

STUDIES UPON THE AMEBAE IN THE INTESTINE OF MAN.*†

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IN 1903 appeared Schaudinn's¹ conclusions regarding the amebae infesting the human intestine. As the result of his researches he clearly differentiated and described two species of amebae parasitic in man, one, pathogenic, producing the lesions of amebic dysentery, which he called *Entamoeba histolytica*; the other, non-pathogenic, to which he gave the name, *Entamoeba coli*.

At the time Schaudinn's publication appeared the writer was in charge of the laboratories of the U. S. Army General Hospital in San Francisco, Cal., where hundreds of cases of amebic dysentery had been under observation and treatment, and where there was always abundant material for the study of amebae. After over a year of constant work upon the subject I published the results of my investigations,² which largely confirmed those of Schaudinn, and which have been in turn confirmed by numerous independent observers. My conclusions at that time were briefly as follows:

The intestine of man may be infested with two species of amebae, one, pathogenic, the other, non-pathogenic; the non-pathogenic ameba (*Entamoeba coli*) was found in 65 per cent of healthy individuals examined, and in 50 per cent of those suffering from diseases other than dysentery; the two species may be distinguished in both fresh and stained preparations; they differ in their method of reproduction; the pathogenic ameba (*Entamoeba histolytica*), whether fed in milk or injected into the rectum produces in kittens the lesions of dysentery as seen in man; the non-pathogenic ameba, whether fed in milk or injected into the rectum, never produces in kittens any lesions whatever; neither feeding experiments nor rectal injections of fecal material, or the bacteria occurring in such material, produce in kittens

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any of the lesions of amebic dysentery, unless *Entamoeba histolytica* be present.

While at the present time Schaudinn's classification of the intestinal amebae in man has been accepted by such zoölogists as Stiles³ and Minchin,⁴ and by almost every investigator of, and writer upon, amebic dysentery, there are some authors who still hesitate to accept the division of the amebae into a pathogenic and non-pathogenic species, preferring to believe that the pathogenicity of these organisms, depends upon their environment or that, as yet, we have not sufficient evidence to warrant us in differentiating species.

In view of this fact any contribution upon the amebae in man, based upon the results of personal observation and careful study, should prove of some value in the elucidation of this question, and it is my purpose, in this contribution, to give in detail the results obtained from the study of the amebae observed in 1,579 cases of amebic dysentery, and from the study of the amebae observed in the feces in health and in diseases other than dysentery, together with the evidence obtained by the experimental production of the disease in animals.

Those who have used cultural forms of amebae as an argument against species have apparently lost sight of the fact that all known protozoa that have been cultivated develop, in cultures, very different forms from those usually observed, as, for instance, in the case of the trypanosomes and the Leishman-Donovan bodies. To compare amebae derived from external sources and cultivated upon artificial media with *Entamoeba coli* and *Entamoeba histolytica* as observed in the feces, and by the results of such comparison to claim on morphological grounds, that it is impossible to differentiate these two species, is surely an unscientific deduction and one that cannot be accepted as fact. In this paper, therefore, the amebae are described as they appear in the feces or in the intestine, and no deductions are drawn from the morphology of cultivated organisms. I am firmly of the opinion that the evidence already at hand regarding the two species of amebae described by Schaudinn is amply sufficient to establish the truth of his observations, but if further evidence is needed, it would appear that the question could be definitely settled by the cultivation, side by side, upon the same media, of *Entamoeba coli* and *Entamoeba histolytica*, an obvious and apparently simple method which appears

to have been overlooked by Schaudinn's opponents, for nowhere in the literature can I find a record of such experiments, although all kinds of amebae have been cultivated from extraneous sources and compared to the species of amebae occurring in man. While *Entamoeba histolytica* appears to have been cultivated, no instance is on record of the cultivation of *Entamoeba coli*, or even an attempt to do so, under similar circumstances, and upon similar media. Personally I have found it impossible to cultivate *Entamoeba coli* upon the media used for other amebae.

However, it is obvious that if it were possible, the cultivation of the amebae found in health and those found in amebic dysentery, with the determination of the pathogenic action of such cultures upon susceptible animals, would settle for all time the question of the existence of pathogenic and non-pathogenic species, but until this has been accomplished, we must accept the accumulated evidence, both morphological and experimental in favor of Schaudinn's classification as sufficient to prove its truth.

Prior to the perusal of Schaudinn's paper I had undoubtedly confused the two species of amebae, regarding the harmless *Entamoeba coli* as an atypical form, or as a stage in the development of *Entamoeba histolytica*. Both species often occur together in the feces of cases of amebic dysentery, and this fact has been one of the principal stumbling-blocks in the identification of the two organisms. If they be studied separately, however, the variation in morphology is so marked as to render their differentiation a matter of but little difficulty in the vast majority of instances. The harmless *Entamoeba coli* is a very common parasite of man and this renders the study of the intestinal amebae of great importance, as it is undoubtedly true that many cases have been erroneously diagnosed as amebic dysentery which were in reality cases of diarrhea due to other causes but in which *Entamoeba coli* was present in the feces and was mistaken for *Entamoeba histolytica*. This fact may account for the wonderfully successful results in the treatment of amebic dysentery claimed by some practitioners in the tropics.

As would be expected, I have seen certain cases infested with *Entamoeba coli* develop true dysenteric symptoms, with *Entamoeba histolytica* in the feces, but in an amebae infected country, such as the

Philippines, it would be strange indeed if certain of the individuals showing the harmless ameba in their feces did not, sooner or later, become infected with the pathogenic variety, especially when, as I shall show, 72 per cent of white men examined harbored the harmless ameba. Those opposed to Schaudinn's classification have tried to use such cases as an argument in favor of the unity of all intestinal amebae, but with the chances of infection so great as they are in the tropics, an argument based upon these cases has but little, if any, scientific value. It should also be remembered that a man might be suffering at the time of examination with amebic dysentery, yet only *Entamoeba coli* be found in his feces, and if only one examination were made the true condition would not be discovered. However, a few isolated cases of this kind, so easily explained, have no weight as a scientific argument against the truth of Schaudinn's classification of the intestinal amebae in man.

HISTORICAL.

Probably the first investigator to observe amebae in the stools of man was Lambl⁵, of Prague, who, in 1860, described organisms occurring in the feces of a child suffering from diarrhea which he interpreted as amebae, although they may have been stages in the development of one of the intestinal flagellates. In 1870, Lewis and Cunningham⁶ found amebae in the feces of nearly 20 per cent of cholera patients studied in India, and again, in 1881, in cholera stools and in those of patients suffering from other diseases and even in those of healthy individuals. These investigators did not consider these organisms of any pathological significance and interpreted them as stages in the development of flagellates.

Aside from the observations mentioned, the first investigator to describe minutely amebae found in the human intestine, and to associate them with the etiology of dysentery, was Loesch,⁷ in 1875, who studied carefully the amebae found by him in the stools of a man suffering from chronic diarrhea. To these organisms he gave the name "*Amoeba coli*," and claimed to have been able to produce dysentery in dogs by the rectal injection of fecal material containing them. Grassi⁸ confirmed the presence of amebae in human dejecta, but considered them as harmless, as he found them in both health and disease. Grassi's paper was followed by those of Sonsino, Norman,⁹ Perroncito,¹⁰ Callandrucio,¹¹ and Blanchard, all confirming the presence of amebae in the stools of patients suffering from diarrhea or dysentery.

In 1883, Robert Koch,¹² while investigating cholera in Egypt, found amebae in the tissues of the intestine in three cases of dysentery, and was able to demonstrate them in sections, situated at the base of the ulcerations, which situation Koch considered as conclusive of their etiological relationship to the disease. Koch's paper resulted in the publication by Kartulis¹³ in 1886, of his investigation of Egyptian dysentery, in which he states that in all of 150 cases of dysentery he was able to demonstrate amebae in the stools; he concludes that the amebae are the cause of the dysentery and in later publications¹⁴ gave the results obtained in the study of 500 cases of the

disease. The work of Kartulis may be said to have finally established the etiological relationship of amebae to dysentery and his results were soon confirmed by numerous investigators in various parts of the world.

Hlava¹⁵ found amebae in 60 cases of dysentery occurring in Prague, and produced dysentery in both cats and dogs by the rectal injection of feces containing amebae; Osler¹⁶ in 1890, was the first to find the parasite in America, observing amebae in the feces, and in the pus from a liver abscess, in a man suffering with dysentery. Other American observers, as Musser,¹⁷ Stengel,¹⁸ and Dock¹⁹ confirmed the presence of these organisms in dysenteric stools. In 1891 appeared Councilman and Lafleur's²⁰ classical work upon amebic dysentery, in which the authors conclude that the disease is a clinical entity, and is characterized by a definite pathology, the lesions of which are due to amebae. They proposed the name "*Amoeba dysenteriae*" for the ameba associated with the lesions they described, and stated that other, and perhaps non-pathogenic amebae might infest the intestine of man. Confirmatory studies appeared from 1891 to 1893 by Cahen,²¹ Lutz,²² Kovacs,²³ and Quincke and Roos,²⁴ and in 1894, Kruse and Pasquale²⁵ published the results of their investigations of dysentery in Egypt, in which they also conclude that amebic dysentery is due to a specific ameba, and that other amebae occur in man which are harmless. The later investigations of Harris²⁶ and the writer,²⁷ in the United States, of Strong and Musgrave²⁸ and Musgrave and Clegg,²⁹ in the Philippines, have all been confirmatory of the etiological relationship of amebae to a characteristic form of dysentery, especially prevalent in tropical regions, and frequently accompanied by abscess of the liver.

CLASSIFICATION AND NOMENCLATURE.

Almost from the time of the first description of the amebae in man the classification and nomenclature of these organisms has occasioned much confusion and difficulty. While all were agreed that they belonged to the Protozoa, almost every writer differed in his classification or in his conception of the biological history of the amebae associated with dysentery, and it was not until Schaudinn's observations were published that a clear and definite classification of these parasites was possible.

The absence of amebae in numerous well-marked cases of dysentery, and their presence in health and in diseases other than dysentery, had gradually led to the grouping of students of the subject into three schools, i. e., those believing that amebae are always harmless commensals, more numerous, perhaps, in the feces of dysenteric patients because of a more favorable environment; those believing that all amebae may be pathogenic if suitable conditions be present; and those believing that harmless and pathogenic species are present in the intestine of man.

Prior to Schaudinn's work several observers had endeavored to

establish a classification based upon morphological differences, but without success so far as the general acceptance of any one classification was concerned. The following is a brief résumé of the attempts made in this direction:

In 1893, Quincke and Roos,²⁴ as the result of their studies, divided the amebae infecting man into three species, as follows:

1. *Amoeba intestini vulgaris*, 40 microns in diameter, with large granules, which is pathogenic for neither man nor cats.

2. *Amoeba coli mitis*, similar in size and appearance to the preceding, but which is pathogenic for man alone.

3. *Amoeba coli* (Loesch) about 25 microns in diameter, with a finely granular endoplasm, which produces dysentery in both man and cats.

This classification, which was based largely upon the results of animal experiments, is not conclusive, as they evidently, from their descriptions of the organisms, were dealing with mixed infections with both the harmless and pathogenic ameba.

Celli and Fiocca³⁰ described no less than six species of amebae infecting the intestine of man. Their classification is as follows: (1) *Amoeba spinosa*; (2) *Amoeba vermicularis*; (3) *Amoeba diaphana*; (4) *Amoeba reticularis*; (5) *Amoeba lobosa*, variety *guttata*; (6) *Amoeba lobosa*, variety *oblonga*. The names sufficiently describe their differential characteristics, and they all differed in size, one species, *A. diaphana*, measuring only 0.5 to 2 microns in diameter. To one who has studied these organisms the description of an ameba measuring but 0.5 micron in diameter may well be viewed with suspicion, when we consider that such an organism would be but little larger than the *Micrococcus melitensis*, which measures 0.4 micron in diameter. The largest ameba described by Celli and Fiocca, *A. spinosa*, did not exceed 10 microns in diameter, which is positive proof that these authors were not dealing with true dysentery amebae and it is more than probable that many of the amebae described by them were really stages in the development of the intestinal flagellates.

In 1894, Kruse and Pasquale³¹ distinguished four varieties of amebae based entirely upon morphological characteristics: (1) A form presenting a very refractive protoplasm, found in normal feces; (2) A form showing irregular and small granules; (3) A form in which the endoplasm consisted largely of vacuoles; (4) A form in which the protoplasm was filled with foreign bodies. The two latter forms were found only in dysenteric feces.

It will at once be seen that the differences in these forms are so slight as to be of no scientific value as a basis of classification, and the fact that these authors describe the form found in normal feces as being very refractive is evidence that they confused the pathogenic and harmless species, although they recognized a pathogenic ameba, *A. dysenteriae*, thus following Councilman and Lafleur²⁰ who, in 1891, objected to *Amoeba coli* as a name for the ameba causing dysentery and suggested the name "*Amoeba dysenteriae*." The latter authors, while not attempting to differentiate species, expressed it as their opinion that under certain conditions a number of species of amebae may inhabit the intestine.

Casagrandi and Barbagallo were the first investigators to describe accurately the species of ameba occurring in the feces of healthy individuals, and in those of patients suffering from other diseases than dysentery, although Grassi⁸ first called attention to

such amebae. They established for this ameba the genus *Entamoeba* which was afterward accepted by Schaudinn. They describe very minutely the morphology of this species and also the method of reproduction, which they state consists normally in simple division and in the formation of cysts in which eight daughter amebae develop. Their investigations directed the attention of other workers to the species of amebae occurring in man, but they were not successful in differentiating a pathogenic and harmless species. Strong and Musgrave³³ recognized two species of amebae as occurring in their cases in Manila and state that with the harmless ameba, which they call *Amoeba coli*, found in normal individuals, they were never able to produce dysentery in cats, while with the pathogenic ameba, *Amoeba dysenteriae*, "we have had no difficulty in producing dysentery and ulceration of the large bowel in cats by injection of the stools or contents of liver abscesses containing motile *Amoebae dysenteriae*."

