seen at six months of age, at which time the weight was 17 pounds and the general condition good. The modified milk had not been increased. The breast milk of fairly good quantity showed the following analysis, before nursing, fat, 2.72%, proteid .79%; after nursing, fat, 11.52%, proteid not obtainable as sample was small.

THE PRESENCE OF *B. DYSENTERIAE*, *B. PROTEUS VULGARIS*, *BACT. WELCHII*, AND *MORGAN'S BACILLUS* No. 1, IN THE STOOLS OF CASES OF INFECTIOUS DIARRHEA.

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THE interpretation of the results of any bacteriological study of a disturbance of the gastrointestinal tract is extremely difficult, owing to the great number and variety of organisms present, and to the fact that under abnormal conditions, some of these may multiply so rapidly as to lead the investigator to believe that they are the cause of the condition rather than the result of it. We all work under more or less of a bias, and if we decide that some one organism is the cause of a pathological condition we are apt to spend most of our time in searching for this one bacterium and in neglecting other factors which may be the true cause of the trouble. We believe that this accounts to a great extent for the importance attached to the several organisms that have been described as the cause of infectious diarrhea in infants. In taking up a study of this condition we decided to examine a large number of stools for four of the organisms that have the best evidence in their favor as being the etiological agent, rather than to attempt a complete bacteriological examination of the stools. These organisms are *B. dysenteriae*, *B. proteus vulgaris*, *Bact. welchii*, and *Morgan's bacillus* No. 1.

The study was made on the Boston Floating Hospital during the summer of 1914, and while we regret that the work has not extended over more than one summer, we feel that our observations should be recorded. The hospital provides an ample supply of material, and a well equipped laboratory. A spirit of coöperation on the part of the staff prevails that goes far towards making the work a success.

Infectious diarrhea is characterized by more or less fever and prostration, tenesmus, and the passage of an increased number of stools. These

stools contain mucus, and frequently blood and pus. A Gram-stained preparation made from the feces shows pus cells and bacteria, the majority of which are short Gram-negative rods lying free or in leucocytes and surrounded by a clear zone resembling a capsule. In addition, the stools have a characteristic odor that is recognized by those who have had considerable experience in examining them. The disease is an important one, in Boston at least, as it is very prevalent in the summer months, and the mortality among the hospital cases rises to between 40 and 50%.

The association of organisms of the dysentery group with these cases was first noted by Duval and Bassett¹ in 1902. They found dysentery bacilli in the stools of forty-two out of fifty-three cases examined. In 1904 was published "The Bacteriological and Clinical Studies of the Diarrheal Diseases of Infancy from the Rockefeller Institute of Medical Research." The laboratory studies were made by several workers under the direction of Dr. Flexner, in a number of eastern cities of the United States, and while the results of the different observers vary considerably, the dysentery bacillus was found in 63.2% of 412 cases examined. The work of Weaver and Tunnicliff,² Wollstein,³ and Kendall⁴ in this country, and of Leiner,⁵ Auché and Compana,⁶ Gildemeister and Baerthlein,⁷ Siegel,⁸ Bauer, Ellenbeck and Fromme,⁹ Weihe and Schürer¹⁰ Baerthlein and Howard,¹¹ and others in Europe, shows that the dysentery bacillus is found widely distributed in association with these cases. Immune substances such as agglutinins and complement fixing-bodies for the dysentery bacillus, have been found in the blood of these cases by a number of observers.

Tissier,¹² Klein,¹³ and Kendall¹⁴ have shown in certain cases which are clinically infectious diarrhea, that spores of organisms belonging to the groups of *Bact. welchii* are present in apparently increased numbers. The last observer found that a certain percentage of cases from which he was unable to isolate dysentery bacilli, passed stools, a small portion of which inoculated into milk and the milk heated for twenty minutes at 80° and then incubated, caused a "stormy fermentation" in this medium. It is stated that stools showing dysentery bacilli and those from normal cases did not give this reaction. These cases are said to improve on a diet of buttermilk and to do badly on a high carbohydrate diet, while the cases of infection with the dysentery bacillus are said to react in the opposite manner. He concludes that organisms of the *Bact. welchii* group are the etiologic factor in cases giving this reaction, and says that the number of these cases varies from year to year. So far as we have been able to learn, no immune bodies have been demonstrated in the blood of these patients and very few cultural studies on the organisms isolated have been published.

