to become familiar with the bacteria found normally in the stools. It is from this point of view that Bienstock, Stahl, and especially Escherich, have made their valuable investigations of the bacteria in normal fæces.* Previous observers confined their

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* Bienstock. *Fortschritte d. Medicin*, 1883, p. 609 (preliminary communication), and *Zeitschrift f. klin. Med.*, Bd. VIII. p. 1. Stahl. Verhandl. d. Congresses f. Innere Medicin 3d Congress, p. 193, 1884. Escherich. *Fortschritte d. Medicin*, 1885, Nos. 16 and 17, and "Die Darmbacterien des Säuglings," Stuttgart, 1886.

Bienstock was the first systematically to isolate and study by the bacteriological methods the bacteria of the fæces. Stahl isolated twenty varieties, concerning which only a brief notice was published. His work, which was undertaken under Koch's direction and which promised much, was cut short by his death.

Individual species of bacteria have been isolated from the fæces by Brieger, Koch, Finkler and Prior, Miller, Kuisl, Weisser, and others.

examinations to the microscopical study of the stools. This method of examination is indispensable, but for reasons which have already been mentioned it has hitherto shed little or no light upon the problem before us. Hence it is that we pass over without further comment the painstaking microscopical examinations of the stools made by Hausmann, Szydlowski, Woodward, Nothnagel, Uffelmann, Baginsky and others.

As the bacteria in the fæces of adults living upon a mixed diet differ greatly in number and in kind from those in the healthy fæces of milk-fed infants, and as our interest is solely with the latter, we pass at once to the consideration of the bacteria present in the stools of healthy sucklings. We owe to Escherich the fundamental investigation of this subject. His researches are of great value and have been made according to the most approved bacteriological methods. These researches have laid the foundation for a fruitful study of the bacteria in the pathological stools of infants.

Escherich found, as had also previous observers, that our present bacteriological methods do not enable us to isolate in the form of pure cultures all of the bacteria existing in the fæces. There is therefore a discrepancy between the results of microscopical and those of bacteriological examination of the fæces, the latter method showing a smaller variety of organisms than the former. For this reason, if for no other, it is plain that the bacteriological cannot displace the microscopical examination. The two methods must be used in combination with each other. Efforts to obtain culture media more suitable than those in common use for the cultivation of a larger number of fecal bacteria have not hitherto been successful.

Escherich proved the correctness of the common belief that the meconium of the newborn infant is entirely free from microörganisms. After a variable period bacteria make their appearance in the meçonium, and usually by the second day after birth they are present in large number. The chief mode of ingress of bacteria to the intestine is through the air, saliva and food which are swallowed, but inasmuch as bacteria are sometimes found in the meconium taken from the rectum three to seven hours after birth, Escherich believes that they may enter per anum. The bacteria found in meconium stools are fewer in number but greater in variety than those in the subsequent milk fæces.

While bacteria are present in enormous number in the fæces of healthy milk-fed infants, it was found that two species of bacteria are constantly present, and that of these one species so greatly preponderates that it is sometimes found almost as a pure culture. The bacteria constantly present are the bacterium lactis aerogenes and the bacterium coli commune. These are designated obligatory milk-fæces bacteria, in distinction from the inconstant bacteria, which are called facultative or potential milk-fæces bacteria. The bacterium lactis aerogenes is present greatly in excess of the colon bacterium in the upper part of the small intestine, but in the lower part of the small intestine and throughout the colon it diminishes in number, and in the healthy stools only comparatively few individuals of this species are found. On the other hand, the bacteria coli commune* becomes more and more numerous toward the end of the intestine and vastly exceeds in number all other varieties of bacteria in the stools.

The obligatory milk-fæces bacteria are capable of growing with the production of fermentation, without oxygen. The bacterium lactis aerogenes causes lactic acid fermentation of milk sugar, with the development of $CO_2$ and H, and there is every reason to believe that it is this organism which causes this species of fermentation, which has been proven to occur normally in the infant's intestine. The colon bacterium, which is found also in the meconium and in fæces from a mixed diet, probably causes

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* Escherich thinks it possible that under this name several closely allied species are included, as he found some differences in the appearances of the colonies on gelatine plates.

some species of fermentation or of decomposition in the secretions of the large intestine, perhaps in the mucus.

The potential or inconstant milk-fæces bacteria are for the most part aerobic, and so far as they develop at all in the intestinal canal, probably grow in the peripheral layer, which contains a small amount of oxygen. The inconstant bacteria are, as a rule, somewhat more numerous in the fæces of infants fed with cow's milk than in the stools of sucklings.

Two facts of the greatest importance are brought to light by Escherich's investigations, viz.: the remarkable and unexpected simplicity and uniformity of the bacterial vegetation in the healthy fæces of milk-fed infants, and the variation in this vegetation which occurs with a change in the quality of the diet.

At first glance it is not easy to understand why, of the manifold varieties of bacteria which gain access to the infant's intestine, only two should develop there constantly and in large number, but this difficulty is less considerable when we consider that an organism in order to supply itself with the necessary oxygen and food in the intestine must be capable of causing anaerobic fermentation of the intestinal contents. In harmony with the simple and uniform food of the milk-fed infant, we find a corresponding uniformity in their intestinal bacteria.

It is not possible to foresee to what clinical and therapeutic uses may be put the knowledge of the constant characters of the bacterial vegetation in the normal milk fæces. It seems a justifiable inference that any marked variation in the quality of this bacterial vegetation is an expression of some disturbance in the alimentary tract.

So long as the normal fæces were believed to contain a chaotic mass of all sorts of bacteria, it seemed a useless and unpromising task to make any especial study of the likewise chaotic mass of bacteria in diarrhœal stools. This point of view, however, is now changed. With definite information concerning the more important and constant bacterial species in the normal stools, it has become a matter of the utmost interest to learn what new species of bacteria appear in diarrhœal stools, and what changes occur in the normal bacterial vegetation.

Such knowledge may prove of value in many ways. Hitherto the study of the fecal bacteria in pathological cases has had especially for its object the hope of discovering some specific forms which might be regarded as the essential cause of the disease. This is, of course, a most important object of research, and one which I have kept in view in my investigations. There is, however, another point of view hardly less important and which is based upon the fact that the kind of bacteria found in the fæces vary with the intestinal contents which serve as food for the bacteria. Thus, we find sharply defined differences in the bacterial vegetations characterizing milk fæces, meconium, meat fæces, fæces from a mixed diet, etc. In cases of summer diarrhœa there are abnormal changes in the contents of the stomach and of the intestine, in consequence of morbid secretions, peristalsis and fermentations, and corresponding to these abnormal intestinal contents we may expect to find abnormal vegetations of bacteria, and it is reasonable to suppose that some definite relations may be discovered between certain forms of bacteria and certain definite changes in the intestinal contents. Such a discovery might be of diagnostic and, perhaps, of therapeutic value.