Strong,³⁴ in a later publication, divides the amebae in man into two species, *Amoeba dysenteriae* and *Amoeba coli*, the first causing dysentery; the second, non-pathogenic. He describes in detail the morphology of these organisms and gives the results of animal experiments.

According to Schaudinn⁴ the first investigator to clearly identify and describe the two species of amebae infecting man was Jürgens³⁵ and much of Schaudinn's work is confirmatory of that of the latter observer. To Schaudinn, however, undoubtedly belongs the credit of the establishment, on scientific grounds, of the two distinct species of amebae which infect the intestine of man.

In describing in detail the morphology of the amebae considered in this paper I shall speak more fully of Schaudinn's results, which lead him to classify the amebae in man into two species under the genus *Entamoeba* established by Casagrandi and Barbagallo. To the amebae occurring in the feces of normal individuals, or in those suffering from other diseases than dysentery, he gave the name *Entamoeba coli*, while to those occurring in dysenteric feces, and causing that disease, he gave the name *Entamoeba histolytica*. Regarding the nomenclature of the amebae in the human intestine Stiles³ has written an exhaustive article in which he clearly states the nomenclatural situation, and concludes that for those who believe that there is but one species of ameba infecting man the correct name to use is *Entamoeba coli*, not *Amoeba coli*, for, as shown by Casagrandi and Barbagallo,³² the amebae of the human intestine differ from the freshwater amebae to which the generic terms "Chaos," later emended by Ehrenberg to "Amoeba" were originally given; while for those who believe in a harmless and pathogenic species, the correct generic terms are *Entamoeba coli* and *Entamoeba histolytica* respectively. In a previous contribution³⁶ I suggested that as the name *Amoeba dysenteriae* had been given by Councilman and Lafleur, the name *histo-*

lytica should give way to *dysenteriae*, but as Stiles has conclusively shown that *dysenteriae* is not a *new* name but merely a *synonym* of *Amoeba coli*, it follows that it cannot be used to designate the pathogenic ameba, and therefore we must accept Schaudinn's name *Entamoeba histolytica* for the parasite causing amebic dysentery.

At the present time Schaudinn's classification has been accepted by most investigators and writers, and by all zoölogists whose writings upon the subject I have seen. Musgrave and Clegg,²⁹ almost alone of those who have had an extensive experience with amebic dysentery, still refuse to accept Schaudinn's classification, although they state, "we do not at all question the multiplicity of both genera and species of amebae, both within and without the intestine of men." They also adhere to the name *Amoeba coli* although, as Stiles has shown, the proper generic term is *Entamoeba*.

The exact zoölogical position of the intestinal amebae of man may be indicated as follows.

THE PROTOZOA.

Class, *Sarcodina*.

Sub-class, *Rhizopoda*.

Order, *Amoebida*.

Family, *Amoebidae*.

Genus, *Entamoeba* (Casagrandi and Barbagallo, 1897).

Species,

Entamoeba coli (Schaudinn, 1902).

Entamoeba histolytica (Schaudinn, 1903).

OCCURRENCE OF AMEBAE IN THE HEALTHY INTESTINE.

Before the publication of Schaudinn's paper a few observers had noted the occurrence of amebae in the intestinal discharges of healthy individuals and this fact gave rise to the opinion that amebae were harmless commensals and only of accidental occurrence in patients suffering from dysentery. However, as evidence of the pathogenic action of amebae accumulated it was found that such an opinion was untenable. In order to explain pathogenesis the theory regarding the effect of environment upon these organisms arose, together with the belief that more than one species of amebae occurred in the human intestine.

Comparatively little work has been done regarding the frequency of the occurrence of amebae in the feces of healthy individuals, and it is remarkable that even Schaudinn's paper seems to have stimulated but little research in this direction. The examination of the feces in health results almost invariably negatively as regards the presence of amebae, unless some drug be given which will produce a slight diarrhea. This undoubtedly accounts for the negative results obtained by some careful observers, for I have examined a large number of healthy men in which no amebae were found unless a cathartic was first administered. Such experiments show that in order to demonstrate *E. coli* in the feces in health it is necessary that a condition of diarrhea be induced by the use, preferably, of a saline cathartic. Negative results obtained by observers who have not followed this procedure are of no value.

Historical summary.—Grassi,⁸ in 1888, was probably the first investigator to demonstrate the occurrence of amebae in the feces of healthy individuals, and he gave a very full and accurate description of the organism now known as *Entamoeba coli*, although Cunningham,³⁷ in 1881, stated that he found amebae in the stools of healthy men, but his descriptions indicate that he was probably observing developmental stages of flagellates. Schuberg³⁸ demonstrated amebae in the feces of ten out of twenty healthy individuals examined, or 50 per cent, while Gasser³⁹ examined the feces of twenty healthy persons and found amebae in 20 per cent of them. Strong and Musgrave³³ found them in only 4 per cent of healthy persons examined, yet felt justified in believing that these amebae were harmless because of the negative results of animal experiments. Dock⁴⁰ examined the stools of 200 healthy individuals and was only able to find amebae in two; he concludes his contribution as follows: "Even if a certain parasite occurs in every case in one locality, it would not follow that the same parasite would also be found as wide-spread elsewhere." Kartulis¹³ examined the stools of several hundred healthy individuals and was able to demonstrate amebae in only three cases, but he neglected to produce a diarrheal condition before the examinations, which undoubtedly accounts for the small positive result. Schaudinn¹ in 1903, published the results obtained by him in the examination of the feces of healthy individuals; he found, at his home in West Prussia, that 50 per cent of the healthy individuals examined among the farming population showed the presence of *E. coli* in their feces, while in Berlin he found that only 20 per cent showed this parasite. Along the shores of the Adriatic he found that in 385 examinations in as many individuals in perfect health, no less than 256, or 66 + per cent, showed the presence of *E. coli*. His observations show that locality and occupation had something to do with the number of individuals infected.

Personal observations.—In 1905, I published² the results obtained in the examination of the feces of American soldiers stationed at San Francisco, Cal., which were confirmatory of those obtained by Schau-

dinn in Europe. My experiments were conducted largely upon members of the Hospital Corps of the U. S. Army, recruited from almost every portion of the United States, who were on duty at the General Hospital at that time, and were under constant observation. I made over 200 examinations in such men, and found that, after the administration of magnesium sulphate in ounce doses, the feces of 65 per cent showed the presence of *E. coli*, but in many cases repeated examinations were necessary in order to demonstrate the parasite. Thinking that geographic distribution might have something to do with the proportion of infected individuals, I inquired into the locality from which the individuals came, but was unable to prove that the proportion of infected cases varied to any marked extent with the locality. This negative result cannot be considered as conclusive, and the question can only be settled definitely when large numbers of persons can be examined, under similar conditions, in different localities. All of the men I examined were in robust health, gave no history of diarrhea or dysentery, and certainly presented no symptoms of either condition. Many of the men showed immense numbers of *E. coli* in the feces, while in others they were very few in number.

An examination of my own feces, and of that of other medical men on duty at the hospital, resulted in the demonstration of this ameba and at the date of this writing, 1908, none of us have developed dysentery. My results, as showing the proportion of American soldiers harboring this parasite, were confirmed by Vedder,⁴¹ who, in 1906, published the results obtained by him in the Philippine Islands in the examination of American and native soldiers. After giving them preliminary doses of magnesium sulphate he examined the feces of 50 healthy American soldiers, and 50 Filipino scouts; of the American soldiers, 50 per cent showed *E. coli* in their feces, while of the Filipino scouts, 75 per cent were infected with this parasite. Regarding the subsequent history of these men Vedder says: "all the men have been under observation for a period of nine months and none of them has developed dysentery." Ashburn and myself, while serving in Manila upon the "Army Board for the Study of Tropical Diseases," examined the feces of 107 healthy American soldiers. The result of our work, published in 1907,⁴² was briefly as follows:

In all we examined 107 healthy men, all members of the Hospital Corps of the Army, and all on active duty at the U. S. Army Division Hospital, Manila, P. I. Of the 107 men, 76 or 71 + per cent were found to be infected with the non-pathogenic *Entamoeba coli*, while two showed the pathogenic *Entamoeba histolytica* in their stools. None of these men, with the exception of the two showing the pathogenic ameba, had diarrhea or dysentery at the time of examination, and all denied ever having suffered from dysenteric symptoms since residing in the Philippines. Of seventy-two men showing *Entamoeba coli* in their feces, one had resided in the Philippine Islands for eight years; four, seven years; one, six and a half years; three, six years; four, five and a half years; one, five and one quarter years; two, five years; four, four years; three, three years; two, two and a half years; ten, two years; one, one year and ten months; two, one year and nine months, nine, one and a half years; thirteen, one year; and the remainder, or seventeen, less than one year.

The two men showing *Entamoeba histolytica* in their stools were apparently in good health but inquiry elicited the information that both were suffering from dysenteric symptoms at the time of examination, and both were later returned to the United States suffering from chronic amebic dysentery. At the time that we examined the feces of these men we knew nothing of the occurrence of the dysenteric symptoms, and our diagnosis was based entirely upon the morphology of the amebae observed in their feces. It will thus be seen, that contrary to the opinion of certain investigators, it is possible to differentiate *Entamoeba coli* from the *Entamoeba histolytica* as they occur in the feces, and therefore, that such differentiation becomes of very great importance in the diagnosis of diarrheal conditions of the intestine.

In order to determine how long infection with *Entamoeba coli* might exist we made the following examinations:

A. Upon Nov. 20, 1906, thirteen men were re-examined who had been first examined upon March 17, 1906, eight months having elapsed since the first examination. Of these thirteen men, eleven showed *Entamoeba coli* in their feces March 17th, and nine, or 81.8 per cent still showed them upon Nov. 20th. During this time not one of these men had suffered from diarrhea and all had been on duty continuously at the hospital.

B. Upon Nov. 20, 1906, seven men were re-examined who were first examined upon May 2, 1906, six months and twenty-two days having elapsed since the first examination. Of these seven men, five were positive for *Entamoeba coli* upon May 2nd, and five were still positive upon Nov. 20th, and none of these men had suffered from diarrhea or dysentery during this time, and were continuously under observation.

C. Upon Nov. 20, 1906, eight men were re-examined who were first examined upon July 10, 1906, four months and thirteen days having elapsed since the first examination. Of these eight men, five were positive for *Entamoeba coli* upon July 10th, and two were still positive upon Nov. 20th. Neither of these men had developed symptoms of diarrhea or dysentery during this time.

As the result of our work in the examination of the feces of healthy men in the Philippine Islands, Ashburn and I concluded that a very large proportion of white men in these islands harbor *E. coli*, which condition, so far as we were able to observe, does not result in symptoms of diarrhea or dysentery, for in many of the cases the amebae

disappeared without producing symptoms, while in the greater proportion of the cases they persisted for as long as nine months, as shown by us, during which time no disease of the intestine resulted. We also concluded that *E. coli* and *E. histolytica* differ greatly as regards morphology, and that it is possible to distinguish these two species of amebae by their morphological characteristics as observed in fresh specimens of feces, and that "latent infection" cannot explain the lack of pathogenicity in persons harboring *E. coli*.

Summing up my observations of 1905, in San Francisco, and those undertaken with Ashburn in Manila, we have the record of 307 examinations of feces from as many healthy men, all American soldiers, with the result that 176 or 58+ per cent showed the presence of the harmless *E. coli* in their feces. These amebae invariably answered to the description of *E. coli* as given by Schaudinn and myself, and the great majority of the men in whom they were found were under observation for from six months to a year or more, during which time no symptoms of dysentery developed. In 1904 an examination of my own feces after the administration of magnesium sulphate resulted, as has been noted, in the demonstration of *E. coli* in them, and repeated examinations since then, a period of nearly four years, has always resulted in finding numerous typical *E. coli*, and not the slightest symptoms of dysentery have developed.

It would appear to me that these observations prove that amebae may be present and multiply in the intestine of man without producing symptoms, for periods longer than the known incubation period of amebic dysentery, for to hold that the incubation period of this disease may extend over a period of years is impossible, especially when we consider the chances of infection in the meantime with the pathogenic species.

Musgrave and Clegg,⁴³ in attempting to disprove the existence of a harmless ameba in man, instance cases in which amebae were found in the feces during apparent health but in which dysentery followed at periods varying from two to six months, but such cases are of no scientific value as proof of their contention for during the entire period which they regard as the period of incubation the patients were continually exposed to infection with *E. histolytica* in an intensely infected region, i. e., the Philippine Islands. In a later contribution⁴⁴

Musgrave and Clegg attempt to refute the observations of Schaudinn and myself as regards the occurrence of amebae in healthy individuals by the results obtained in the examination of inmates of the hospitals and prisons of Manila. They admit that the examinations "undertaken to determine the prevalence of amebae in the intestine have not dealt with the question of whether or not this situation is a normal one." Their results were briefly as follows:

Of 587 cases in Bilibid Prison, 26 + per cent showed amebae, but these examinations are not said to have been upon healthy men. In another series of 100 cases, 39 per cent showed amebae. Of 318 American patients in the Civil Hospital, most of them suffering from intestinal disturbances, amebae were found in 14 + per cent; of 143 stools examined at St. Paul's Hospital, 44 per cent were positive, and of 30 examinations made at the Biological Laboratory "from stools sent in by private patients suffering from diarrhea or dysentery, 7 were positive or 23 + per cent."

From the data given it will be seen that these observers have compared with the results of Schaudinn and myself, in the examination of the stools of healthy individuals, their results in the examination of the stools of patients, the majority of whom they admit were suffering from diarrhea or dysentery. In a critical paper Vedder⁵⁸ has analyzed these results and I can do no better than quote him in showing that no scientific deductions can be safely drawn from such a comparison. He says:

The examinations of amebae detailed by the authors cannot possibly afford any conclusions as to the presence or absence of amebae in healthy individuals. They examined 587 cases from Bilibid Prison, but they say nothing as to how many cases were normal or how many suffered from disease, or were under treatment for diarrhea or dysentery. If these individuals were normal, the observations of Craig and Vedder are thereby confirmed. If they were the subjects of dysentery, by what possible logic can it be inferred that amebae could not be found in healthy individuals.