In 1897, Booker,¹⁵ working in Baltimore, studied a series of these cases and decided that *B. proteus vulgaris* was the cause of the disturbance, and for the last few years Metchnikoff¹⁶ and his pupils have been working on this organism as the cause of summer diarrheas in infants. They give only a very brief description of their cases and state that blood and pus were present in the stools in only a few instances, so it is evident that many of their cases cannot be classified as infectious diarrhea as it is understood in this country. They seldom find *B. proteus vulgaris* on plates of Conradi-Drigalski medium made directly from the feces, but isolate it by making a stab culture from the stool into gelatine, and within from twenty-four to forty-eight hours transferring a loop of the liquefied gelatine to the condensation water of an agar slant. The greater motility of *B. proteus vulgaris* enables it to climb onto the surface of the slant more rapidly than the other organisms, and if it is present, it is often isolated in pure culture at the end of twenty-four hours. Metchnikoff's work has extended over a period of five years, and during this time he has examined two hundred and eighteen stools from cases of summer diarrhea and has isolated *B. proteus vulgaris* in two hundred and four cases. He only mentions having examined six normal stools and found this organism present in two of these. In thirty-three stools that were slightly abnormal, he found it present in eighteen. Rabbits only a few days old and young chimpanzees, when fed the stools from diarrheal cases, developed a severe diarrhea, and cultures from their intestines showed *B. proteus vulgaris* to be present in great numbers. Metchnikoff does not mention having isolated the dysentery bacillus from any of his cases, but Bertrand,¹⁷ who studied fifty-five diarrheal stools and found *B. proteus vulgaris* in all, isolated the dysentery bacillus in fifteen cases. From the stools of twenty-four cases whose digestive tracts were apparently normal, the latter observer isolated *B. proteus vulgaris* only twice and the dysentery bacillus four times.

Morgan¹⁸ has studied a very interesting organism which he regards as a factor and perhaps the most important one in the infectious diarrheas found in England. From one hundred and twelve cases that were studied, this organism, known as *Morgan's bacillus No. 1*, was isolated on MacConkey's bile salt neutral red agar plates in forty cases, while dysentery-like organisms were found only five times. Agglutinins for the Morgan bacillus were not found in the blood of cases from which it was isolated. When cultures of this organism were fed to young rats, a severe diarrhea, followed by death, resulted.

With this brief review of some of the more important papers bearing on the organisms under consideration, we will give the results ob-

tained in our search for them in the series of cases studied.

The stools were passed into clean but not sterile pads, and as soon as possible were sent to the laboratory, where the various media were inoculated at once.

B. dysenteriae. For the isolation of the dysentery bacillus a suspension of the stool was made in bouillon and streaked over the surface of previously prepared Endo plates containing two per cent. of agar. After from sixteen to twenty hours' incubation, the plates were examined and suspicious colonies transferred to bouillon, and after five hours' incubation, transfers from the latter were made to two tubes of Hiss semi-solid media, one containing lactose, and the other mannit. Lactose was used, since occasionally cultures from colonies resembling dysentery on the Endo plates were found to produce acid in lactose, these being as a rule cocci. After these tubes had been incubated over night, the bouillon cultures whose sub-cultures showed growths characteristic of dysentery, were used for agglutination. The macroscopic test was used and was made by mixing one cubic centimeter of the culture with one cubic centimeter of a 1-500 dilution of anti-dysentery "Flexner" (mannit-fermenting strains) horse serum obtained from the Rockefeller Institute for Medical Research. This serum in a dilution of 1-1000 caused a complete agglutination of the mannit-fermenting dysentery bacilli after the tubes had been incubated for two hours and then allowed to stand in the cold for twenty hours.

The colonies of the dysentery bacillus on Endo plates are circular, translucent, and resemble drops of paraffine from one to two millimeters in diameter. While these organisms apparently grow readily on this medium, the number of colonies found in any one case was never very great, and oftentimes we failed to get them after repeated trials, though we were practically convinced from the appearance of the case that they were present in the feces. Repeatedly, the diagnosis was made on sub-cultures from the only dysentery-like colony on the plate. In two instances we were unable to isolate dysentery bacilli from the feces, in four separate attempts, yet they were obtained with ease at autopsy from the mucosa of the large intestine. In another case that clinically was infectious diarrhea and where the blood culture showed dysentery bacilli, we were unable to isolate these organisms from the feces though seven attempts were made.

Duval and Bassett¹⁹ state that for the isolation of the dysentery bacillus, the media should be *distinctly acid to litmus* and they further say, "Should the reaction be neutral to litmus, the difficulty of isolation of *B. dysenteriae* is very much increased; and in the presence of an alkaline reaction, isolation is rendered almost impossible." Should this be true, it is not surprising that in this day of selective media (that are always alkaline to litmus) the dysentery bacillus

is so seldom found. This, together with the apparent scarcity of the organisms in the feces and the fact that a negative report is often given after one examination, may account for their being missed in a certain number of cases. It has been our experience that while this bacillus is very difficult to isolate from the stools, with the method we used, we could readily isolate it from the mucosa of the large intestine after death. We made a few attempts to isolate it directly from the mucosa of the rectum during life by the help of the proctoscope, but were not successful. A careful comparative study of the methods of isolation of the dysentery bacillus and an attempt to get some good "enrichment medium" is greatly needed, and we regret that the pressure of other work prevented our taking up this problem.