It is evident that we must be very cautious in assuming that any causative relation exists between strange forms of bacteria in the fæces and the existing disease. Various interpretations of such a coincidence are possible. First: it may be that the new forms of bacteria are to be regarded simply as the necessary accompaniment of the altered intestinal contents, and do not influence in any way the disease. Even upon this supposition of their harmless saprophytic nature the study of these bacteria may prove of diagnostic and therapeutic value, for reasons which have already been mentioned. In the second place, the new forms of bacteria, while not the primary cause of the disease, may, by their presence and growth in the intestine, cause a continuance and aggravation of the disease. For instance, we can readily suppose that a gastric or intestinal catarrh, or some abnormality in peristalsis or secretion, induced primarily by error in diet, or by heat, or by some constitutional cause, or by unhygienic surroundings, may be kept up and aggravated by the presence of microörganisms whose continued existence in the intestine is first rendered possible by some abnormality which would otherwise be transitory. In the third place, the bacteria may begin their work outside of the body, by developing in the milk or other food taken by the infant and causing abnormal products of fermentation or decomposition, possibly poisonous ptomaines. In the fourth place, one or more of the species of bacteria found in the stools of infants affected with summer diarrhœa and not found in the normal stools may be the essential and specific cause of the disease in the same sense that the typhoid bacillus is of typhoid fever, or the anthrax bacillus of anthrax. It must be admitted that without further proof of any given case the presumption is against this last supposition.

In view of the very peculiar qualities which bacteria must possess in order to adapt themselves to the conditions of growth in the healthy infant's intestine, and in view of the preoccupation of the field by other bacteria, it is not likely that bacteria which enter the normal intestine will be able to displace those normally existing there. That preparation of the soil which we vaguely call predisposition doubtless plays a most important rôle in the class of diseases now under consideration. Given a favorable soil, such as that resulting from gastric or intestinal catarrh, or from abnormalities in peristalsis or secretion, then bacteria which would fail to gain lodgment in the healthy intestine may grow, and by their presence become the most serious factor in the disease.

The foregoing considerations have led me to undertake the biological investigation of the bacteria found in the dejecta of infants affected with summer diarrhœa.

## METHODS PURSUED IN THIS INVESTIGATION.

(*a*) For collecting material: A small, slightly pointed glass tube, sterilized in the flame of a spirit lamp, was introduced into the rectum, and immediately after withdrawing it the contents were emptied into a sterilized test tube plugged with sterilized cotton wadding. As only the contents of the tube were put in the test tube, and the room in which the work was carried on was comparatively free from germs (as shown by the small number of colonies, often none, growing upon agar plates exposed for a considerable time), the purity of the cultures may be reasonably assured. Agar plates were made at once, and all colonies showing the slightest difference were planted in agar tubes. The cultures were then transferred to the pathological laboratory of the Johns Hopkins University, where the further isolation and study of the bacteria were carried on under the general supervison of Prof. Welch and Dr. Meade Bolton, the assistant in the Bacteriological Department.

(*b*) For the study of the morphological and biological characters of the bacteria the modern methods introduced by Koch were employed. The usual solid and fluid nutritive media, such as nutrient gelatine, nutrient agar, steamed potatoes and bouillon were used. As a rule, in place of the old plate method, Esmarch tubes were employed to isolate different species of bacteria, to test the purity of cultures and to observe the characters of the colonies. The investigation of the fermentative properties of the organisms was carried on chiefly with milk, as will be described hereafter.

(*c*) For testing pathogenic properties: The animals used were guinea pigs, young kittens, rabbits, white mice and rats. The experiments consisted in: (1) feeding pure cultures in milk which had been previously sterilized one hour for three successive days in the steam sterilizer. It was fed to mice in small sterilized glass dishes, and small pieces of bread were crumbled in it when the mice would not take the milk culture by itself; 3–8 cc. of the milk was given daily until death occurred, or for five to eight days, when no effect was produced. A similar quantity was fed to the guinea pigs and kittens, through a sterilized pipette. The animals otherwise were fed as usual during the experiments. (2) Hypodermatic and intra-venous injections: The injections were made with a sterilized Koch's syringe. The fluid used for injection was sterilized water or bouillon impregnated with the bacteria and injected at once. (3) In other cases bouillon was inoculated and allowed to stand for several weeks, and then sterilized according to Tyndal's method of fractional sterilization. Hypodermatic injections were made in rats and mice, at the root of the tail; in guinea pigs and kittens about the middle of the abdomen. The intra-venous injections were made in rats in the jugular vein, and in rabbits in the outer vein of the ear.

(*d*) To test if the bacteria were pathogenic in very small quantities, a pocket was made in the skin with sterilized scissors, after trimming off the hair, and by means of a straight platinum needle dipped into a fresh culture a very minute quantity of the culture was taken up and introduced into the pocket under the skin.

Autopsies were made as soon after death as possible, and cultures and cover slip preparations made from different cavities and organs of the body. Before opening the abdomen the hair was singed or cut off, and the skin washed with sublimate. In making cultures from the organs of the body, the needle was introduced through an opening made with heated scissors, so that it could only come in contact with the interior of the organ.

*Condition of the children from whom the cultures were obtained.*—From 1500 to 2000 children affected with summer diarrhœa are sent every summer from Baltimore city to the Thomas Wilson Sanitarium, ten miles out in the country. Since the opening of the Sanitarium, in 1884, only one case of cholera infantum, according to the classical description, has been brought to it, though a modified form of the disease is not uncommon.* The great majority of the children have acute or chronic gastro-enteric catarrh, and simple diarrhœa, and about one per cent. have dysentery.

The children from whom the fæces were taken for examination were chosen with special reference to the severer forms of these affections, with one perfectly healthy child for control, and one just beginning with a mild diarrhœa, in all, seventeen children.

## DESCRIPTION OF THE INDIVIDUAL CASES.

CASE I.—Three months old; fed exclusively on breast milk; has decided stupor, and is extremely emaciated; evacuations frequent, watery, and yellow or greenish in color.

CASE II.—Eleven months old; chiefly cow's milk diet; has high fever, stupor, and very frequent vomiting and purging; evacuations watery.

CASE III.—Nine months old; chiefly cow's milk diet; is emaciated, has slight fever, drowsiness, frequent vomiting and purging; evacuations watery.

CASE IV.—Eight months old; chiefly breast milk diet; sick only a few days; has stupor, vomiting and purging; evacuations rice colored, watery, and mixed with considerable fecal matter.

CASE V.—Eight months old; cow's milk diet; has stupor and persistent vomiting immediately after anything is taken into the stomach; evacuations are not frequent, and are semi-fluid and of yellow color.

CASE VI.—Eleven months old; chiefly cow's milk diet; has frequent vomiting and purging and decided stupor.

CASE VII.—Six months old; condensed milk diet; is emaciated; has drowsiness, frequent vomiting and purging; evacuations green and watery.

CASE VIII.—Five months old; breast milk diet; frequent vomiting and purging.