I believe that it is evident, from the observation given, that in a certain proportion of healthy individuals, in certain localities, an examination of the feces will demonstrate that the intestine harbors a harmless ameba, and that this organism answers to the description given by Schaudinn for *E. coli*. Schaudinn found this ameba present in 50 per cent of healthy individuals examined in West Prussia, in 20 per cent examined in Berlin, and in 66 per cent of those examined along the shores of the Adriatic; Craig in 65 per cent of healthy American soldiers at San Francisco; Ashburn and Craig in 71 per cent of healthy American soldiers in Manila. P. I.; and Vedder in 50 per cent of

American soldiers, and in 72 per cent of Filipino scouts in Mindanao, P. I. While it is obvious that the percentage of infections varies in different localities, probably depending on local conditions and the presence of a source of infection, the fact remains that infection with *E. coli* is very common and that it is not difficult to demonstrate this organism in the feces of healthy individuals after the administration of a saline cathartic.

OCCURRENCE OF AMEBAE IN DISEASES OTHER THAN DYSENTERY.

As regards the occurrence of *E. coli* in the feces of patients suffering from other diseases than dysentery, several observers agree in having demonstrated amebae in diseases such as chronic enteritis and typhoid fever, and also in conditions in which the lesions were not confined to the intestine.

Cunningham³⁷ found amebae in the intestinal contents of cholera cases; Celli and Fiocca³⁹ in many infants suffering from intestinal inflammation; Berndt⁴⁵ in cases of typhoid fever; Normand⁹ in chronic colitis; Massiutin,⁴⁶ working in Kiev, in the feces of five cases suffering from intestinal catarrh, typhoid fever, and diarrhea; Grassi⁸ in diarrhea; Peroncito¹⁰ in chronic diarrhea; Babes in cases of hepatitis, Casagrandi and Barbagallo³² in various diseases, as well as Kruse and Pasquale,³¹ Quincke and Roos²⁴ Kartulis,¹³ Shuberg,³⁸ Gros,⁴⁷ Sternberg,⁴⁸ and Ijima.⁴⁹

In my investigations I have not confined myself to examinations of the feces of patients suffering from diarrhea, but have examined the excreta in all cases, whatever the diagnosis, and I have found that of 250 such cases, 49 per cent showed *E. coli*. It is significant that a smaller percentage of cases of disease show this ameba than of healthy individuals, which would appear to indicate that *E. coli* finds a more congenial environment for its development in the intestine of normal individuals. Table I, giving the results obtained in the examination of 30 cases, well illustrates the variations in disease from which the patients suffered, and also the different localities from which the patients came. In all the cases where diarrhea was not present a saline cathartic was given before the examination. From a consideration of this table it is evident that none of these cases were suffering from diarrhea or dysentery, and that they were under observation in the hospital for periods varying from 10 days to 5 months, during which time no dysenteric symptoms developed; it cannot then be said that these patients were cases of latent infection or that the symptoms indicated an inflamed intestinal tract; indeed I have found that

E. coli occurs fully as frequently in the feces of patients suffering from other diseases not diarrheal in character as it does in those cases presenting diarrhea provided a saline cathartic be given before the examination. The occurrence of such a large number of instances of the presence of *E. coli* in patients suffering from other diseases is of great practical importance, especially in those localities where amebic dysentery is endemic or in patients coming from such regions. An examination of the feces in this class of cases, if diarrhea were present, would

TABLE I.
EXAMINATION OF PATIENTS FOR *E. Coli*.

No.	Diagnosis	Residence	Dysentery	Diarrhea	In Hospital	Bowel Movements	<i>E. Coli</i>
1.....	Anemia, secondary	England	No	No	70 days	2 daily	Present
2.....	Abscess of leg	Illinois	"	"	5 months	2 "	"
3.....	Abscess of axilla	"	"	"	20 days	2 "	"
4.....	Diabetes insipidus	Georgia	"	"	40 days	1 "	"
5.....	Fracture	Alabama	"	"	40 days	2 "	"
6.....	Gastritis, chronic	"	"	"	2 months	2 "	"
7.....	"	"	"	"	2 weeks	1 "	"
8.....	Gastritis, acute	California	"	"	1 week	1 "	"
9.....	Gonorrhea, acute	Tennessee	"	"	1 month	1 "	"
10.....	"	California	"	"	1 month	1 "	"
11.....	"	Jamaica	"	"	10 days	2 "	"
12.....	"	Pennsylvania	"	"	20 days	1 "	"
13.....	" Chronic	Kentucky	"	"	35 days	2 "	"
14.....	"	"	"	"	1 month	1 "	"
15.....	"	Pennsylvania	"	"	1 month	1 "	"
16.....	Hemiplegia	California	"	"	10 days	1 "	"
17.....	Pharyngitis, acute	"	"	"	10 days	1 "	"
18.....	Malarial Fever	Connecticut	"	"	2 weeks	1 "	"
19.....	"	California	"	"	2 weeks	2 "	"
20.....	"	Virginia	"	"	2 weeks	1 "	"
21.....	"	"	"	"	2 weeks	1 "	"
22.....	Otitis media	Texas	"	"	3 months	1 "	"
23.....	Measles	"	"	"	40 days	1 "	"
24.....	"	"	"	"	4 months	2 "	"
25.....	Pemphigus	Arkansas	"	"	5 months	1 "	"
26.....	Poliomyelitis	Mississippi	"	"	4 months	2 "	"
27.....	Mitral regurgitation	Pennsylvania	"	"	10 days	2 "	"
28.....	Stricture	California	"	"	2 weeks	1 "	"
29.....	Sciatica, chronic	Missouri	"	"	3 months	1 "	"
30.....	Varicocele	Ohio	"	"	3 months	2 "	"

result in the finding of amebae which the inexperienced observer would probably regard as *E. histolytica*, and a diagnosis of amebic dysentery would be made. Since I have been investigating this subject I have seen numerous cases diagnosed as amebic dysentery, because of the finding of *E. coli* in the stools after a dose of salts, which were in reality some other disease, and the clinical history of these cases was conclusive evidence that the diagnosis of amebic dysentery was erroneous. I am convinced that a considerable proportion of the cases returned from the Philippine Islands, during 1900 and 1901, and perhaps later, diagnosed as amebic dysentery were, in reality,

cases of enteritis showing *E. coli* in the feces, and the clinical histories bear out this belief. It is certainly necessary, then, that we recognize the fact that non-pathogenic amebae do occur both in health and disease in the intestine of man, and that we should be able to differentiate between *E. coli* and *E. histolytica*; for this reason I have endeavored in the following descriptions of these organisms to indicate the principal differential features but it will be found that considerable experience will be required in the examination of the feces both in health and in amebic dysentery before such a differentiation will become easy, and it should be remembered that no merely superficial study of these organisms will result in success in this direction.

METHODS OF EXAMINATION.

The amebae infesting the intestine of man may be studied in both fresh and stained specimens of the feces; for the study of the vital activities of these organisms the fresh specimens are best, while for the study of the exact structure and the methods of reproduction the stained specimens are necessary. With either method a differential diagnosis of *E. coli* and *E. histolytica* can be made.

Fresh preparations.—A very small portion of a freshly passed stool should be placed upon a microscopic slide and covered with a cover-glass, gentle pressure being used to spread the specimen. In making the preparation take preferably a drop of the liquid portion of the stool, and if this is impossible, select a fragment of mucus or of any blood-stained material. The preparations should be examined with a one-sixth-inch lens and a one- or two-inch eyepiece. For the finer details regarding the structure of the protoplasm and the nucleus it is necessary to use the one-twelfth-inch objective. The use of a very weak solution of neutral red is often of great service in those cases in which the amebae are few in number; this solution is very quickly absorbed by the amebae, coloring them a fine pink, and if it is not too strong it will not interfere with the movements of the organisms, so that it is very easy to distinguish them, as the only other organisms that take up the stain are the flagellates and these are easy to differentiate from either *E. coli* or *E. histolytica*. Even when the amebae are motionless it is not difficult to distinguish them by the use of this reagent. If the feces have to be kept for any time the vessel containing

them should be placed in another containing water at a temperature of at least 100° F., which temperature should be maintained. However, it is often possible to render the amebae motile, even in feces kept at room temperature for hours, by gently warming the slide before placing it upon the microscope. Many observers claim that a diagnosis of the presence of amebae in feces should never be made unless the organisms be motile. While this is valuable advice for the novice in this line of work, it is certainly true that by one who has studied these organisms, they can be recognized easily when motionless, provided that the feces have not been kept so long as to have lead to degenerative changes in the amebae.

Staining methods.—So far as gross staining is concerned, both *E. coli* and *E. histolytica* may be stained by solutions of many of the anillin dyes, as methylene blue, Borrel blue, carbol-fuchsin, thionin, etc., but the only stain that I have found satisfactory in demonstrating the exact structure of these organisms and their method of reproduction, is Wright's modification of the Romanowsky stain, made as directed by Oliver, of San Francisco. With this stain the chromatin of the nucleus is differentiated in some specimens very beautifully, as well as the granular structure of the protoplasm. Both species of amebae stain with some difficulty with this stain, and sometimes scores of specimens will have to be examined before a perfectly stained one is found; for this reason the use of stained preparations in the differentiation of these organisms is not to be recommended for general work, as it requires time and detail of technic not possessed by the inexperienced observer. Löffler's solution of methylene blue, carbol-fuchsin, Borrel blue, and thionin are good general stains but they are really of little diagnostic value. The thionin method, recommended by Mallory and Wright,⁵⁰ and Heidenhain's iron hematoxylin and the Borrel stain, as recommended by Woolley and Musgrave⁵¹ give the best results in the staining of amebae in the tissues.

Method of preparing spreads for staining.—Several cover-glasses are thoroughly cleaned and placed in a row upon the table. With a sterilized platinum loop a small portion of the material to be examined is picked out of the feces and placed upon the cover-glass. A clean cover-glass is placed over this, and the two slid carefully apart, using gentle pressure, and allowed to dry. By proceeding in this way a dozen or more even, thin smears can be made in a very few minutes. If any other method than Wright's is used the preparations should be placed smeared side up in equal parts of

alcohol and ether and allowed to remain for from 15 to 20 minutes in order to fix the specimen. If Wright's method be used it is not necessary to thus fix the specimen, if the feces are warm many of the amebae will be fixed while undergoing ameboid motion. After fixing, the smears are ready to stain.

As I shall only describe the staining reactions of *E. coli* and *E. histolytica* with Oliver's modification of Wright's method it is unnecessary for me to give in detail the various steps in the application of other methods. The stain I have used is prepared as follows:

Add 0.5 gm. of sodium bicarbonate to 100 c.c. of distilled water, dissolve thoroughly, and add 1 gm. of methylene blue (Grubler's); heat the mixture for one hour in an Arnold sterilizer after the steam is up. After heating allow the solution to cool. Make a 1 to 1,000 solution of yellow aqueous eosin (Grubler's) and add this, while stirring, to the cooled methylene blue solution in the proportion of 500 c.c. of the eosin solution to 75 c.c. of the blue solution. This should be done in a large white porcelain dish and the eosin solution added until a marked metallic scum appears upon the surface, even though more of the eosin solution has to be added than is recommended. After letting the mixture stand for a period of from 10 to 20 minutes, filter it through one small filter paper, save the precipitate on the paper, dry in a hot air oven, and use to make the staining solution. This powder will keep well and can be used as stock material.

To prepare and use the staining solution, proceed as follows:

Take 0.3 gm. of the powder and add it to 100 c.c. of pure methylic alcohol (Mercks reagent alcohol is best, although any methylic alcohol absolutely free from acetone will do) filter, and to 80 c.c. of the filtrate add 20 c.c. of methylic alcohol, or enough to bring the amount up to 100 c.c. The staining solution is now ready for use and will keep unimpaired for several weeks.

To stain,

add a few drops of the staining solution to the preparation of feces without preliminary fixation, and let stand for five minutes; then add enough distilled water to cause a slight metallic scum to form on the surface of the preparation; let stain for 10 minutes and wash thoroughly in running distilled water. If it is desired to preserve the specimens do not use a cover-glass for making the smear but a slide, and do not mount in Canada balsam, with a cover-glass, but preserve the slide as it is.

For the diagnosis of amebae in the stools, staining methods should never be resorted to, as the examination of the fresh specimen is altogether superior and safer. Stained preparations are only of use in the study of certain morphological characters and the methods of reproduction.

DESCRIPTION OF ENTAMOEBA COLI.

General description.—*E. coli* consists of a mass of protoplasm, containing a well-defined nucleus, and in the majority of instances, one or more nucleoli. A vacuole, non-contractile in character, is

present in some instances, but multiple vacuoles are seldom, if ever, observed. The protoplasm of the organism is divided into two portions, an outer, or ectoplasm, and an inner, or endoplasm, but this differentiation cannot be made out, in most instances, even when the organism is in motion. While possessing the power of motion *E. coli* is very sluggish as compared to *E. histolytica*, and, as a rule, may be said to be almost immotile. Reproduction occurs, under favorable conditions, by simple division, while if unfavorable conditions arise, reproduction occurs by encystation, with the formation within the cyst of eight daughter-cells, which are liberated and become young amebae. The following details in the morphology of this organism have been determined from the study of thousand of specimens, but it should be remembered that not every ameba of this species presents all the details described, but that the description is a composite picture, true for the vast majority of organisms examined.

Size.—*E. coli* is never as large as the largest specimens of *E. histolytica*, but while this is so, it is not at all uncommon to find *E. coli* that approximate the size of the majority of *Entamoeba histolytica* that are usually observed. Almost every writer upon the amebae of man have differed in the measurements given for these organisms as is well shown by the following summary:

Kartulis¹³ gives the size of the ameba as from 12 to 30 microns in diameter; Quincke and Roos,²⁴ from 15 to 25 microns; Celli and Fiocca³⁰ 0.5 to 10 microns; Councilman and Lafleur,²⁰ 6 to 35 microns; Kruse and Pasquale,³¹ from 10 to 50 microns; Osler,¹⁶ from 10 to 20 microns; Sodre,⁵² from 15 to 35 microns; Marshall⁵³ from 10 to 40 microns; Fletcher,⁵⁴ from 12 to 26 microns; Zorn,⁵⁵ 14 to 22 microns; Strong and Musgrave,²⁸ 10 to 50 microns, and Musgrave and Clegg,²⁹ 3 to 40 microns.