During the summer, ninety-one cases were diagnosed clinically as infectious diarrhea, but in twelve of these we did not have an opportunity to make a careful bacteriological examination of the stool. Of the remaining seventy-nine cases, fifty-four, or sixty-eight per cent. showed dysentery bacilli. Many of the cases that were negative entered the hospital after they had been sick some days, and in some instances two weeks after the onset of the disease. We feel certain that many of them had dysentery bacilli in their stools, though we were unable to isolate them. The blood of these cases should have been tested for agglutinins, but lack of time prevented our making these tests.

Not a single culture of the Shiga bacillus was isolated. All the infections were due to organisms of the mannit-fermenting group of dysentery bacilli and the majority of the cultures, sixty-eight per cent., belonged to the Hiss Y type. That is, they formed acid in media containing dextrose and mannit, but not in lactose, saccharose, maltose, and dextrin. The remaining cultures were about equally divided between the Strong and the Flexner types. This differentiation of the mannit-fermenting dysentery bacilli into groups is very unsatisfactory, as the cultures vary so in their ability to attack the higher carbohydrates. Intermediate forms between each two of the groups are common and it is difficult to classify them correctly. The organisms that ferment saccharose and maltose tend to lose this property and then would come under the Hiss Y group. This has occurred with several of our cultures and also with the "Flexner-Harris" culture that has been in our laboratory for a number of years.

The stools of twenty-eight cases, with a diagnosis of diarrhea due to indigestion, were all negative for dysentery bacilli. In marked contrast to the cases of infectious diarrhea, the Gram-stained preparations from these stools showed few or no leucocytes and a predominance of organisms that were positive to Gram.

The evidence in favor of the dysentery bacillus as the cause of infectious diarrhea in infants

is its association with the majority of cases, the abundance of these organisms in the mucosa of the large intestine in acute cases, and the development of immune bodies to this organism in the blood of patients with this disease. While it has occasionally been found in the stools of individuals with an apparently normal digestive tract, these persons usually give a history of a previous diarrhea or an association with a case of this disease. The fact that it is apparently absent in a certain percentage of typical cases may be accounted for in some of the cases by the difficulty of isolation of the organisms, the use of unfavorable media, or to the examination being made rather late in the course of the disease.

Bacterium Welchii. The medium used in determining the presence of this organism was sterile fat-free milk in test tubes, the depth of the fluid being approximately six centimeters. A loop of the feces was transferred to the milk and emulsified by rubbing against the sides of the tube, after which the tube was immersed in a water bath at 80° C. for twenty minutes, and then incubated at 37° C. for twenty hours. At the end of this time the milk in some tubes was unchanged, in which case the reaction was said to be negative; in others, there was a solid coagulum, a smear from which showed organisms having the morphology of *Bact. welchii* when the reaction was said to be questionable, or there was the so-called "stormy fermentation," which was said to be a positive reaction. We fully realize that this is at best only a qualitative test for spores of gas-producing organisms, and while the organisms may have the morphology of *Bact. welchii* they should be isolated and studied, but we had neither the time nor the facilities for doing this work.

Simonds²⁰ has studied a number of strains of *Bact. welchii* and subdivides them into four groups, according to their action on glycerin and inulin. In the few cultures from infants' feces that were studied, those from diarrheal stools do not differ from those of the normal infants.

The results of the tests made on the stools from cases of infectious diarrhea and on those from various other cases are given in Table I.

TABLE I.

TESTS FOR SPORES OF ORGANISMS GIVING THE REACTION IN MILK THAT IS CHARACTERISTIC OF *BACT. WELCHII*.

Clinical Diagnosis.	Number of Cases.	REACTION.			
		Negative.	Doubtful.	Positive.	
		No. %	No. %	No. %	
Infect. diarrhea. (Dysentery bacilli found.)	53	35 66	12 23	6 11	
Infect. diarrhea. (Dysentery bacilli not found.)	37	19 51	11 30	7 18	
Indigestion with diarrhea	34	16 47	9 26.4	9 26.4	
Malnutrition	14	5 36	5 36	4 29	
Normal	13	2 15	6 46	5 38	

It will be seen that while this reaction in milk is produced by a certain number (11%) of cases showing dysentery bacilli, it is given by a greater number of cases of indigestion (26.4%), and by a still greater number (29%) of cases of malnutrition. In normal stools it was found with the greatest frequency, and while the number here recorded is small, the observations are confirmed by others made some years ago by one of us working under the direction of Dr. Theobald Smith and by the work of Knox and Ford,²¹ who found *Bact. welchii* in the stools of eighteen children with normal digestive tracts. This organism is an inhabitant of the digestive tract of normal man and many animals, is frequently found in milk, and has been found by us to be present in the "Eiweiss" milk that has been recommended as a treatment for these cases. It is difficult to understand how an organism having such a wide distribution and being an inhabitant of the normal digestive tract can be the cause of this condition, and we agree with Knox and Ford that sufficient evidence that *Bact. welchii* is the cause of infectious diarrhea in children has not been produced.