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* True cholera infantum and the modified form of the disease will be classed together in this article as cholera infantum.

CASE IX.—Ten months old; chiefly breast milk diet; slightly emaciated; has vomiting and purging; evacuations watery.

CASE X.—Eight months old; cow's milk diet; extremely emaciated; evacuations white and watery, with small quantity of fecal matter.

CASE XI.—Six months old; cow's milk diet; extremely emaciated; evacuations greenish fluid with mucus and white lumps mixed through it.

CASE XII.—Six months old; cow's milk diet; extremely emaciated; has sallow complexion; evacuations greenish and watery.

CASE XIII.—Eight months old; milk diet; is emaciated, restless and has frequent vomiting and purging. Evacuations watery.

CASE XIV.—Eight months old; breast milk diet; the rectum is covered with a croupous deposit; evacuations very frequent and containing a large quantity of pus, sometimes mixed with blood.

CASE XV.—Eight months old; cow's milk diet; emaciated; evacuations frequent and composed of bloody mucus or pus, with a small quantity of fecal matter. The dysentery is secondary to catarrhal enteritis.

CASE XVI.—Seven months old; breast and cow's milk diet; evacuations semi-fluid and have a whitish-yellow color; commencing with a mild diarrhœa; no previous digestive disturbance.

CASE XVII.—Four months old; breast milk diet; has one evacuation daily; the fæces are formed and have a light brown color; has never had any digestive disturbance.

*Summary.*—The children were from three to eleven months old. Twelve were fed exclusively upon milk and the others chiefly upon milk. Case II had been sick for four or five days with a mild diarrhœa, and true cholera infantum only 24 hours. Cases I to VII inclusive, excepting Case II, had been affected with a modified form of cholera infantum from three days to two or three weeks. In these the nervous symptoms were especially prominent. Cases VIII to XIII inclusive were cases of chronic gastro-enteric catarrh. Case XIV had primary dysentery (croupous), which was confined chiefly to the rectum. Case XV had secondary dysentery. Case XVI, previously healthy, was just beginning with a mild diarrhœa. Case XVII was perfectly healthy.

### BACTERIA SEPARATED.

Eighteen different varieties of bacteria have been isolated. The differentiation was made by the morphology of the organisms, by the characters of the growth upon various nutritive media, particularly gelatine, agar and potato, by the action on milk and by the effects of inoculating animals. All the varieties belong to the bacilli except one, which is a coccus. Three varieties of bacilli liquefy gelatine, the others do not liquefy it. The greatest number of varieties were found in the cases of cholera infantum; a larger number in gastro-enteric catarrh than in dysentery; and the smallest number in the healthy child; only one variety being found in it. Eight different varieties, the largest number isolated in any single case, were found in each of two cases. Table I * shows the distribution of the bacteria.

The difference in the number of varieties found in the fæces does not necessarily indicate a similar difference in the intestinal cavity. In the diarrhœal fæces, which come from the upper as well as the lower intestine, and are discharged at such short intervals that no considerable delay can occur in any part of the canal, the conditions are more favorable for obtaining representations from all portions of the canal,

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* The table is not yet complete, as not all the cultures from Cases IV, VI, VII, XI have been completely investigated, and, owing to the present imperfect means for differentiating bacteria the identity of some varieties is not positive; this is especially the case with bacillus C.

than is the case in the dysenteric discharges, which come chiefly from the lower intestine, and in the healthy fæces which are voided once in 24 hours, and are probably retained in the colon sufficiently long to cause the death of many bacteria which cannot exist for a long time in the large intestine. Nor is it claimed that the cultures obtained represent absolutely all the varieties of bacteria contained in the fæces, as the bacteria may not be equally distributed through the fæces, and their colony growth, especially upon agar, to which we are limited in this climate during the greater portion of the summer for plate cultures, is not always distinctive enough to be recognized. Moreover, not all the bacteria present in the fæces will grow in our ordinary culture media.

## TABLE I.

### SHOWING THE DISTRIBUTION OF THE BACTERIA.

| | Cholera Infantum. | | | | | | | Catarrhal Enteritis. | | | | | | Dysentery. | | Beginning Diarrhœa. | Healthy. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bacteria.......... | I | II | III | IV | V | VI | VII | VIII | IX | X | XI | XII | XIII | XIV | XV | XVI | XVII |
| Bacillus A......... | + | + | | + | | + | | | | | | | | | | | |
| " B......... | + | + | + | + | + | | | | + | + | + | + | + | | | | |
| " C......... | + | + | + | + | + | + | + | + | + | + | + | + | + | | | + | + |
| " D......... | + | + | + | | | | | | | | | | | | | | |
| " E......... | | | + | | | | | | | | | | | + | | | |
| " F......... | + | | + | | | | | | | | | | | | | | |
| " H . | | + | | | | | | | | | | | | | + | | |
| " K......... | + | | | | | | | | | | | | | | + | | |
| " L......... | | | | | | | | | | | | | | | | + | |
| " M......... | | + | | | + | + | | + | | | | + | | | | | |
| " N......... | | + | | | | | | | | | | | | | | | |
| " O......... | | | | | | | | | | + | | | | | | | |
| " P......... | | | | | | | | | | | | | | | + | | |
| " Q......... | | | | | | | | | | | | | + | | | | |
| " R......... | | | | | | | | + | | | | | | | | | |
| " S......... | + | + | | | | | | | | + | | | | | | | |
| " T........ | | | | + | | | | | | | | | | | | | |
| Micrococcus V... | + | | + | + | | | | | | + | + | | + | | | | |

### CONSTANCY OF THE BACTERIA.

The bacterium coli commune, or varieties of bacteria so closely resembling Escherich's description of it that positive separation has not yet been successful, was found in all the cases except the two of dysentery. It was present in smaller numbers in the more serious cases, especially in cholera infantum, but was found apparently as a pure culture in the fæces of the perfectly healthy child.

One variety was found nearly constantly, and in large quantities in cholera infantum, and not in the dysenteric or healthy fæces. It resembles the description of the b. lactis aerogenes, but the resemblance is not sufficient to be regarded as an identity. A liquefying bacillus, possessing marked pathogenic properties, was found in four cases of cholera infantum, and not in other cases. The variety of micrococcus was found in three cases of cholera infantum and three cases of catarrhal enteritis, and was not found in the dysenteric or healthy fæces. The bacteria found in dysentery were also found in cholera infantum and catarrhal enteritis, but many varieties appeared in the latter which were not found in dysentery.