The measurements given do not indicate inaccuracy upon the part of the observer but rather that amebae of various size occur in almost every specimen of feces examined. Schaudinn¹ gave the size of *E. coli* as varying between 8 and 50 microns, but it is very seldom that one of these organisms is observed exceeding 25 to 30 microns in diameter, and as a rule this organism measures from 10 to 20 microns in diameter. This average is smaller than for *E. histolytica*, the majority of which measure from 25 to 35 microns, and is, therefore, of some diagnostic importance in distinguishing between them, but the mere difference in size cannot be depended upon in such differentiation, for as I have stated, *E. coli* approximates *E. histolytica* in size.

Therefore, as I have before contended, the separation into species of the amebae of the human intestine, based alone upon size, is altogether unscientific and erroneous. When encysted, *E. coli* measures on the average from 10 to 15 microns, and is always smaller than before encystment.

Those authors who claim that the pathogenic amebae is always larger than the non-pathogenic are surely in error for I have repeatedly seen small *E. histolytica* predominating in feces from well-marked cases of amebic dysentery, and an examination of the feces of this disease will show that amebae of large size are by no means always present, and that generally both large and small amebae are present. It follows, then, that the size of an ameba is of little value in specific diagnosis, although the vast majority of *E. coli* average smaller than *E. histolytica*.

The variation in size of these organisms is, of course, explained by the fact that various stages of development occur simultaneously in the stools; the fact that the greater number approximate each other in size indicates that reproduction of the parasite occurs at definite periods of time and is followed by uniform growth. As yet we have no data which justifies a classification of amebae based upon the measurements of the organisms, and the most that we can say is: that *E. coli* is generally considerably smaller than *E. histolytica*.

Shape.—When not in motion *E. coli* is invariably perfectly spherical in shape. When in motion the shape is very variable, depending upon the contour of the extruded pseudopodia, which are always rounded, and never spinose, as are the pseudopodia of so many of the amebae cultivated from external sources and used, by some authors, in comparison with this parasite. *I have never seen an ameba in the feces with spinose pseudopodia, and yet, in cultures, this is the prevailing type, thus indicating that if these cultivated amebae are really E. coli or histolytica they undergo very marked changes in morphology during cultivation.*

Color.—*E. coli* is always of a peculiar dull grayish color and this is true of the organism whether the feces contains blood or whether the intestine is normal. The color of this organism is of value in distinguishing it from *E. histolytica*, especially in those cases of dysentery in which these two varieties may occur together. Musgrave and

Clegg⁵⁶ considers that color simply means environment, the amebae becoming greenish in color as the stool becomes bloody, due to absorption of blood serum containing hemoglobin, but I have yet to see an ameba answering to the description of *E. coli* of a greenish color, and experimentally I have demonstrated that the addition of blood to feces containing this organism does not result in the absorption of either dissolved hemoglobin or red blood corpuscles. It is extremely difficult to describe the exact color of this organism but to one who has studied both species the difference in the coloring of *E. coli* is very characteristic. Irrespective of the greenish coloration of *E. histolytica*, so often observed, there is a marked difference in the color of these two organisms.

Protoplasm.—As pointed out by Schaudinn¹ it is impossible to differentiate the ectoplasm and endoplasm of *E. coli* when the organism is motionless, and difficult even when it is in motion, although the protoplasm is thus divided. My observations confirm his as regards this, and also as to the ectoplasm being much less refractive to light than the endoplasm, when the two can be distinguished. When distinguishable the ectoplasm presents a perfectly homogeneous appearance, it being impossible to demonstrate any structure even with the highest power objective. I have not noticed that the ectoplasm was more distinct in the larger amebae of this species than in the smaller, so that I think that it may be safely said that the ectoplasm of this parasite is difficult to distinguish at all stages of its growth. In the encysted organisms the ectoplasm is replaced by a slightly wrinkled, refractive cyst wall, which is impervious to staining solutions and evidently of very dense structure. In some instances definite layers of very refractive material may be seen to compose this wall.

The endoplasm, which constitutes the greater portion of the protoplasm, presents a finely granular appearance and an examination with high power shows that it is composed of a delicate network and minute granules which appear to float in a fluid medium inclosed in the network of fibrils. Bacteria are generally observed in the endoplasm, as well as other bodies to be described later. As the majority of *E. coli* do not show any differentiation into ecto- and endoplasm, the whole protoplasm most commonly appears finely granular

as described for the endoplasm. The very young amebae of this species appear homogeneous throughout.

When in motion the pseudopodia, formed by the ectoplasm, are scarcely to be distinguished from the endoplasm, except that they are slightly less refractive to light than is the endoplasm, and a single pseudopodium often resembles a veil-like membrane extending from some portion of the periphery of the organism, and only visible upon careful focusing.

Nucleus.—Almost invariably a well-defined nucleus is demonstrable in *E. coli*, situated a little to one side of the center of the organism, and possessing a thick, easily distinguished, nuclear membrane. The nucleus is large, spherical in shape, and contains a hyalin, grayish substance, embedded in which is a large amount of chromatin in the form of refractive grains and strands, together with one or more small, spherical nucleoli. The nuclear membrane is very refractive when focused upon in some lights and contains several very minute, bright, round granules which are probably chromatin. Schaudinn gave the diameter of the nucleus as from 5 to 8 microns, with which I agree. Not infrequently the nucleus is observed to be distinctly oval in shape, and the parts described cannot always be easily distinguished. When the organism is moving the nucleus tends to retain its relative position and does not change its shape under pressure as does the nucleus of *E. histolytica*. The nucleolus is generally to be distinguished, consisting of a very refractive oval or spherical body or bodies, if more than one is present, situated within the nuclear membrane.

Vacuoles and contained bodies.—A very large proportion of *E. coli* present a finely granular protoplasm in which no vacuoles are present; in the thousands of examples of this species of ameba that I have studied I have not observed more than one vacuole in but a very few specimens, and generally even one could not be distinguished. The vacuoles, when present, are of small size, dim in contour, and never contractile. The absence of vacuoles in this species is in striking contrast to the numerous vacuoles observed in *E. histolytica*.

In stained specimens bacteria are generally to be observed in the endoplasm and infrequently crystals of various kinds obtained from the feces. The further study of this species of ameba has convinced me that it is only very infrequently that they will engulf red blood

corpuscles, and experimentally it is almost impossible to make these organisms engulf the erythrocytes when blood is added to feces containing them, although in the case of *E. histolytica* the engulfing of red blood corpuscles is of very frequent occurrence. In the very few instances in which I have observed red blood corpuscles in *E. coli*, there has never been more than one or two, and while in a previous communication⁵⁷ I stated it as my belief "that the greater frequency with which red blood corpuscles are seen in *E. histolytica* depends very largely upon the fact that in amebic dysentery the feces contain much blood, whereas in infections with *E. coli* there is very little, if any, blood in the feces," I am now inclined to believe that the ingestion of blood by *E. coli* is an abnormal process occurring but very seldom, even when the feces contain much blood. This fact alone, however, does not warrant us in differentiating species, but, taken with the variation in morphology and method of reproduction, it is of some assistance in this direction.

Motility.—*E. coli* may be described as a sluggishly motile organism, in which this property is often absent, and when present, is of slight duration and very limited in extent. Motion is rendered possible by the extrusion of pseudopodia formed of the ectoplasm. Two forms of motion are most common; the first consists in the extrusion of the ectoplasmic pseudopodia, into which flows the endoplasm, and the consequent production of a very sluggish motion; the second form consists in the extrusion of pseudopodia from different portions of the periphery of the organism simultaneously, causing a change in the shape of the organism but no progressive motion. When progressive motion is present it is generally so slow that the organism has to be observed for some time before it can be detected.

The pseudopodia are small and rounded in contour, and are less refractive to light than is the endoplasm; sometimes they are so minute as to be distinguished with difficulty, which is never true of *E. histolytica*. I have never observed the long, finger-like pseudopodia in this organism that are so frequently observed in the pathogenic species, and it is always difficult to distinguish the boundary between the ecto- and endoplasm even when motility is most marked. The motility of this species of ameba is most apparent in freshly voided

feces, and it is never observed in feces which have stood at room temperature for more than 15 or 20 minutes.

Staining reactions.—The methods of staining *E. coli* have already been mentioned, Wright's method being the only one that I have found of value in differentiating this species from *E. histolytica*. With this method *E. coli*, when stained, is seen to consist of three distinct portions: The ectoplasm, the endoplasm, and the nucleus.

The ectoplasm stains very dimly, the color being a light blue, and generally appears quite structureless; in a few instances, careful examination with a high-power lens (one-twelfth) will show that this portion of the organism consists of very minute granules, so small as to appear like dust, which take the stain very slightly.

The endoplasm stains very intensely a dark blue or violet, and is seen to be composed of deeply stained, well-defined granules, some of which are of considerable size. Scattered among these granules may be seen bacteria of various kinds, and sometimes a few small crystals. If a vacuole, or vacuoles, be present, they are observed within the endoplasm, and do not contain within them any of the staining solution.

The nucleus stains a bright red or crimson, because of the large amount of chromatin that it contains. When division is not occurring the nuclear membrane is very distinct, staining more intensely than any other portion of the nucleus. Within the nuclear membrane the chromatin may be seen as short strands or spherical grains, stained a brilliant red or violet, separated by minute, unstained spaces. The nucleolus stains a very dark violet, but is often indistinguishable in stained specimens. When the nucleus is undergoing division, the chromatin may be seen arranged in two separate masses; spread uniformly throughout the protoplasm; or arranged irregularly, according to the stage of division. The extrusion of the chromatin from the nucleus at certain stages or reproduction, described by Schaudinn, is often observed in stained specimens, small groups of granules of chromatin being seen in the endoplasm of the ameba. The encysted forms, so easily distinguished in the fresh feces, cannot be studied in stained preparations, for the reason that it is impossible to stain them.

Methods of reproduction.—Until Schaudinn's researches but little

was known regarding the method of reproduction of the amebae in the human intestine; a few observers, working with both species, have described roughly certain reproductive phenomena, but as they were unable to distinguish the two organisms, their descriptions are naturally of but little value to the student of this subject. Celli and Fiocca³⁰ working, in all probability, with the harmless species, describe the life-cycle as consisting of an ameboid stage, a resting stage, and an encysted stage; Cassigrandi and Barbagallo³² were the first to describe accurately the methods of reproduction in *E. coli*, consisting in simple division, and the formation, under certain conditions, of cysts, in which are developed eight young amebae. To Schaudinn,¹ however, we owe the clearest description of the methods of reproduction of this species of ameba. He found that in the liquid stools simple division was the common mode of reproduction, the nucleus first dividing, followed by division of the protoplasm, thus forming two amebae. When the stools became semi-formed or formed, he found that simple division ceased and reproduction within a cyst occurred. This process is complicated, and is best studied in a glass cell, kept at an even temperature (about 75° to 80° F.) and shielded from the light as much as possible. I have been able to follow almost every stage in the process, as described by Schaudinn, and the following description is based upon that given by Schaudinn and my own observations:

E. coli when about to encyst, becomes perfectly motionless, and soon, if carefully observed, is seen to develop apparently from its periphery (or ectoplasm) a very refractive hyaline membrane, at first having a single outline but gradually acquiring a double outline, and finally appearing mamilated or irregularly striated. During the development of this cyst wall, the organism has contracted, and when it is fully formed, it appears at least a third smaller than before it became encysted, thus leading us to believe that the cyst-wall is formed of materials secreted by the ameba. Within the cyst-wall is situated the protoplasm and nucleus, both of which undergo changes as development proceeds. The protoplasm, at first granular in appearance, becomes hyalin and homogeneous, the foreign bodies, such as bacteria, having been extruded during the formation of the cyst wall. After the protoplasm has become hyalin, the nucleus undergoes very

complicated changes which result in the formation of eight daughter-nuclei. The changes are briefly as follows:

The original nucleus divides into two portions, each portion being surrounded by a portion of the protoplasm, so that at this stage the cyst-wall contains within it two well-defined masses consisting of a nucleus and protoplasm. Each new nucleus now resolves itself into chromidia, a portion of which, with the remains of the original nucleus is extruded. Each mass of protoplasm now contains a small amount of chromidia, from which a new nucleus is formed; each new nucleus now divides into two, one-half of each being extruded; this process is repeated and finally ceases, two mature nuclei being the result. At this stage the two masses of protoplasm fuse, and the cyst-wall becomes much thicker. Reproduction now commences by the division of the two nuclei, two daughter-nuclei being formed, called the active and passive pronuclei; the active pronuclei fuse with the passive pronuclei, forming two synkarya, each of which divides into four, so that the cyst contains eight nuclei. Under favorable conditions the cyst-wall ruptures, and the eight small amebae, each consisting of a nucleus and a portion of protoplasm, are liberated.

It requires hours of study to follow the changes described, but it is not difficult, by studying the encysted forms in the feces at varying periods of time, to observe many of them. The mature cysts are often observed, consisting of a thick cyst-wall, a homogeneous protoplasm, in which the nuclei of the young amebae are embedded; these appear as small, spherical, very refractive bodies, in many instances very sharply outlined.

Reproduction by simple division I have frequently observed, the process being essentially as follows: The nucleus becomes elongated, the nuclear membrane appearing to become thinner and less refractive, while the minute granular contents of the nucleus (the chromatin) appear to concentrate at each pole as the elongation occurs. After the nucleus has become much elongated a constriction occurs near the center and eventually two new nuclei are formed by division, a considerable amount of chromatin being extruded into the protoplasm at the time of division. Coincident with the elongation and division of the nucleus, the protoplasm of the ameba becomes less granular, and a constriction appears, which, after the division of the nucleus,

deepens and finally becomes complete, two new amebae being thus formed. In the stained specimen the chromatin can easily be distinguished and the nuclear changes studied, but much patience is required in the search for amebae undergoing division, especially if every step of the process is to be studied.