A rough comparison was made between the healthy and pathological fæces, by studying cover-slip preparations and making cultures from the smallest quantity that could be taken up from the fæces by a straight platinum needle, and then estimating the difference in the number of the colonies. This estimate can only be approximative, as the solid fæces are more adhesive than the liquid fæces, and a larger quantity is taken up by the needle when introduced the same depth. Cover-slip preparations from the fæces of the perfectly healthy child showed almost a pure culture of a bacillus identical with Escherich's drawing and description of the b. coli commune (Die Darmbakterien des Säuglings). Table II, Fig. 1. The bacilli were in immense numbers and the fæces appeared to be composed almost entirely of them. There were, besides these, a few short bacilli joined in twos and resembling Escherich's drawing of the b. lactis aerogenes. No other forms were seen. From the cultures made from the fæces only the form resembling the b. coli commune was obtained. Cover-slip preparations from a child just beginning with a mild diarrhœa showed the b. coli commune and two or three other forms, but the number of bacilli in this case were not so great as in the perfectly healthy child. The cover-slip preparations from the diarrhœal fæces varied in different cases; in some the bacteria were in immense quantity, in others they were not so numerous, but in all the preparations more than two different forms could be recognized. In the plate cultures made it was difficult to distinguish any difference in the number of colonies growing from the healthy and pathological fæces; but always a greater variety of colonies could be seen in the cultures from the diarrhœal fæces.

## DESCRIPTION OF THE INDIVIDUAL SPECIES OF BACTERIA.

*Bacillus A.*—Found in cholera infantum.

Morphology.—Varies somewhat in different stages of growth. In fresh cultures the bacilli are narrow, with rounded ends and varying in length, sometimes growing into long rods or dividing into twos. Fig. 1, Table II. The average size in agar culture twenty-four hours old is about three to four $\mu$ long and seven $\mu$ wide. In older cultures the bacilli are shorter and smaller. The bacilli show active motion in hanging-drop preparations.

Gelatine Growth.—They liquefy gelatine rapidly at ordinary temperatures; the colonies grow in twenty-four hours to a size large enough to be seen by the naked eye; under the microscope they appear nearly colorless; after reaching a certain size liquefaction begins. The gelatine in stick culture is completely liquefied in three or four days.

Agar Growth.—In stick cultures the surface is covered in a few days with a nearly colorless skim; in the depth is a luxuriant and delicate stalk corresponding to the line of insertion of the platinum needle. The colony growth is characteristic; the colonies are bluish to the naked eye, and have an indistinct halo around them which shades off imperceptibly into the surrounding agar; slightly magnified, the colonies are light brown and the borders indistinct. The surface of the colony has a delicate, wavy appearance.

Potato Culture.—The growth is luxuriant, a dirty brown color, raised slightly above the surface and has well-defined borders.

Blood Serum.—In twenty-four hours a small, white patch is noticed; this gives place, in a few days, to a white semi-fluid sediment around the edge of the tube; in six weeks about one-third of the stick culture is liquefied.

Action on Milk.—Milk is coagulated into a gelatinous mass with alkaline reaction. In a few days the milk is separated into a clear fluid upper stratum and a lower thick gelatinous stratum; as the culture grows older the fluid stratum increases at the expense of the lower thick stratum. In some cultures in which milk was acid when inoculated the reaction afterward became alkaline, but a sufficient number of experiments have not yet been made to be conclusive upon this point.

*Pathogenic properties tested by experiments upon lower animals:—*

(*a*) Feeding with milk cultures which were from one to six weeks old :—

*Experiment 1.*—Three mice and three young guinea pigs.

Results.—All died; death occurring in from one to eight days. Autopsies were made in each case as soon after death as possible. No abnormal changes were noticed in any case, except a certain degree of emaciation.

Cover-slip preparations showed, in the stomach and intestines, among other bacteria, a bacillus identical in microscopic appearance with the original, and usually in large numbers. From the blood of the heart, spleen, liver, kidneys and peritoneal fluid the bacilli were sometimes found, but not always.

Esmarch tubes from the stomach, intestines and kidneys gave chiefly liquefying colonies. Cover-slip preparations and potato cultures from the colonies were identical with the original. As a rule they were in greater numbers in the small than the large intestines. In the kidneys they were generally pure and in large numbers. From the heart, spleen and liver negative results, except in one case from the heart and one from the liver pure liquefying colonies were obtained.

Only one of the animals, a mouse, lived longer than three days. On the fourth day it was noticed to have frequent discharges, which were soft, of a whitish yellow color and contained some mucus. Esmarch tubes, made from the discharges, had the appearance of being almost pure of the original, and cover-slip preparations from the liquefying colonies showed the same. The diarrhœa continued four days, when the animal died, being very much emaciated. There was no diarrhœa in the other animals.

Control.—Two guinea pigs of the same age as those used were fed, one upon the customary food alone, and the other upon this and sterilized milk. There was no difference between these two, and both continued healthy. Mice were fed with sterilized milk without being affected.

*Experiment 2.*—A young kitten was fed with milk cultures in the same manner as the guinea pigs, without results; it was then given croton oil until diarrhœa was produced, and fed with the culture without results.

*Experiment 3.*—Mouse. Milk culture, fourteen days old, sterilized by the *interrupted* method, was given six days without results. The animal was then fed on the infected unsterilized milk without being affected, except a slight diarrhœa which lasted two days. In this experiment the milk was slightly acid when it was inoculated, and the casein was not liquefied to the same extent as happened when it was alkaline at the time of inoculation.

(*b*) Intra-venous injections.

*Experiment 1.*—Three rats. Bouillon culture, 18 days old and sterilized by the interrupted method. Sterilization was proved by the failure to obtain cultures from it. .5 cc. was injected into a jugular vein of each rat. Results: all died. Death occurring in two and a half to five hours. Autopsies show no changes, except that the blood vessels on the posterior part of the brain appeared congested.

Control.—A rat was injected in a similar manner, with the same quantity of pure sterilized bouillon, without being affected.

*Experiment 2.*—Rat. A small quantity of an eight-days' agar culture was put into one cc. of distilled water, and .5 cc. of this injected at once into a jugular vein; no results.

*Experiment 3.*—Rabbit. Milk, treated in the same manner as in Experiment 1, was filtered in a sterilized filter, and two cc. of it injected into the ear vein without results.

(*c*) Hypodermatic injections.

*Experiment 1.*—Three kittens. One cc. of freshly inoculated bouillon was injected into each kitten.

Results.—Two recovered after being somewhat dull and stupid for several days. The third died on the fifth day.

Autopsy.—The organs of the body appeared healthy. There was suppuration for a space of two centimetres around the point of inoculation. Cover-slip preparations from this point showed a large number of bacilli identical with the original, and Esmarch tubes from it and the peritoneal cavity gave pure cultures of the original.

*Experiment 2.*—Mouse. .3 cc. of liquefied gelatine culture injected.

Result.—Death in twenty-four hours.

Autopsy.—On the back, just above the point of inoculation, the skin was puffed up with air about the size of a hazel nut. The skin around the point of inoculation was red and appeared to be inflamed. Nothing else abnormal noticed. Cover-slip preparation and Esmarch tubes from the point of inoculation, liver, spleen kidney, peritoneal fluid and heart appeared to be pure cultures of the original.

*Experiment 3.*—Two rats. .5 cc. of liquefied gelatine culture injected into each rat.

Results.—In one the skin on the posterior half of the back was gradually separated by a process of necrosis. Complete separation, leaving the under surface bare, was effected in two weeks. The edges were clean cut and there was no suppuration. In the other rat a similar but much smaller separation of the skin took place on the side of the thigh and about one inch from the point of inoculation.

*Experiment 4.*—Mouse. A straight platinum needle dipped into a fresh potato culture was inserted under the skin.

Result.—Death on the sixth day.

Autopsy, immediately after death.—The organs of the body appeared healthy. Cover-slip preparations and cultures from the spleen, heart, liver and peritoneal fluid gave negative results.

SUMMARY.

*Bacillus A* was found in four cases, one of which was in the stage of stupor of acute cholera infantum, and the others were more chronic cases of a modified form of cholera infantum in which the nervous symptoms largely predominated. It was not among the most numerous bacteria found in the discharge in either case, and owing to this fact it may have been overlooked in other cases. It produces a gelatinous coagulation in the milk, which is afterward more or less completely liquefied, and changed into a light brown color. It leaves the oil globules unaffected. Its pathogenic properties appear to reside in the power of producing injurious products in albuminous compounds, and its action seems to vary according to the manner it is introduced into the blood. Milk cultures fed to mice and guinea pigs proved fatal, while the sterilized milk cultures had no effect when fed. When introduced directly into the blood a small quantity of sterilized bouillon cultures proved rapidly fatal, while the unsterilized fresh cultures injected into the veins of rats had no effect. In all the animals to which it was given more or less drowsiness or stupor was produced. Only one had pronounced diarrhœa, and one a mild diarrhœa. Besides being found in the elementary canal of all the animals that died after being fed upon the culture, it was also found in some cases in other organs, especially the kidneys.

*Bacillus B.*—Found nearly constantly in cholera infantum and gastro-enteric catarrh.

Morphology.—Short, thick bacilli, with slightly rounded ends, often joined in pairs. The average size is 2–3 $\mu$ long and 1 $\mu$ wide. Thick, long filaments were often seen. Some of the bacilli and nearly all the long filaments contained a clear, glistening spot, which had every appearance of a spore, but cultures containing these apparent spores were not more resistant to heat than were others. In cultures eight days old the bacilli are longer than they are in fresh cultures, and many of them show when stained with gentian violet a deep violet centre with purple or clear poles. The bacilli often show capsules. The bacilli show no motion of their own in hanging-drop preparations.

Gelatine cultures.—The colonies develop rapidly; the superficial ones are raised and spread out; they are generally round with even borders, though often irregular in shape and white to the naked eye. When seen under the microscope they are loose and granular, and have a yellowish-brown centre with a white border; by the third day this becomes a nearly uniform light brown color, and a few days later a dark central zone displays itself with a light brown peripheral zone. Older cultures show a number of concentric rings of different shades of brown. The deep colonies remain small and have a yellow or brown color. In stick cultures the surface growth is raised, white, and extends about half over the surface; in the depth it has a solid and uniform stalk without any marked end swelling.

Agar cultures.—The colonies develop rapidly, and in forty-eight hours reach a considerable size. They are white to the naked eye, and are round and dome-shaped. Under the microscope they have a light-brown color and are not compact. Neighboring colonies run together without any mark of separation at the point of junction. The deep colonies are small and dark, and have a granular appearance.

Potato cultures.—Cream or pale yellow color, with a raised and nodulated surface, well defined and notched borders and sloping edge. Gas bubbles appear over the surface in two or three days. There is but slight moisture about the growth at first. But slight, if any, difference can be noticed between the cultures on old and new potatoes.

Action on milk.—Milk is coagulated with acid reaction, and sometimes with evolution of gas.

On blood serum a small whitish patch appears in a short time, but instead of continuing to grow as a solid growth, it gives place to a whitish semi-fluid substance seen around the sides of the tube.

*Pathogenic properties tested by experiments upon lower animals:—*

(*a*) Hypodermatic injections.

*Experiment 1.*—Mouse. .5 cc. of a fresh bouillon culture injected.

Result.—Death in forty-eight hours.

Autopsy, at once.—Skin around the point of inoculation was red and appeared to be inflamed. Organs of the chest and abdominal cavities appeared healthy. Cover-slip preparations from the point of inoculation, peritoneal cavity, spleen and right auricle, showed a short, thick bacillus identical with the original. Cultures were obtained from the point of inoculation and from the peritoneal fluid.

*Experiment 2.*—Guinea pig, injected with two cc. of fresh bouillon culture.

Result.—Death in twenty-four hours.

Autopsy.—Slight œdema and redness of the skin around the point of inoculation. The peritoneal cavity contained about ten cc. of a grayish-white viscid fluid which began to escape as soon as the cavity was opened. There was general peritonitis and the stomach and intestines were congested. The other organs of the abdominal cavity and those of the thoracic cavity appeared healthy. Cover-slip preparations from the peritoneal fluid showed an enormous quantity of pus cells and a pure culture of the bacillus; some of the bacilli were around the pus cells and some appeared to be in the cells. Cover-slip preparations from the right auricle also showed a pure culture of the original. Cultures from the peritoneal fluid, spleen and right auricle gave pure cultures of the original.

*Experiment 3.*—Rat and mouse. Five-tenths to one cc. of bouillon culture, obtained from the right auricle of the guinea pig, was injected into each.

Results.—The rat died in forty-eight hours and the mouse on the fourth day.

Autopsy of the rat showed a small inflamed spot at the seat of inoculation, and the body somewhat emaciated. The organs of the body appeared healthy. Cover-slip preparations and cultures made from the heart, spleen, kidney, peritoneal fluid and point of inoculation only succeeded from the spleen, peritoneal fluid and point of inoculation: in these the original bacillus was obtained pure.

Autopsy of the mouse.—The body was very much emaciated and the skin was separated, by sloughing, from the abdomen and right thigh. The lymphatics of the mesentery were white and distended and the receptaculum chyli was very large and filled with a white fluid. The organs of the body appeared healthy. Cover-slip preparations from the abscess and receptaculum showed a large number of bacilli identical with the original.

*Experiment 4.*—Rat injected with .5 cc. and a kitten with one cc. of fresh buillon culture, with negative results.

*Experiment 5.*—Two mice. A straight platinum needle was dipped into a fresh potato culture and inserted under the skin.

Results.—One mouse was unaffected; the other died on the sixth day.

Autopsy showed nothing abnormal, and cover-slip preparations and cultures were negative.

(*b*) Feeding with milk cultures.

*Experiment 1.*—Three mice.

Results.—Death in two to four days.

Autopsies.—No pathological lesions were noticed in any case. Cover-slip preparations from the stomach and duodenum showed a larger proportion of short, thick bacilli, resembling the original, than the lower part of the ileum. In some preparations the cover-slip preparations from the stomach and duodenum looked like almost pure cultures of the original. A bacillus identical with the original was also found in the cover-slip preparations from the heart, spleen, liver and kidney; in these it was generally the only bacillus found, and pure cultures were obtained from these organs. Esmarch tubes made from the contents of the stomach and intestine gave a large proportion of the colonies in every way resembling the original, and this proportion appeared to be greater in the upper than in the lower intestine.