Relation to disease.—While I am firmly of the opinion that the data already given regarding the presence of *E. coli* in the feces of healthy individuals for months and even years without producing symptoms of disease, is positive proof of the harmless nature of this organism, it may be of value to detail the experimental evidence we possess of this fact, evidence which it seems to me is incontrovertible.

Kartulis²³ experimented with the amebae obtained from the healthy intestine, by injecting them into the intestinal canal of cats, and was not able to produce any pathological lesions or symptoms. Kruse and Pasquale,²⁵ working with amebae obtained from the feces of healthy individuals, were not able to produce any of the pathologic lesions of dysentery in cats. Celli and Fiocca³⁰ use as one of their strongest arguments against the pathogenic action of amebae that they were not able to induce dysentery in animals with the amebae from healthy individuals or those from dysenteric patients. These authors were undoubtedly working with *E. coli*, as all of their experiments gave negative results. Kovacs²³ was unable to produce dysentery in cats with the amebae found in healthy individuals. Strong and Musgrave²⁸ were unable to produce dysentery in cats by the injection of amebae from healthy individuals, and their comment regarding their work is of interest. They say, "One of these cases which has been under our observation for several months has had these harmless amebae in his stools constantly during that time, yet he has no dysentery and no history of any, and he has no intestinal trouble. We have repeatedly injected large numbers of these non-dysenteric amebae (*amoeba coli*) while motile in the stools, into the rectum of cats, but with no effect. We have neither been able to produce dysentery with them nor any lesions of the large bowel. . . . On the other hand we have had no difficulty in producing dysentery and ulcerations of the large bowel in cats by injection of the stools or contents of liver abscesses containing motile amebae dysenteriae."

Jürgens³⁵ found that *E. coli* was not able to penetrate the normal mucous membrane of the intestine, and that it had no pathogenic action upon this membrane. He considered that this was due to the slight strength of the ectoplasmic pseudopodia, which, as has been said, is of very delicate structure, so delicate as to be almost invisible in many instances. Schaudinn¹ confirmed Jürgens' observation regarding the pseudopodia, and was also unable to produce dysentery or any lesion of the bowel in animals by feeding experiments or the rectal injection of material containing *E. coli*. He twice inoculated himself by swallowing the encysted forms, and numerous ameba were afterward found in the feces, but in neither instance did any symptoms of diarrhea or dysentery develop, and the amebae finally disappeared.

I have made a large number of experiments as to the pathogenic properties of *E. coli*, using young kittens as the animals experimented

upon, injecting feces containing this organism into the rectum, as well as feeding with milk containing the infested feces. I have injected into the rectum of kittens fecal material containing the encysted forms of *E. coli*, as well as the motile form, and have never been able to produce the least symptom of diarrhea or dysentery by such injections, although 50 per cent of the kittens given rectal injections of feces containing the pathogenic *E. histolytica* developed severe dysentery, of which most of them died. These injections have been repeated upon the same cat from five to ten times, and in no case has there been a diarrhea produced or any evidence of intestinal inflammation. I have repeatedly fed kittens with milk containing large amounts of fecal material with the cystic and vegetative forms of *E. coli* and in not a single instance were there produced any symptoms of diarrhea or dysentery, although these feedings were repeated at frequent intervals. In kittens fed with milk containing feces infected with *E. histolytica* over 65 per cent developed severe dysentery, as will be described later.

It would appear certain from the experiments of the various observers mentioned, and from my own, as well as the observations of the very frequent occurrence of this species of amebae in the stools in health and in diseases other than dysentery that have been detailed in previous sections of this report, that *E. coli* is a harmless parasite which is present in a large proportion of individuals in many parts of the world. The non-pathogenic character of *E. coli* is as fully proven as is the pathogenic character of *E. histolytica* and I believe that we are fully justified in accepting Schaudinn's classification as regards this common and harmless species.

DESCRIPTION OF ENTAMOEBA HISTOLYTICA.

General description.—*E. histolytica*, like *E. coli*, consists of a mass of protoplasm containing a nucleus. One and generally several vacuoles are present, which are not contractile. The shape varies with its movements, but it is always spherical when motionless. The protoplasm consists of two very distinct portions, an outer, or ectoplasm, and an inner, or endoplasm. The nucleus is situated in the endoplasm, and contains a nucleolus, which, in most instances, cannot be distinguished in the fresh specimen. The organism possesses active

motility by means of pseudopodia. Reproduction occurs by simple division and sporulation or gemmation. The following detailed description is the result of the study of many thousands of these organisms, and refers only to the morphology of the amebae as they are observed in the feces. There can be no doubt but that the morphology varies greatly upon cultures, and for this reason we can not use cultural characteristics as a basis for the differentiation of this organism from *E. coli* as it appears in the feces.

Size.—In the majority of instances *E. histolytica* is larger than *E. coli*, and here, as in the case of the latter organism, various authorities have given very diverse measurements, for the reason that amebae of the same species vary in size according to the period of growth at which they are observed, and because most authors have confused the two species of amebae most commonly found in the intestine of man. I am of the opinion that the size of *E. histolytica* is generally under- rather than overstated by most authorities, and from my observations I believe that *E. coli*, in the vegetative stage, is very seldom less than 10 microns in diameter, and generally much larger. When we consider that the normal red blood corpuscle averages 7 microns in diameter, it is at once apparent that an ameba of this species is very seldom observed as small as a red cell. As is well known, many amebae of this species are observed to contain red blood corpuscles, even the smaller organisms containing one or two, while it is not uncommon to observe organisms containing six or eight red cells, without being distended by them, thus proving that the average size of this amebae is considerably in excess of that of a red blood corpuscle. I have often seen amebae of this variety containing from 20 to 30 red blood corpuscles, which would make their diameter at least 50 microns, and I have repeatedly seen individuals measuring as much as 60 to 70 microns. As the result of my observations I believe that the great majority of individual *E. histolytica* measure at least 35 microns in diameter, a much larger average measurement than that of *E. coli*. These remarks do not apply to the young, free spores of this species, which measure about 5 microns in diameter, and are easily distinguishable from the vegetative form of the organism.

The size of *E. histolytica* is of importance both from a diagnostic and etiologic standpoint. As I have stated, while the size of *E. coli*

approximates that of the pathogenic species, I have never seen it equal the larger specimens of *E. histolytica* which are often observed, and the average is much smaller. If this fact be remembered, together with the differences in morphology, it is not a difficult matter to differentiate the two species. Some authorities have gone so far as to found the species distinction entirely upon the size of the amebae, the larger being classed as pathogenic, the smaller as non-pathogenic. With such a classification I cannot agree. Careful examination of the feces in cases of amebic dysentery will show that amebae of large size are not always present, while in other cases both large and small amebae are found, belonging to the pathogenic species, so that the most we can say regarding size in the differentiation of these two species is that, in the vast majority of instances, *E. histolytica* averages larger than *E. coli*.

Shape.—The shape of the organism varies with its movements, but when it is at rest it is spherical or slightly oval. Kartulis,¹³ in his description of the amebae occurring in amebic dysentery, states that when at rest the organism is oval, but observation will demonstrate that the spherical shape is altogether the more common. When in motion the organism presents most extreme variations in shape, owing to the changes in the form of the pseudopodia.

Color.—I have spoken of the peculiar dull-grayish color of *E. coli*; as a rule *E. histolytica* is almost colorless, the ectoplasm being perfectly so, appearing like a piece of ground white glass. The endoplasm is of a very light-gray color in most instances, but when blood is present in the feces, there is often observed a slight greenish tint in this portion of the organism. This color I believe to be due to hemoglobin liberated during digestion of red blood corpuscles by the ameba; this explanation has been questioned by some authors, but there can be no doubt that *E. histolytica* does engulf and destroy these cells, and anyone can convince himself of this fact who will spend the time necessary to demonstrate it. I have time and again watched this ameba engulf red blood cells, and traced the process of digestion, the cells gradually losing their color and breaking up into fragments. I have repeatedly seen an ameba cross the microscopic field and engulf a red blood cell, just as the leucocytes engulf the malarial plasmodia; it would indeed be strange if such a manifest example of phagocytic activity did not

result in the destruction of the engulfed body, and I believe that this phenomenon proves that *E. histolytica* is capable of destroying red blood corpuscles, which is not true of *E. coli*, for in the few instances in which I have observed red blood cells within this organism, I have never seen any evidence that the ameba was able to destroy them.

Protoplasm.—The appearance of the protoplasm in *E. histolytica* varies somewhat with the age of the organism. In the small or young amebae, the protoplasm is finely granular in appearance, and unless the organism is in motion it is very seldom that the ectoplasm and the endoplasm can be distinguished. The nucleus is, as a rule, invisible, but when visible, is situated to one side of the center of the endoplasm. As the organism enlarges the ectoplasm and endoplasm become differentiated, and in the large amebae these two portions of the protoplasm may be differentiated, in many instances, even when the organisms are motionless. In the moving organism the distinction is always very clear and unmistakable.

The ectoplasm comprises about one-third of the protoplasm and appears perfectly hyalin and glass-like, although an examination with the highest power lenses demonstrates that it is composed of some dense material and innumerable very minute granules. This portion of *E. histolytica* is very refractive to light, much more so than the endoplasm, the opposite of which is true in *E. coli*. One has but to compare the firm-appearing, well-defined ectoplasm of *E. histolytica* with the delicate, indefinite ectoplasm of *E. coli*, to be convinced of the truth of Schaudinn's and Jürgen's assertion that the secret of the pathogenic action of *E. histolytica* lies in the ability of the ectoplasm to penetrate the mucous membrane of the intestine, a property which the ectoplasm of *E. coli*, by reason of its delicate structure, does not possess.

The endoplasm, comprising about two-thirds of the body of the fully developed organism, is light grayish in color, and is composed of granular material, the granules being of considerable size, and more or less refractive. The endoplasm of this species appears much more granular and coarser than the endoplasm of *E. coli*, and is not as refractive to light.

While the granules comprise the greater portion of the endoplasm of this ameba, one, and generally more than one, vacuole, is always

present, and in many of the largest amebae, the entire protoplasm appears vacuolated, a change which is probably degenerative in character. Besides the vacuoles, the endoplasm may contain red blood cells, bacteria, crystals, and peculiar oval bodies, the nature of which will be explained later.

Nucleus.—A nucleus is, of course, always present in *E. histolytica* but it is generally very difficult to distinguish, in contradistinction to *E. coli*, in which nearly every organism shows a distinct and easily observed nucleus. When it can be seen, the nucleus is situated, as pointed out by Schaudinn, eccentrically in the endoplasm, and it is often seen flattened against the boundary between the ecto- and endoplasm. It contains but little chromatin compared to the nucleus of *E. coli*, is circular in shape, and may contain a definite nucleolus. The nuclear membrane is very indistinct, and the structure of the nucleus difficult to ascertain. When the organism is in motion the nucleus continually changes its relative position, unlike the nucleus of *E. coli* which tends to retain its relative position in the endoplasm. The nucleus of this species is not very refractive, is colorless, and the nucleolus, when it can be distinguished, is less refractive than the nucleus and situated to one side of the center of that body. The size of the nucleus varies with the stage of growth of the organism but averages about 5 to 6 microns when fully developed. In the large amebae the nucleus is not visible, as a rule, because during reproduction by sporulation the nucleus is distributed, and thus becomes invisible in all but stained specimens. Where reproduction occurs by simple division the nucleus is always visible, situated near the center of the organism.

Vacuoles and contained bodies.—The endoplasm of *E. histolytica* always presents one or more vacuoles. In the smaller amebae only one is visible but in the fully developed organisms the vacuoles may vary in number from one to ten, or even more. When only one vacuole is present it is of large size, but when more than one is present, the size varies. In certain organisms the entire protoplasm appears to be replaced by vacuoles, and in such I have considered the process as of a degenerative nature. In many instances the vacuoles contain within them dissolved hemoglobin, thus having a greenish tint, bacteria, very small crystals, and peculiar, very refractive granules, in

active motion. The vacuoles are never contractile, and are perfectly spherical in shape when the organism is not in motion. When in motion the vacuoles change position with the movement of the endoplasm.

There can be no doubt but that *E. histolytica*, unlike *E. coli*, is normally phagocytic for the red blood corpuscles of man. This can be easily demonstrated by adding blood to feces containing these organisms and watching the result. Red blood corpuscles are very commonly seen within this organism, not alone because the feces in amebic dysentery frequently contains blood but because this ameba is normally a red corpuscle destroyer. In those cases of amebic dysentery in which both *E. histolytica* and *E. coli* are found in the feces, and such cases are far from uncommon, it is but very seldom that *E. coli* is seen to contain red corpuscles, although almost every specimen of *E. histolytica* may be filled with them. In the great majority of cases where these two species occur together, not a single specimen of *E. coli* will be seen to have engulfed red corpuscles, while numerous examples of such action by the pathogenic ameba can be demonstrated.

Generally not more than two to six red cells are observed in the endoplasm but it is not rare to see *E. histolytica* containing so many red cells that but little of the structure of the ameba can be distinguished; such amebae, if kept at the body temperature and carefully observed, will be seen to gradually break up these red cells, the hemoglobin coloring the organism a light green. The cells are not extruded but are very evidently digested, and the extrusion of red cells, which sometimes occurs, is an abnormal process induced by unfavorable changes in the environment, as, for instance, observation of the amebae without the use of a warm stage maintained at the right temperature. Besides red blood corpuscles, bacteria of various kinds, pigment granules, and crystals of various kinds are observed within the endoplasm of *E. histolytica*, as well as small oval bodies, which will be described in speaking of the methods of reproduction of this organism.

Motility.—In recently passed specimens of feces from cases of dysentery *E. histolytica* possesses very active motility, and if a warm stage be used these organisms preserve their motility for a long time. There can be no doubt but that this species of ameba is much more

actively motile than is *E. coli*, and that this is a valuable distinguishing feature between the two organisms. Musgrave and Clegg⁵⁶ do not agree with this statement and attempt to disprove it by comparing the rate of movement of various amebae upon culture plates with the movement observed in the feces. Such a method of comparison is of little value, as the environment is not the same, neither were the authors mentioned using pure cultures of *E. coli* or of *E. histolytica*, so far as their experiments indicate. As I already pointed out we cannot draw conclusions regarding the amebae observed in feces by what occurs during the artificial cultivation of amebae from various extraneous sources.