*Experiment 2.*—Mouse, young guinea pig and kitten. Negative results. Artificial diarrhœa was produced in the kitten, with croton oil, and the milk culture again fed to it with negative results.

*Summary of Bacillus B.*—Found nearly constantly in cholera infantum and catarrhal enteritis, and generally the predominating form. It appeared in larger quantities in the more serious cases. It was not found in the dysenteric or healthy fæces. It resembles the description of the b. lactis aerogenes, but the resemblance does not appear sufficient to constitute an identity and, in the absence of a culture of the latter for comparison, it is considered a distinct variety, for the following reasons: Bacillus B is uniformly larger, its ends are not so sharply rounded, and in all culture media long, thick filaments are seen, and many of the bacilli have the protoplasm gathered in the centre, leaving the poles clear. There is some difference in their colony growth on gelatine, and in gelatine stick cultures bacillus B does not show the nail-form growth with marked end swelling in the depth. In potato cultures the b. lactis aerogenes shows a difference between old and new potatoes, while bacillus B does not show any difference.

Bacillus B possesses decided pathogenic properties, which was shown both by hypodermatic injections and feeding with milk cultures.

*Bacillus C.*—Found nearly constantly in the stools of cholera infantum, catarrhal enteritis and the healthy suckling. It appeared in diminishing quantity the more serious the case of diarrhœa. It answers so fully to the description of the b. coli commune that the two are regarded as identical. The difficulty which Escherich met with in being always able to consider his culture of the b. coli commune as pure, and yet unable to separate it into more than one variety, was experienced by myself with the cultures of this variety obtained from different children. Cultures of bacillus *C* taken from different children would sometimes, on the same medium, appear identical, and at other times, with all the conditions for the two cultures as nearly alike as it was possible to have them, each would appear sufficiently different to constitute distinct varieties if the difference had remained constant. It is highly probable, as Escherich suggested, that instead of this being one variety it may prove to be a group of varieties, or a family of closely allied organisms.

*Bacillus D.*—Found in cholera infantum and chronic gastro-enteric catarrh.

Morphology.—Small, narrow bacilli, varying in length and thickness at different stages of growth. In the fæces, and in fresh blood serum cultures they are narrow and have very small, round, clear spaces throughout the body of the cells, which give a granular appearance when seen with Zeiss homogeneous immersion objective one-twelfth and eye piece I. In fresh gelatine cultures the cover-slip preparation has the appearance of being mixed; large rods with vacuoles and staining deeply, and small, narrow rods which do not stain so deeply being seen; but repeated plating failed to separate them into two varieties. The bacilli show no motion of their own in hanging-drop preparations.

Agar stick growth.—A small, round, white patch on the surface, with a thick stalk in the depth.

Gelatine stick growth.—A colorless, slightly raised patch, with irregular borders on the surface, and a delicate stalk in the depth.

Gelatine colony growth.—The surface colonies on the second day, small, white and beginning to spread; on the third day they have nearly reached their full growth, and are white or bluish white to the naked eye. Microscopically, at first they have a dead white appearance, and as they grow older the central part changes to a light yellow color, and is slightly raised; the borders are irregular and notched and remain white. The old colonies have a uniform light yellow color. The deep colonies remain small and have a uniform light yellow color and granular appearance.

Agar colony growth.—Small, pale yellow colonies, not characteristic.

Potato growth.—Characteristic. A bright golden yellow color, raised and dry surface, and well-defined border. There is no difference in the growth on old and new potatoes.

Action on milk.—No change is produced for three or four days; after this time it becomes more decidedly alkaline, but never coagulates. In six to eight weeks the milk has a brown, transparent appearance.

*Pathogenic properties:—*

(*a*) Hypodermatic injections.

*Experiment 1.*—Five mice, two rats and two guinea pigs. Buillon cultures used; .5 cc. injected into the mice, one cc. into the rats, and two cc. into the guinea pigs. Results.—The rats and three mice were unaffected, and two mice and the guinea pigs died, death occurring in 24 hours to four days. Autopsies.—Nothing abnormal was noticed beyond a limited inflammation at the point of inoculation, which was suppurative in the guinea pigs. Cover-slip preparations and cultures from the point of inoculation, peritoneal fluid, spleen and heart in the mice gave a pure culture of a bacillus identical with the original, and the same from the peritoneal fluid and point of inoculation in the guinea pigs.

*Experiment 2.*—Two mice. A straight platinum needle was dipped into the pure cultures obtained from the mice that died, and inserted under the skin, with negative results.

(*b*) Feeding with milk cultures. Four mice and two kittens.

Results.—Three mice and the kittens were unaffected, and one mouse died on the seventh day.

Autopsy.—Body emaciated; nothing else abnormal was noticed. Cover-slip preparations from the peritoneal cavity, stomach, intestine, heart and liver were mostly negative. Esmarch tubes from the stomach, duodenum and ileum gave in the second tubes about 100 colonies from each. The great majority of the colonies were identical with the original. Potato cultures made from the colonies were also identical with the original.

*Summary.*—Bacillus D was found in only three cases of cholera infantum, and was not found in large numbers in any case.

Its pathogenic properties are not strong, as more than half the animals in which it was tested were unaffected by it.

*Bacillus E.*—Found in cholera infantum, gastro-enteric catarrh and dysentery; it was not found in many cases.

Morphology.—In fresh gelatine cultures they are short, small bacilli, with rounded ends, many joined in twos and some in long filaments. In potato and milk cultures they are longer and some have small, clear spaces in the ends.

Potato cultures have straw or yellow color, raised surface and the borders sometimes defined and sometimes not well defined.

Gelatine plate culture.—The surface colonies are white and not much spread out; under the microscope they have a uniform lemon color. The deep colonies remain small and have a light yellow border with a brown centre. Gelatine stick culture has a slightly raised, smooth and nearly colorless patch on the surface, and a luxuriant stalk with fringed border in the depth.

In agar the colonies are bluish to the naked eye and uniform grayish-white under the microscope. The stick culture has a smooth, white, raised patch on the surface, and a thick stalk in the depth.

Only negative results were obtained in the experiments made upon lower animals with this variety, and milk is apparently unaffected by it.

*Bacillus F.*—Found in only one case of cholera infantum.

Morphology.—In fresh cultures they are small, short bacilli, which do not stain deeply with a few longer and thicker forms which take a deeper staining; in old cultures small, clear spaces are seen throughout the body of the cell. In some cultures, especially milk, very long filaments are seen.

Potato growth.—A moist, dirty brown and sometimes glossy surface, but slightly raised, with borders generally defined.