In *E. histolytica* motility may be very marked and when present is always more marked than the most active motility seen in *E. coli*. The mere extrusion of the pseudopodia is more rapid, even though progressive motion may not occur, while the division between the ectoplasm is very distinct. Three forms of motility may be distinguished; active progressive motion, the extrusion of pseudopodia without resultant progression, and movements of the endoplasm.

The character of the progressive motion of *E. histolytica*, in freshly passed feces, is generally rapid for ameboid organisms, and especially so when it is compared to the progressive motion of *E. coli*. As the feces containing the amebae loses the heat imparted to them by the body, the amebae gradually lose their progressive motion, and finally become motionless. The organism advances by throwing out pseudopodia, composed entirely of the clear, glass-like ectoplasm, into which flows the endoplasm. In cooled specimens the motion may be so sluggish as to require careful and prolonged observation to distinguish it, but in specimens kept at the temperature of the body *E. histolytica* exhibits a comparatively rapid progressive motion. The pseudopodia of this species of ameba vary considerably in size and general appearance, but are always larger, more distinct, and more commonly observed than in *E. coli*. The shape varies from a broad, rounded mass of ectoplasm to long, finger-like processes, with rounded extremities. I have never observed pointed pseudopodia in this species in the stools, although such forms are said to occur upon cultures. The long slender pseudopodia are most frequently observed in those organisms which are moving rapidly, while the broader and

more rounded pseudopodia occur in ameba which are more sluggish in their motion. When first extruded the pseudopodia are always hyalin in appearance, being composed entirely of ectoplasm, but the endoplasm quickly flows into them, and a granular appearance is thus produced; in very rare instances amebae of this species are observed in which the motion is so rapid and continuous that the distinction into ectoplasm and endoplasm cannot be made, but if such organisms be observed for a sufficiently long time, motion becomes slower and then the two portions of the protoplasm can be differentiated. I have spoken of the very gradual flowing in of the endoplasm into the pseudopodia of *E. coli*, this phenomenon occurring so slowly, and the two portions of the protoplasm of that organism being so similar in appearance as to render the observation of it almost impossible. This is not so in *E. histolytica*. In this organism the flowing into the ectoplasmic pseudopodia of the endoplasm occurs very rapidly as a rule, it often appearing as though the periphery of the endoplasm ruptured, thus allowing the remainder of this portion of the protoplasm to rush into the pseudopodia. In many instances the pseudopodia appear constricted near the boundary of the endoplasm, and when this occurs the endoplasm may be seen to pass slowly through the constriction, the contents, as the nucleus, vacuoles, and red blood corpuscles, being compressed as they pass through the narrowed portion. When motility is marked, as in fresh specimens of feces, the amebae are often seen to progress in a definite direction, as toward a blood corpuscle, or other substance, or to cross the microscopic field without cessation of progressive motion; in other instances motion will occur in one direction, quickly followed by progression in nearly the opposite direction, so that it may be many minutes before the ameba will pass out of the field of a one-sixth-inch objective.

In specimens which have been exposed to room temperature for some time, the second form of motion is frequently observed. This consists in the active extrusion of pseudopodia, but no progressive motion, the processes of ectoplasm being continually projected from the periphery of the ameba, and as quickly withdrawn. In such instances the endoplasm does not flow into the pseudopodia, as a rule, and when it does, a new pseudopodium is projected from some other portion of the periphery of the organism, thus neutralizing what would

otherwise result in slight progression. This form of motility is only observed in amebae which are exposed to unfavorable conditions, as lowered temperature, and mild solutions of certain chemical substances.

In rare instances, what may be called an intraprotoplasmic form of motion is observed, produced by currents in the protoplasm of the ameba. In such instances the contents of the protoplasm are seen to be in motion in a circular manner within the ectoplasm, the motion being most apparent toward the periphery of the organism. The motion may be slow or so rapid as to be hardly distinguishable; the nucleus, if visible, the vacuoles, red blood cells, bacteria, and crystals being whirled about within the protoplasm of the ameba. An undulatory motion of the border of the organism is often present along with this movement of the protoplasm.

As to the significance of this form of motion we are ignorant. It is very similar to that occurring in *Balantidium coli* when encystment begins, preparatory to reproduction, but although such ameba have been observed for hours, encystment does not occur, but, on the contrary, the intraprotoplasmic motion ceases, and progressive motion may be resumed.

Staining reactions.—With the modification of Wright's stain described, *E. histolytica* presents definite staining reactions serving to distinguish it from *E. coli*. In the larger organisms the ectoplasm stains very intensely, while the endoplasm stains dimly, the opposite of which is true in *E. coli*; in the smallest organisms this distinction cannot be as easily made, but every specimen of feces containing this species of ameba will present numerous individuals in which this distinctive staining of the ectoplasm and endoplasm will be found. The nucleus of this ameba stains very poorly as compared to that of *E. coli*, on account of the small amount of chromatin present in it, as well as from the fact that in many of the ameba the nucleus is undergoing division prior to sporulation. With Wright's stain the ectoplasm stains a very intense blue or violet, the endoplasm a light blue, and the chromatin of the nucleus a delicate pink or red. The staining reaction of the forms undergoing reproduction will be described in the next section. As I have already stated, it is difficult to properly stain these organisms for the purpose of morphological study, and many

preparations will have to be examined before one can expect to obtain suitable material for this purpose.

Methods of reproduction.—To Schaudinn we owe the first accurate description of the method of reproduction of *E. histolytica*, upon which he based very largely his classification of the species, and which the opponents of this classification have never disputed. The difference in the method of reproduction of *E. histolytica* and *E. coli* alone amply suffices to establish the two species.

E. histolytica reproduces in two ways: by simple division and by a process of budding or sporulation. Simple division of an ameba into two parts occurs in the feces under favorable conditions, and the process can frequently be observed if a warm stage is used and the organisms be watched for a sufficient period of time. It does not differ from the process already described for *E. coli*, the nucleus first dividing, followed by the division of the protoplasm, and the formation of two motile amebae.

Prior to Schaudinn's observations regarding the method of reproduction of this species of ameba I published⁵⁷ a description of the organism in which I described what I considered might be spores which were situated in the endoplasm and stained dimly with methylene blue. In concluding this paper I said:

There occur in all but the degenerative forms of ameba small, round, or oval, dimly stained areas, uniform in appearance, and most numerous in the large, full grown forms, being entirely absent in the vacuolated shells of amebae. These areas resemble similar areas in stained segmenting malarial plasmodia, and which are in them due to the young spores. Reasoning from analogy it may be that these areas in the amebae are also spores.

Schaudinn confirmed my opinion regarding these bodies. He found that under certain conditions nuclear division does not occur as in simple division but that the nucleus distributes its chromatin to the protoplasm, while the remainder of the nucleus is absorbed or extruded. This form of nuclear distribution only occurs when conditions arise in the host (man) unfavorable to the vegetative existence of the organism, and marks the first stage in the evolution of the resistant form or spore. The distributed chromatin gradually collects in small oval masses toward the periphery of the ameba, and finally becomes situated in the ectoplasm, from which the masses of chromatin, each surrounded by a portion of protoplasm, are separated, and

become free as spores. These spores Schaudinn regarded as the infective stage of the organism and proved that they were capable of producing dysentery in cats.

I have been able to confirm Schaudinn's description of this mode of reproduction and the following description is the result of the study of this process as observed in living amebae and in stained specimens.

It is not infrequent to observe amebae of this species in the feces, in which the protoplasm is seen to contain numerous brightly refractive granules and minute rod-like bodies which almost fill the organism. These bodies are the chromidia, which have been liberated in the protoplasm by the breaking-up of the nucleus. Other amebae are seen in which these refractive particles are collected into small clumps, arranged irregularly in the protoplasm or about the periphery, even distending the ectoplasm. This appearance is due to the grouping of the chromidia and the collection of these groups at the periphery, just before their liberation as spores. The final stage consists in the separation of these masses of chromidia, together with a small amount of protoplasm. Since the publication of my original papers upon this subject I have been so fortunate as to be able to follow this method of reproduction in its entirety in living specimens of *E. histolytica*, and thus to confirm Schaudinn's original description. In the feces of cases of amebic dysentery, especially in those cases in which by reason of the natural resistance of the system, or because of therapeutic measures, an unfavorable environment has been produced, the spores of *E. histolytica* occur in enormous numbers, and may be easily studied. They are round or oval in shape, have a yellowish membrane, while the contents appear homogeneous. They measure from 3 to 6 microns in diameter, the average being about 4 microns. On account of their yellowish or brownish color they resemble red blood corpuscles, under a low power objective, but can be easily differentiated from them with a one-sixth-inch objective. In stained specimens of feces containing this species of ameba, this method of reproduction can be traced by examining a large number of preparations at various periods of time. The larger amebae should be selected for study, and if Wright's method be used, the following forms illustrating various stages in the process will be observed:

1. Amebae in which the chromatin of the nucleus is stained a light

pink, and is arranged in the form of rods and granules. Compared with *E. coli* the chromatin is small in amount and does not stain so intensely.

2. Amebae in which the chromatin is partly within the nucleus and partly outside, somewhat increased in amount, and staining more intensely. This form illustrates the beginning of the distribution of the chromidia to the protoplasm and the destruction of the nucleus.

3. Amebae in which the chromatin is distributed in very faintly stained grains and granules throughout the protoplasm, and the original nucleus has entirely disappeared.

4. Amebae in which the chromatin rods and granules are collected into small clumps situated in the endoplasm and near the periphery of the organism in an irregular manner. These organisms are almost ready to sporulate.

5. Amebae in which some of the masses of chromatin are arranged in the ectoplasm causing it to project slightly, and in which one or more masses may be partially separated from the parent ameba. These various forms are illustrated in Plate 3.

In some amebae an unstained area may be seen surrounding the masses of chromatin and dimly stained protoplasm, thus showing the outline of the spore. It will thus be seen that in specimens stained by the method mentioned every stage of the process or reproduction described by Schaudinn may be observed. The spores I have not been able to stain. After they are liberated the resistant membrane surrounding them is formed and this effectually prevents the action of the staining solution. They do not in the least resemble the encysted forms of *E. coli*.

From the observations of Schaudinn, which I have confirmed in full, I conclude that reproduction in *E. histolytica* occurs in two ways: by simple division or fission and by the process of sporulation which I have described. The latter method of reproduction definitely differentiates this species from *E. coli*, and this fact, taken with the occurrence of *E. coli* in health and in diseases other than dysentery, and the experimental evidence of the effect of the two species upon susceptible animals, certainly proves beyond doubt the truth of Schaudinn's classification.

Relation to disease.—At the present time almost all authorities are

agreed in believing that a certain form of dysentery is caused by amebae, for it seems to me that if there is one fact absolutely proven in pathology, it is that amebic dysentery is due to the pathogenic action of the amebae invariably associated with the lesions of that disease. I shall not consider here the great mass of experimental evidence that has justified this belief, but shall consider only the evidence proving that *E. histolytica* is the species concerned in the etiology of this form of dysentery. I have already described the negative results obtained by Schaudinn and myself in experiments upon kittens with *E. coli*, experiments which definitely prove that this species has no pathogenic action upon these animals, and which can easily be repeated by anyone interested in the problem of amebic infection. I shall now describe certain experiments which show, it would appear, that *E. histolytica* is capable of causing in kittens all the lesions of amebic dysentery observed in man, and that no other organism occurring in the feces with the amebae possesses like power when separated from them. Schaudinn experimented upon cats and concluded that, in feeding experiments, the spores only of *E. histolytica* were capable of causing dysentery in these animals, and he therefore regards the spores as the infective agents in amebic dysentery.

One of Schaudinn's experiments, showing the infectivity of the spores, was as follows: He dried, in the air, the feces from an amebic dysentery case in which numerous spores were demonstrated upon microscopic examination. After thorough drying enough water was added to make about 20 preparations, using a cover-glass of 18 × 22 mm. These preparations were all examined carefully for *E. coli*, or its encysted stage, and were demonstrated to be free from this species of ameba, but the spores of *E. histolytica* were present in large numbers. The cover-glasses were removed from 10 of these preparations, the material washed off, and enough distilled water added to make 1 c. c. This was fed to a young, healthy cat in milk and upon meat. Upon the evening of the third day after feeding, the cat passed some bloody mucus, which, upon examination, was found to contain large numbers of *E. histolytica* in the vegetative stage of development. The cat died upon the afternoon of the 14th day, and section of the intestine showed the characteristic ulcerations. Another experiment which Schaudinn believes demonstrates that only the spores of *E.*

histolytica are able to produce infection, is as follows: A cat was fed upon material containing the vegetative stage of this organism only, and observed for a period of four weeks, during which time she remained healthy, and an examination of her stools at the end of this time demonstrated the absence of amebae. She was then fed with material containing only the spores of *E. histolytica* with the result that after an incubation period of six days she developed dysentery with bloody stools, which contained numerous amebae of this species. Death occurred at the end of two weeks and the intestine presented the characteristic lesions of amebic dysentery.

These experiments of Schaudinn throw a flood of light upon the interpretation to be given the negative results of other observers. Those who have obtained only negative results in their experiments were undoubtedly working with the non-pathogenic *E. coli*, while those who have obtained only a certain proportion of positive results in working with *E. histolytica* probably used feces in which the spores of this organism were absent in those cases in which they obtained negative results.

Personal observations.—I have already detailed the negative results I obtained in kittens by feeding experiments and rectal injections with feces containing the vegetative and encysted stages of *E. coli*, and I have repeated these experiments, using the feces of dysenteric cases in which both the vegetative stage, and the spores of *E. histolytica* were present, with the result that in 65 per cent of the kittens experimented upon by feeding, typical amebic dysentery developed, and in 50 per cent of those in which rectal injections were used the same result was obtained. Control tests were made with the bacteria occurring in the feces, and with feces containing *E. coli*.

Rectal injections.—Half-grown kittens were used in these experiments, about 5 c. c. of feces containing the ameba being injected into the rectum. This is the method which has been employed by most investigators of this subject. I have found that 50 per cent of the kittens so injected developed dysentery. The negative cases may be explained by the absence of the spores of *E. histolytica* from the stools, for while motile amebae were present in all of the material used we, were not certain of the presence of the spores as at the time of the

experiments I was inclined to believe that the vegetable stage of this ameba could produce the infection.

In dysentery produced by the rectal injection of the infective material, the lesions tend to be localized in the rectum and are not so general or so severe, as a rule, as when the infection is acquired through the mouth. The incubation period varied from six days to nearly two weeks, and was generally longer than in the feeding experiments. The lesions produced were typical of those occurring in amebic dysentery in man, and varied in extent and severity with the length of time the infection lasted.

As an example of the character of the lesions produced by the rectal injections of material containing *E. histolytica*, the following autopsy report is given of a kitten injected October 19 and killed November 21, the first evidence of infection having appeared about October 30.

Kitten 1.—Body that of a half-grown kitten, very greatly emaciated. The abdomen is greatly distended with gas. The mucous membrane of the anus appears swollen and a considerable amount of blood-stained mucus is adherent to it. The sub-cutaneous fat has almost entirely disappeared and the muscles appeared dry and atrophied. The pleural cavities are free from fluid and the lungs appear normal. The heart is greatly congested and contains red clots in all the chambers. The liver is hypertrophied, deeply congested, and marked albuminoid degeneration is present but there is no trace of abscess formation. The kidneys are congested and upon section present the usual lesions of an acute parenchymatous nephritis. The omentum contains a small amount of fat and is not inflamed. The bladder is filled with urine.

The intestines are greatly dilated with gas and fluid. Upon external examination the large intestine appears swollen, is grayish in color, with small, darker colored areas scattered along it. Upon opening the large intestine the mucous membrane of the rectum is found considerably swollen and inflamed, but no ulcerations are present. Above the rectum for a distance of about 10 cm. the mucous membrane is very much swollen and edematous, bright red in color, and between the folds a considerable amount of pus can be seen. For a distance of about 4 cm. from the upper end of the large intestine, the mucous membrane is inflamed, being red, swollen, and edematous. In this area there are numerous ulcerations, covered in with bloody mucus; they are small size, somewhat irregular in shape, and extend, in most instances, to the sub-mucosa, although there are a few which extend to the muscular coat of the intestine; the edges are undermined and many of the ulcers are covered with necrotic tissue, brownish yellow in color, which has to be removed in order to expose them. A few of the ulcers communicate beneath the mucous membrane. The small intestine shows a rather severe acute enteritis and the stomach an acute gastritis.

This case was unusual in that the greater number of the ulcerations occurred near the ileo-cecal valve, while the rectum escaped, whereas in other successful experiments by rectal injection, the lesions were

almost confined to the rectum and the intestine for a short distance above the rectum.

The amebae, actively motile, were demonstrated in smears made from the intestine in this kitten, being most numerous where the lesions were most severe and least numerous in that portion where no ulcerations were present, but every part of the large intestine showed infection with *E. histolytica*. Smears made from the small intestine, immediately above the valve, did not show any amebae.

The clinical symptoms present in this case were those observed in all of the kittens that developed dysentery, and were similar to those occurring in man. The first symptom was invariably diarrhea, the stools being frequent, at first free from blood, but soon becoming bloody and filled with mucus, while numerous amebae were present in them. Emaciation occurred rapidly, and some fever was generally present. After persisting for several days, the diarrhea, in this kitten, ceased and a period of constipation intervened, in which two or three days passed without a bowel movement, but after a week the dysenteric symptoms recurred, and from that time until the animal was killed, the bowel movements varied in number from six to ten a day, and the animal became almost a skeleton. *E. histolytica* could always be demonstrated in the feces after the initial symptom of diarrhea appeared.

Feeding experiments.—While rectal injection of infective material results in the production of dysentery in 50 per cent of the kittens I have experimented with, the most successful results have been obtained by feeding kittens with infected fecal material. In this way I have produced typical dysentery, amebic in type, in 66 + per cent of the kittens experimented upon (8 out of 12) and have demonstrated *E. histolytica* in the feces of all and in sections of the diseased intestines.

My method of feeding was as follows: The kittens were starved for 24 hours, and at the end of that time were given milk containing about 5 c. c. of the infected feces, containing motile amebae of this species and their spores; the animals do not object to taking the mixture, and an almost equal amount of feces may be mixed with the milk without the kittens appearing averse to eating it. After the animals were fed they were observed carefully and the symptoms noted.

In all the successful cases the symptoms consisted in diarrhea with the passage of blood-stained, mucous stools, containing multitudes of motile *E. histolytica*, rapid emaciation, loss of appetite and strength, severe tenesmus, the kittens appearing much distressed while voiding the feces, and finally death from exhaustion.

The period of incubation varied considerably, the shortest being seven days, the longest eleven days; most of the animals showed diarrhea, with the passage of blood-stained stools, by the 8th day, so that the period of incubation is shorter by this method than by the use of rectal injections. Short periods of constipation were observed in two of the animals, lasting a day or two, but were always succeeded by profuse diarrhea with the passage of almost pure blood and mucus.

As illustrating the pathological lesions found in these animals, and produced by feeding with material containing *E. histolytica* the following autopsy records are inserted:

Kitten 3.—This kitten was fed once with feces containing *E. histolytica* and seven days later developed diarrhea, the feces containing blood and mucus, as well as numerous motile amebae. At the end of two weeks it died, having presented severe symptoms of amebic dysentery during this time.

Autopsy.—Body that of a half-grown kitten, very greatly emaciated. Subcutaneous fat entirely absent, and muscles dry and much atrophied. The abdominal cavity is free from fluid and the intestines appear normal externally. The pleural cavities are free from fluid and the heart and lungs appear normal. The liver is brownish red in color externally, with irregular yellow motilings. There is a small abscess present at the dome of the right lobe, measuring 0.25 cm. in diameter, showing very distinctly through the capsule of the organ. Upon section of the liver the cut surface appears greatly congested, the lobules are distinct, and no abscesses are found other than the one mentioned. The gall bladder appears normal. The kidneys appear enlarged and congested and upon section show an acute congestion, with some thickening of the cortex. Externally the large intestine appeared slightly, if at all, congested, although the walls were markedly thickened. Upon opening the large intestine it was found filled with fecal material mixed with a large amount of pus, and blood-stained mucus. About 1 cm. from the anus, which was blood-stained and covered with mucus, there was an area measuring 4 cm. in length, presenting the typical lesions of amebic dysentery, as they are observed in man. The entire mucous membrane was swollen, congested, and edematous. Numerous nodular areas projected into the lumen of the intestine, which, when incised, were found filled with a glairy material containing hundreds of *E. histolytica*. There were also numerous ulcerations, more or less irregular in shape, with thickened and undermined edges; many were covered in with necrotic tissue, which, upon being removed, showed that the floor of the ulcer was formed by the muscular coat of the intestine. Many of these ulcers communicated with one another beneath the mucous membrane, and most of them had penetrated to the muscular coat. The remainder of the large intestine presented numerous ulcera-

tions, typical of those seen in the intestine of patients who have died of amebic dysentery. The lesions were most marked just below the ileo-cecal valve, where large areas of the mucous membrane had been destroyed, the muscular coat of the intestine being exposed.

Kitten 5.—This kitten was fed with milk containing *E. histolytica* several times before dysentery developed. The period of incubation was eight days from the date of the last feeding, but from that time, until it was killed, three weeks afterward, the animal presented the symptoms of amebic dysentery, there being gradual loss of appetite, emaciation, and a diarrheal discharge, containing blood and mucus, with numerous motile *E. histolytica*.

Autopsy.—Body that of a half-grown kitten, much emaciated. Subcutaneous fat entirely absent and muscles much atrophied. The pleural cavities were free from fluid and the lungs and heart appeared normal save for congestion. Upon opening the abdominal cavity the small intestine appeared congested externally. The liver is hypertrophied and greatly congested. The kidneys are congested and enlarged and upon section showed the lesions of an acute parenchymatous nephritis. The large intestine was dark gray in color externally, and was considerably thickened, especially toward the rectum. Upon opening the intestine it was found to contain much fecal material, mixed with blood, mucus, and pus. Commencing at the rectum and extending for about half the length of the large intestine, the mucous membrane was greatly swollen, bright red in color, and contained numerous ulcers. The majority of the ulcers were spherical in shape, the edges were undermined and greatly thickened, and many were covered in with necrotic tissue. Upon removing this necrotic material the base of the ulcer is found to be formed by the muscular coat of the intestine. The ulcers present were typical of the amebic ulcerations seen in the intestine of man in every respect. The remainder of the large intestine was black in color and gangrenous, the mucous membrane having been almost entirely destroyed, exposing the muscular coat throughout this portion of the intestine. About 4 cm. below the ileo-cecal valve there was a small perforation measuring about $\frac{1}{8}$ cm. in diameter.

From the protocols of the autopsies given I believe that it is evident that *E. histolytica* produces in kittens the typical lesions of amebic dysentery as they are observed in man. All of the kittens experimented upon were examined prior to the experiments as to the presence of amebae in their feces, and all were found negative in this respect. The examination of sections of the intestines presented the same microscopic pathology observed in sections from dysenteric intestines from man and the amebae could be demonstrated in the tissues. It is a significant fact that the amebae observed in the feces and in the intestine of every kitten in which dysentery resulted from either the injection of infected material, or from the feeding of such material, presented the morphological characteristics of *E. histolytica*, no organisms answering to the description of *E. coli* being observed.

Control experiments.—That *E. histolytica* produced the lesions of

dysentery observed in the infected kittens, and not the bacteria occurring with the amebae, was conclusively proven by using cultures obtained from the feces for feeding and injections in the same manner in which the feces containing the amebae were employed. Mixed cultures of all the bacteria that could be cultivated were used in the controls and no evidence of dysentery was produced. While there were probably bacteria present that could not be cultivated, I think that the conclusion is justifiable that *E. histolytica* is the cause of amebic dysentery. Further proof of the etiological relation of this species of ameba to the lesions produced is found in the fact that in the abscess of the liver which occurred in one of the infected kittens the pus was sterile save for a few of the amebae, and these organisms were also demonstrated in sections made of the abscess.

As I have already noted, the controls made with feces containing *E. coli* resulted negatively in every case.*

DIFFERENTIAL DIAGNOSIS OF ENTAMOEBA HISTOLYTICA AND ENTAMOEBA COLI.

The differential diagnosis of *E. histolytica* and *E. coli* rests upon the study of their morphology and of their methods of reproduction. From a practical standpoint the diagnosis must be made from differences in the appearance of the two species as they are observed in the feces, and such a diagnosis can be made when material is available and the two organisms can be studied together. I am firmly of the belief that anyone who has so studied these organisms will be forced to admit that they vary greatly in morphology, so much so as to render a differential diagnosis possible in every case. I have demonstrated to several investigators the differences in the morphology of the two species, and, while at first they had not accepted the plurality of species, careful study convinced them of the truth of Schaudinn's classification. In discussing the subject of the differential diagnosis of the amebae infecting the intestine of man in a previous paper, I said:

I am convinced that many cases have been diagnosed amebic dysentery, which in reality presented the harmless *Entamoeba coli* in the feces, this organism being mis-

* From the results obtained in the experiments of Schaudinn and myself, it is evident that the term "amebiasis" used by some recent authors to indicate amebic dysentery, is incorrect, and should be discarded. The term might be used in a general sense to indicate the presence in the intestine of both species of amebae but its use as a synonym of amebic dysentery cannot be recognized by those who accept the classification of amebae into a pathogenic and non-pathogenic species.

taken for *Entamoeba histolytica*. This mistake might easily be made in patients suffering from acute enteritis, in which it is more than probable that the majority would present *Entamoeba coli* in the feces, and this fact undoubtedly explains the numerous instances of so-called amebic dysentery with rapid and complete recovery.

From my experience there is no disease so resistant to treatment and in which a prognosis is so discouraging as amebic dysentery. Everyone is familiar with the fact that amebic dysentery recurs even after long periods of time, and it is very important, both to the patient and the physician, to know absolutely that the disease being treated as amebic dysentery is in reality due to *Entamoeba histolytica*, and that *Entamoeba coli* has not been mistaken for this organism.

During nearly four years' further study of the amebae occurring in the intestine of man, I have seen no reason to change my belief as expressed in the quotations given; indeed, further experience has only strengthened it, and while I have been able to add little to my original description of the morphology of these organisms, I am convinced that a differential diagnosis of *E. histolytica* and *E. coli* can be made with little difficulty upon morphological data alone.

The following are the principal differential features between two species as they are observed in freshly voided feces. It should be remembered that the differential diagnosis is arrived at from a consideration of *all* the data given, and not, as a rule, from the presence of a single feature. It is too much to expect that every individual organism will present all the points of difference enumerated, but many of them will, and in those that do not, enough will be present to render a diagnosis possible. To illustrate: not every large, motile ameba, without a distinct nucleus, is an *E. histolytica*, but if to these characteristics be added very marked motility, a clearly differentiated and highly refractive ectoplasm, and, perhaps, the presence of red blood corpuscles within the endoplasm, we may rest assured that we are dealing with *E. histolytica* and not *E. coli*. Bearing in mind, then, that our differential diagnosis must depend upon the presence of several morphological features, rather than any one, the following are the chief points in which *E. histolytica* differs from *E. coli*, as these organisms are observed in the feces.

Size.—As a rule *E. histolytica* is considerably larger than *E. coli*, but we cannot base a differential diagnosis upon size alone. This factor is only of value when other morphological data are considered with it, except in rare instances in which the very large size of the

ameba is enough in itself to enable us to designate the species, for *E. coli* never reaches the size of the largest *E. histolytica*.

Color.—The color of the two organisms is of some assistance in distinguishing them. In *E. coli*, the ecto- and endoplasm are both grayish in color and there is never observed the greenish color which is not uncommon in *E. histolytica*; in the latter species the ectoplasm is always colorless and hyaline in appearance, while the endoplasm is grayish or greenish. These observations apply only to the amebae as they appear in the stools.