Gelatine growth in stick culture.—A dry, light brown patch on the surface, with a very delicate growth in the depth. The growth is very slow and never very extensive on the surface. The colonies also grow very slowly and seldom reach a large size. Microscopically, the surface colonies are white, glistening and coarsely granular; as they grow older they show a light yellow centre surrounded by darker concentric rings; the deeper colonies remain small and are yellow and granular.

Agar growth in stick culture.—Small, moist, glass-colored patch, slightly raised and lobulated surface, with well-developed stalk in the depth.

Colony.—The surface colonies are large and round, and have a ropy consistency. Microscopically, they are yellow and have a wavy surface.

It renders milk more alkaline without coagulation. Old milk cultures have a brown, transparent appearance.

*Pathogenic Properties:—*

(*a*) Hypodermatic injections.

*Experiment 1.*—Two mice. .5 cc. of melted gelatine culture injected.

Results.—Death in 24 and 48 hours.

Autopsies showed nothing abnormal in either case. Cover-slip preparations and cultures from the point of inoculation gave an enormous quantity of bacilli identical with the original.

*Experiment 2.*—Two mice injected with .5 cc. of bouillon culture, with negative results.

(*b*) Intra-venous injection.

*Experiment 1.*—Rabbit. One cc. of bouillon culture injected into the outer vein of the ear, with negative results.

(*c*) Feeding with milk culture.—Four mice and one kitten. Negative results. Two of the mice were then given croton oil and again fed on the milk culture.

Results.—Death in 24 and 36 hours.

Autopsies.—No abnormal changes noticed. Cover-slip preparations and cultures from the intestines gave a large number of bacilli and colonies which closely resembled the original. Potato cultures made from the colonies were identical with the original.

*Bacillus H.*—Found in one case of cholera infantum and one case of dysentery.

Morphology.—In fresh agar cultures they are small bacilli with rounded ends. In fresh potato cultures they are larger and longer, and the ends are more pointed.

Potato growth.—Bright yellow-colored growth, but slightly raised, with dry surface and defined borders.

Gelatine growth.—The stick cultures have a small, colorless patch, but slightly raised above the surface, and a well-developed growth in the depth. In plate cultures the surface colonies are bluish and spread out. Microscopically, they show a white, irregular and notched border, with a yellow, slightly raised centre.

Agar growth.—The stick cultures have a smooth, nearly colorless and but slightly raised patch on the surface, and a thick stalk in the depth. In plate cultures the colonies are round and white, with flat surface. Microscopically, they have a uniform brown color and are not compact.

Action on milk.—Coagulates milk with reaction.

In the few experiments with this variety upon lower animals only negative results were obtained.

*Bacillus K.*—Found in one case of cholera infantum and one case of dysentery.

Morphology.—Short, small bacilli, varying in size. The growth on potato is not uniform. Sometimes several days elapse before anything is noticed; at other times the culture develops more rapidly and is yellow, defined, raised and dry.

In gelatine plate cultures the colonies are whitish, or nearly colorless, and but slightly spread out. Under the microscope they are uniform, smooth and have a straw color. The stick culture has a small colorless patch on the surface, with a delicate stalk in the depth.

In agar plate cultures the colonies are round and white. Under the microscope they have a uniform light yellow or straw color. In agar stick culture the surface is moist and glassy, with a delicate stalk in the depth.

It renders milk acid, and coagulates it only after standing in the thermostat for five or six days. Pathogenic properties not tested.

*Bacillus L.*—Found in one case of mild diarrhœa.

Morphology.—Short, thick bacilli; many in the form of the figure eight; a few long filaments sometimes seen. It resembles somewhat the b. lactis aerogenes, but differs from it in important particulars.

In gelatine plate cultures the colonies are usually not spread out, but are thick, with a flat surface, and white to the naked eye. Microscopically they have a uniform light yellow color, or a flat, straw colored, central zone, which is raised above a white border.

On potato the culture is lemon colored, dry and not well defined. It renders milk acid, and coagulates it only when kept in the thermostat at 38° C. for five or six days. Pathogenic properties not tested.

*Bacillus M.*—Found in cholera infantum and gastro-enteric catarrh.

Morphology.—Solid, thick bacilli, about two $\mu$ long and one $\mu$ wide. Some are joined in twos and some are in long filaments. The colony growth in gelatine is changeable. The surface colonies at times are spread out, white or bluish white, and often showing a concentric arrangement to the naked eye. Microscopically they have the appearance of a large yellow colony placed in a larger white colony with only the irregular border of the latter showing, or they show a concentric arrangement, some of the rings being lobulated and some fringe-like or homogeneous. At other times the colonies are nearly colorless and show under the microscope, at first, a uniform greenish-yellow color with fine concentric markings. As the colonies grow older they show a yellow central zone with fine bar-like markings, and an outer white zone. The deep colonies are small and yellow.

On potato, the growth has a golden-yellow color with a moist, glistening surface, and well-defined borders.

Milk is rendered acid, but is not coagulated. As far as tested it showed no pathogenic properties.

Bacillus M resembles the b. coli commune in many respects, but differs sufficiently to be considered a separate variety. It is larger, and never shows the protoplasm gathered into the centre of the cell with the poles clear. On potato, it has a brighter yellow color, and the colony growth in gelatine, though often resembling, is never identical with the b. coli commune.

*Bacillus N.*—Found in one case of cholera infantum.

Morphology.—Exceedingly small and short bacilli.

Potato growth.—A faint straw or light yellow-colored growth, with a dry and slightly raised surface and defined border; in some cultures nothing is seen upon the potato.

Gelatine plate cultures.—The colonies are small and glassy or colorless, and are taken up entire with the needle.

Agar growth.—In stick cultures the surface has a small raised patch with a rough surface, and a well developed growth in the depth. The surface growth is drawn in ropes with the needle. In plate cultures the colonies are small. The superficial colonies are bluish to the naked eye; microscopically, a lemon-colored centre which gradually tapers to a white border; the deep colonies are uniform light yellow and remain very small. As far as tested it showed no pathogenic properties.

*Bacillus O.*—Found in one case of catarrhal enteritis.

Morphology.—Small, short bacilli, many joined in pairs. The potato cultures are dry, yellow, raised and defined, sometimes having a bright yellow color, at others a dark yellow. In gelatine plate cultures the colonies are spread out and have a grayish-white centre with a bluish outer zone. Microscopically they have a yellow centre with a white border. In gelatine stick culture the surface has a small, colorless, slightly raised patch with smooth surface. In the depth is a luxuriant stalk with fringe border. In agar plate culture the colonies are large, round and white. Microscopically they have a light yellow color, and gradually taper from the centre to the border.

It coagulates milk rapidly into a solid, firm clot with acid reaction. Pathogenic properties not tested.

*Bacillus P.*—Found in one case of dysentery.

Morphology.—Small bacilli with rounded ends, some in long filaments. Potato cultures are yellow, with dry raised surface and well defined borders.