Protoplasm.—The very marked distinction between the ectoplasm and endoplasm in *E. histolytica* is one of the most important features which differentiate this species from *E. coli*. This distinction can always be made in the motile amebae and generally in those which are not moving, the ectoplasm being visible at some portion of the periphery of the organism. In *E. coli* the ecto- and endoplasm can with difficulty be distinguished in the moving organisms, but never in the quiescent ones, and there is never present in this species the glass-like, perfectly hyaline appearance of the ectoplasm invariably observed in *E. histolytica*.

In *E. histolytica* the ectoplasm is very strongly refractive to light, much more so than the endoplasm; in *E. coli* the endoplasm is most refractive, but neither the ectoplasm or the endoplasm is as refractive as is the ectoplasm of *E. histolytica*.

Nucleus.—In *E. histolytica* the nucleus is generally invisible, and when visible is situated near the periphery of the organism, contains but little chromatin, is small, and possesses a very poorly defined nuclear membrane. In *E. coli* the nucleus is almost invariably visible, is situated near the center of the organism, contains a great deal of chromatin, is large, and possesses a very thick, well-defined nuclear membrane.

Vacuoles and contained bodies.—In *E. histolytica* a vacuole is always present, except in the smallest individuals, and generally there is more than one. In *E. coli* a vacuole is generally absent, and more than one is of very rare occurrence. In *E. histolytica* the endoplasm is very often observed to contain one or several red blood corpuscles, while in *E. coli* red blood corpuscles are very, very rarely observed in the endoplasm, and experiments by adding blood to the feces containing

these amebae show that they do not engulf the red cells, while the same experiment demonstrates that many red blood cells are engulfed by *E. histolytica*.

Motility.—In *E. histolytica*, in freshly voided feces, the motility is marked, the organism progressing quite rapidly in a more or less definite direction. *E. coli*, under the same circumstances, possesses very sluggish motility, and often none at all, while it is almost never seen to progress in a definite direction. The motility alone will serve to distinguish these organisms, if both be observed in freshly voided feces, for it will invariably be found that the amebae exhibiting the most active form of motility will present morphological features which prove that it belongs to the pathogenic species.

To sum up: if, in a freshly voided specimen of feces we observe large, motile amebae, showing a clear, hyaline ectoplasm, the distinction between the ecto- and endoplasm being marked; an absence of a nucleus, or a nucleus situated near the periphery of the endoplasm, small in size, poor in chromatin, and having a dimly defined nuclear membrane, with two or more vacuoles, and with or without red blood corpuscles in the endoplasm, we may be sure that the organism observed is *E. histolytica*, for the amebae occurring in the feces of healthy individuals or in those of patients suffering from diseases other than amebic dysentery do not present the above characteristics.

Stained specimens.—In well-stained preparations the two species of ameba can be distinguished by the difference in the staining reactions of the ecto- and endoplasm. In *E. histolytica* the ectoplasm stains more intensely with Wright's stain than does the endoplasm, while in *E. coli* the opposite is true. This applies to specimens observed in the feces. In stained preparations showing reproductive forms *E. histolytica* can easily be distinguished by the occurrence of the various stages of sporulation described.

Method of reproduction.—To one who cares to devote the time and study necessary, the investigation of the methods of reproduction of the two species of amebae described will prove a certain method of differentiating them. Both *E. histolytica* and *E. coli* reproduce by simple division when conditions are favorable for a vegetative existence; when unfavorable conditions arise, *E. histolytica* reproduces by spore formation, a method totally different from that of *E. coli*, which,

under similar conditions, proceeds to encystment, and the formation within the cyst, of eight daughter-amebae. Both these methods have been described, but it should be insisted upon that this difference is all that is necessary to prove the existence of two species of amebae in the intestine of man.

CONCLUSIONS.

From my further study of this subject I see no reason for changing the conclusions arrived at in a former communication, and which are given briefly at the beginning of this paper. I believe that at least two species of amebae infest the intestine of man, one, pathogenic, the other, a harmless commensal. To the pathogenic species the name *Entamoeba histolytica*, given by Schaudinn, should be applied, while for the non-pathogenic species the name *Entamoeba coli*, also given by that investigator, must be retained. My belief regarding the existence of the two species of amebae is based upon the following facts:

First, the occurrence of amebae in approximately 50 per cent or more of healthy individuals and of individuals suffering from other diseases than dysentery.

Second, the occurrence of amebae in every case of amebic dysentery.

Third, the marked morphological differences between the amebae found in health and in diseases other than dysentery, and the amebae found in dysentery.

Fourth, the diversity in the method of reproduction of the amebae found in health and in diseases other than dysentery, and of the amebae found in dysentery.

Fifth, the production of typical amebic dysentery in kittens by the amebae present in dysentery, and the total absence of pathogenic action in kittens of the amebae occurring in health and in diseases other than dysentery.

But one conclusion is possible when the data given in this paper are considered: viz., that the intestine of man harbors at least two species of amebae, differing in their morphology, in their method of reproduction, and in their effect upon susceptible animals.

NOTE.—While correcting proof my attention has been called to an article by Walker, published in the *Jour. Med. Res.*, 1908, 17, p. 379, entitled "The Parasitic Amebae of the Intestinal Tract of Man and Other Animals," in which the author

from a study of cultures of various amebae, concludes that Schaudinn's classification is incorrect. Walker has fallen into the error already referred to, of comparing the morphology and reproduction of amebae in cultures with the morphology and methods of reproduction of amebae in the feces. While he calls attention to the erroneous conclusions which may be formed by a study of cultural forms, and states that control observations should be made of the organisms in their natural habitat, it does not appear that he has observed the development of *E. histolytica* in the feces in a single instance, his only material being a single culture of an ameba isolated from a dysenteric stool by Musgrave and Clegg. All of his other observations were made upon cultures of ameba from other animals than man, and from water and dead leaves. This author, while claiming that the morphological differences noted by Schaudinn between *E. Coli* and *E. histolytica* are trivial, does not hesitate to describe several new species which are based upon similar and, in some instances, even more trivial morphological differences. He was not able to cultivate ameba from the feces of six men, two of them suffering from amebic dysentery. His description of spore formation in the cultures of ameba studied is not inconsistent with the description given by Schaudinn, except that in *E. histolytica* the spores are liberated by a process of budding or gemmation. Walker states that Schaudinn's results have never been confirmed, despite the fact that my paper confirming them was published as long ago as 1905, and almost every systematic worker upon the subject has confirmed them since then. He states that he has not been successful in staining ameba with ordinary stains, but he was working with cultured organisms, and I have never been able to stain any ameba grown upon cultures with Wright's stain, which would appear to indicate that grave structural changes occur during cultivation. I believe that it is obvious that the author's conclusions regarding Schaudinn's work are unjustified, when it is remembered that he did not study *E. coli* at all, and that the only dysenteric ameba studied by him, so far as his paper indicates, was an organism sent him in culture by Musgrave and Clegg, and isolated by the latter authors from a dysenteric stool. It is evident that such an organism, cultivated for several months, and studied under cultural conditions, is an unsafe guide in the study of *E. histolytica*, as observed in the feces of dysenteric cases.

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EXPLANATION OF PLATES.

PLATE 2. FIGS. 1 TO 12. ENTAMOEBA COLI.

FIG. 1.—*Entamoeba coli*, showing nucleus with heavy nuclear membrane. No ectoplasm visible.

FIG. 2.—*Entamoeba coli*, showing a small vacuole, and large nucleus, with well-marked nuclear membrane.

FIGS. 3, 4, 5, and 6.—*Entamoeba coli*, motile form, showing well-marked nucleus and lack of distinction between the ectoplasm and endoplasm. The pseudopodia, composed of ectoplasm, resemble very closely in structure the endoplasm.

FIG. 8.—*Entamoeba coli* showing nucleus and crystals in protoplasm.

FIGS. 8, 9, 10, 11, and 12.—Stages in the reproduction by encystment of *Entamoeba coli*.

FIG. 8.—Primary division into two masses, containing protoplasm and nucleus.

FIG. 9.—Formation of double cyst-wall and division into active and passive pronuclei.

FIG. 10.—Fusion of the active and passive pronuclei within the cyst.

FIG. 11.—Division of the synkarya, formed by the fusion of the active and passive pronuclei, into eight young amebae. Note character of cyst wall.

FIG. 12.—Thinning of cyst wall preparatory to liberation of the young amebae.

FIGS. A TO H. ENTAMOEBA HISTOLYTICA.

FIG. A.—Diagram of *Entamoeba histolytica*. 1, Ectoplasm; 2, Endoplasm; 3, Vacuoles; 4, Crystals; 5, Red blood corpuscles; 6, Bacteria; 7, Nucleus.

FIG. B.—*Entamoeba histolytica*, showing two vacuoles and well-defined, hyaline ectoplasm. In this and the succeeding figures note the clear distinction between the ectoplasm and the endoplasm, as shown in the perfectly hyaline pseudopodia, which are composed entirely of ectoplasm.

FIG. C.—*Entamoeba histolytica*, showing small nucleus with thin membrane and two engulfed red blood corpuscles.

FIGS. D, E, and F.—*Entamoeba histolytica*, showing absence of nucleus, vacuoles, and red blood corpuscles in the endoplasm.

FIG. G.—*Entamoeba histolytica*, showing vacuolar degeneration.

FIG. H.—*Entamoeba histolytica*, showing vacuoles, red blood corpuscles, and nucleus.

PLATE 3. FIGS. A TO H. ENTAMOEBA COLI, STAINED BY WRIGHT'S METHOD.

FIG. A.—*Entamoeba coli*, showing deeply stained protoplasm and nucleus.

FIGS. B and C.—*Entamoeba coli*, showing deeply stained endoplasm and more lightly stained ectoplasm. Also large nucleus, rich in chromatin.

FIG. D.—*Entamoeba coli*, showing staining reactions of ectoplasm, endoplasm, and nucleus. A small vacuole is present.

PLATE 2.



1.



2.



3.



4.



5.



6.



7.



8.



9.



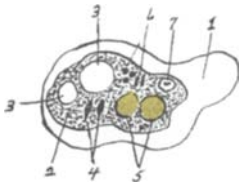
10.



11.



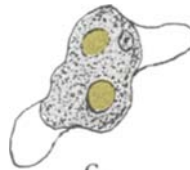
12.



A.



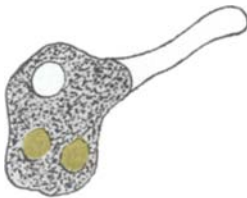
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C.



D.



E.



F.

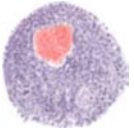


G.

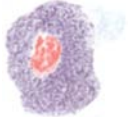


H.

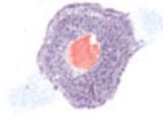
PLATE 3.



A.



B.



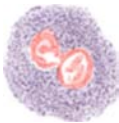
C.



D.



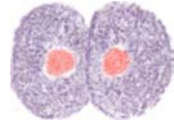
E.



F.



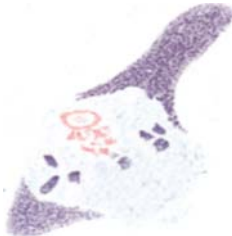
G.



H.



I.



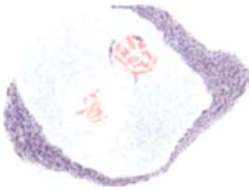
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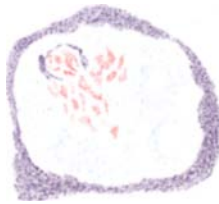
3.



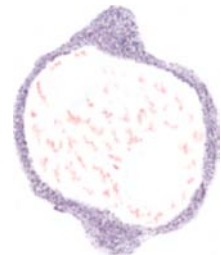
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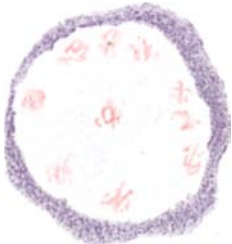
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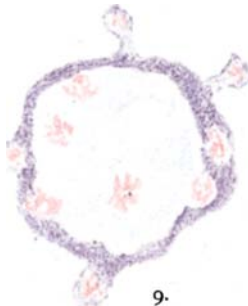
6.



7.



8.



9.



10.

FIGS. E, F, G, and H.—Various stages in reproduction by simple division of *Entamoeba coli*.

FIGS. 1 TO 10. *ENTAMOEBIA HISTOLYTICA* STAINED BY WRIGHT'S METHOD.

FIG. 1.—*Entamoeba histolytica* showing staining reactions of ectoplasm, endoplasm, and nucleus. Note the light staining of the endoplasm, the deep staining of the ectoplasm, and the smaller nucleus, less rich in chromatin, than the nucleus of *Entamoeba coli*.

FIG. 2.—*Entamoeba histolytica*, showing deeply stained pseudopodia, nucleus with some free chromatin, bacteria, and dimly stained endoplasm.

FIG. 3.—*Entamoeba histolytica*, showing absence of nucleus, characteristic staining of ectoplasm and endoplasm, and three red blood corpuscles.

FIG. 4.—*Entamoeba histolytica*, showing numerous vacuoles, and characteristic staining.

FIGS. 5, 6, 7, 8, and 9.—Stages in the reproduction of *Entamoeba histolytica* by spore formation.

FIG. 5.—Organism showing chromatin of nucleus arranged in rods and granules. The vacuole present contains some stained material.

FIG. 6.—Organism showing escape of the chromatin from the nucleus.

FIG. 7.—Organism showing distribution of the chromatin throughout the endoplasm, in the form of rods and granules.

FIG. 8.—Organism showing the collection of the chromatin into clumps about the periphery near the ectoplasm.

FIG. 9.—Organism showing the collection of the chromatin in the ectoplasm and the separation of the spores from the parent ameba by a process of budding.

FIG. 10.—Spores of *Entamoeba histolytica* as seen in the feces. The spores are bile stained and cannot be stained by Wright's method.

NOTE.—All of these drawings were made from specimens observed under the microscope using a 1-inch eye-piece and a $\frac{1}{8}$ -inch objective.