Gelatine plate cultures.—The superficial colonies are spread out and bluish-white. Under the microscope they have a light yellow centre with a white border. As the colonies grow older they have a uniform light yellow color. In agar plate cultures the colonies are large, round and white. Under the microscope they have a light brown centre with a white rim. It coagulates milk more slowly than bacillus *O*, and the coagulation is not as solid or as firm. It renders milk strongly acid. Pathogenic properties not tested.

*Bacillus Q.*—Found in one case of catarrhal enteritis.

It resembles bacillus B in many respects, but is not identical with it. It is a short, thick bacillus, having a similar form to bacillus B, but does not stain clean like the latter; its borders show fine, fuzzy processes, and the intermediate spaces between the bacilli are stained, showing a peculiar network.

The colony growth in gelatine resembles that of bacillus B, only it is darker and more of a grayish-brown color under the microscope. The potato culture resembles bacillus B, but is more luxuriant and juicy. The most marked difference is in its action on milk. Bacillus Q renders milk acid, and coagulates it only when kept in the thermostat, at 38° C., for five or six days.

*Bacillus R.*—Found in one case of catarrhal enteritis.

It resembles bacillus Q in its action on milk but resembles bacillus B more closely in other respects. In morphology bacillus R is shorter, smaller, and more rounded ends, often having the appearance of diplococci.

In its growth upon potato and gelatine it resembles bacillus B very closely; the only difference observable is that the colonies are more compact in bacillus R.

The pathogenic properties of bacilli Q and R were not tested.

*Bacillus S.*—Found in one case of cholera infantum and one case of catarrhal enteritis.

Morphology.—Small bacilli, varying in length, some joined at the ends, forming long chains. On potato the culture is dirty yellow, slightly raised and has well-defined borders.

It liquefies gelatine more slowly than bacillus A, and coagulates milk with an acid reaction.

In agar plate cultures the colonies are blue to the naked eye; under the microscope the colonies are indistinct and whitish.

Pathogenic properties were not tested.

*Bacillus T.*—Found in one case of cholera infantum.

Morphology.—Plump, oval bacilli.

On potato it has a pretty pink growth with well-defined borders, and the surface is raised, in places, into large gas bubbles. On old potato culture the growth eats into the potato and is lower than the surrounding border. It liquefies gelatine rapidly and coagulates milk with acid reaction. Pathogenic properties were not tested.

*Micrococcus V.*—Found in cholera infantum and catarrhal enteritis.

Morphology.—Small round cocci, some joined together, forming diplococci.

Nothing is seen, in potato cultures, on the surface of the potato

Gelatine cultures.—Both the plate and stick cultures develop very slowly. In stick cultures a small dry patch is seen on the surface, and limited to the point of inoculation after a considerable time; the growth in the depth is more flourishing. In plate cultures the colonies cannot be seen with the unaided eye before the seventh or eighth day. They are round and have a bluish appearance; under the microscope a uniform lemon or light brown color. The colonies never reach a large size. In agar the growth is also slow. In stick culture the surface growth is limited to a small patch at the point of inoculation. The growth in the depth is not extensive. In plate cultures the colonies remain small and have a bluish appearance. Microscopically, they have a light yellow, granular appearance. It produces no apparent change in milk, and, as far as tested, has shown no pathogenic properties.

## GENERAL CONSIDERATIONS.

Regarding the work in its present condition as too incomplete to admit of positive conclusions, the following considerations are given as showing the tendency of the results thus far obtained:—

The bacterium coli commune does not disappear in the diarrhœal fæces of infants, but appears to diminish in number, according to the severity of the disease. It, or a variety or varieties closely resembling it, appears to be constantly present, but not as a pure culture or in the same largely predominating form as in the healthy milk fæces.*

It was not found in the dysenteric discharges.

No variety of bacteria has been found which bears the relation of constant or obligatory bacterium to the diarrhœal and dysenteric discharges that the colon bacterium bears to the healthy milk fæces.

One variety was found nearly constantly and generally in the predominating form in cholera infantum and catarrhal enteritis, but was not found in the dysenteric or healthy fæces. This variety resembles the description of the b. lactis aerogenes, but differs from it in certain particulars, and in the absence of a positive culture for comparison, has been regarded as distinct. The identity of the two, however, is rendered more probable by the fact that the b. lactis aerogenes is a constant or obligatory bacterium, and the predominating form in the healthy upper intestine of sucklings, while it gradually diminishes in number or altogether disappears in the lower intestine and fæces; and it is easy to conceive that with the frequent and rapid passage of the diarrhœal discharges through the intestine, a large number of the b. lactis aerogenes might be contained in them and in a suitable condition for obtaining cultures. Should the separation into a distinct variety be confirmed by proper comparisons, then we have a new form possessing many of the characteristics of the b. lactis aerogenes, with marked pathogenic properties, nearly constant in cholera infantum and catarrhal enteritis, which has not been found in the contents of the intestine of the healthy suckling, or their fæces. Should the two forms prove identical, then we have the b. lactis aerogenes occurring more frequently and in much larger quantity in the diarrhœal than in the healthy stools.

The number of varieties of bacteria in the diarrhœal fæces exceeds that of the dysenteric and healthy fæces.

The actual number of individual bacteria in the healthy fæces is as great as in that of the diarrhœal fæces.

The number of varieties of bacteria as well as the number of individual bacteria is less in the dysenteric than in the diarrhœal fæces.

The bacteria found in the dysenteric fæces were also found in the diarrhœal, but a number of varieties were found in the diarrhœal fæces which were not found in the dysenteric.

The bacteria differ from the inconstant varieties found by Escherich in the contents of the healthy intestine of sucklings and their fæces.

The number of varieties of micrococci, liquefying and chromoginic bacteria was less than that found by Escherich in the healthy suckling.

Considerable difference was manifest in the effects produced upon lower animals by the different varieties of bacteria with which experiments were made.

Two varieties appeared to possess marked pathogenic properties; in others the pathogenic properties were less marked and some appeared entirely non-pathogenic.

A liquefying bacillus which was found frequently but not constantly in cholera

* It is probable that some of the cultures which have been considered as identical with the b. coli com. would prove, with better means for the differentiation of bacteria than we have at present, to be different varieties.

infantum, and not in other forms of summer diarrhœa or healthy fæces, effected changes in albuminous compounds which proved rapidly fatal when injected in small quantity into the veins of rats, and milk cultures of the same bacillus generally resulted in death when fed to mice and guinea pigs.

Diarrhœa was not a prominent symptom in the animals in which the experiments were made. A pronounced diarrhœa was noticed in only one case.

It was shown by experiment that none of the bacteria were capable of multiplying in ordinary hydrant water, and cultures could not be obtained from the water in any case forty-eight hours after it had been inoculated, and in the great majority twenty-four hours afterward.

All the varieties of bacteria found are capable of thriving in milk. Some varieties produce coagulation of the milk with acid reaction, some render milk acid without coagulation, some render milk alkaline without coagulation, some have no apparent effect upon milk, and one variety coagulates milk with alkaline reaction.