In order to say that *Bact. welchii* is a possible cause of infectious diarrhea one should be able to show repeated negative examinations for the dysentery bacillus in the stools and negative immune-reactions for this bacillus in the serum. In addition, demonstration should be made by plate cultures that the spores or the vegetative forms of *Bact. welchii* were present in greatly increased numbers, and immune-bodies to this organism should be found in the blood. It might also be that cultural differences could be found between the organisms from cases of infectious diarrhea and those from normal individuals.

The obtaining of a "stormy fermentation" in milk inoculated with feces and then heated does not indicate that *Bact. welchii* is present in increased numbers, for we have shown that eight spores will produce this change in milk and a milligram of feces from a normal infant often shows many times this number of spores of this organism.

Early in the disease of infectious diarrhea the reaction for *Bact. welchii* is usually absent, but it appears after the acute stage has passed. This may account for the statement that cases showing this reaction usually recover, or it may be that this organism has an action antagonistic to the dysentery bacillus.

B. Proteus Vulgaris. For the isolation of this organism, stab cultures were made into gelatine from the suspension of the stool in bouillon, and after several days' incubation, provided the gelatine was liquefied, attempts were made to isolate it by transferring a loop of the fluid medium to the condensation water of slanted semi-solid media. Thirty-one cases showing dysentery bacilli were examined in this way and in twenty-one of these there was no liquefaction of the

gelatine, so we have no evidence that proteus was present. In the ten cases where the gelatine was liquefied the organisms isolated in three cases belonged to the group of *B. coli*; in three to the *B. cloacae* group; and in four to the *B. proteus vulgaris* group. We, therefore, conclude that *B. proteus vulgaris* is frequently absent in cases of infectious diarrhea, and we have no evidence that it plays any part in the process. Its association with the more simple diarrheas is a subject that we will not attempt to discuss.

Morgan's Bacillus No. 1. This organism was isolated from Endo plates three times where dysentery bacilli were also present and six times where we were unable to isolate the latter organism. It is an extremely interesting organism from a bacteriological point of view as it attacks the hexoses forming acid and gas, but has no action on the di- or poly-saccharides or on the alcohol mannit. While it may play some part in the disease, we have no evidence that it is the primary cause of the trouble.

The same may be said of the organisms of the para-typhoid group and of pyocyaneus and the streptococci that are encountered so frequently. They must modify the disease of infectious diarrhea in one way or another, but we regard them as secondary invaders that multiply on account of the abnormal conditions produced by the dysentery bacillus. The part they play in the disease can be determined only by the most careful quantitative examinations of the stools and of the contents of the digestive tract at various levels, immediately after death, correlated with careful clinical observations. The study of the spontaneous diarrheas that occur in the lower animals, especially the disease that so frequently affects monkeys, should also throw light on this disease of children.

CONCLUSIONS.

1. In order to isolate the dysentery bacillus from the stools of cases of infectious diarrhea, repeated attempts often have to be made, and the number obtained may be very few.
2. Dysentery bacilli, of the mannit-fermenting type, were isolated from sixty-eight per cent. of the seventy-nine cases studied.
3. Organisms of the *Bact. welchii* type are occasionally associated with dysentery bacilli, but they are found more frequently in the cases of simple diarrhea and still more frequently in normal stools.
4. We have no evidence that *Bact. welchii*, *B. proteus vulgaris*, *Morgan's bacillus No. 1*, the para-typhoid bacilli, or *B. pyocyaneus* are the cause of infectious diarrhea, though they probably influence the course of the disease that is apparently started by the dysentery bacillus.

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A NOTE ON THE INVASION OF THE BODIES OF INFANTS BY *B. DYSENTERIAE*.

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THE isolation of dysentery bacilli from the blood culture made from an infant suffering from an acute attack of infectious diarrhea, made it seem worth while to study a series of cases, in order to determine the frequency of the invasion of the body by this organism. In the text-books one finds the statement that this is a rare occurrence, but in the literature there are a number of cases reported of positive cultures from the blood or from the organs.

Rosenthal,¹ in fifteen autopsies on adults, found dysentery bacilli in the liver and heart's

FINDINGS IN CULTURES MADE AT AUTOPSY

Hospital Number.	Age.	Duration of Disease.	Clinical Diagnosis.	Dysentery Bacilli Isolated from Stools before Death.	Autopsy.	
					Time Post-mortem.	Pathological Diagnosis.
1641	10 mos.	35+ days	Inf. diar.	Yes	9 hrs. 45 min.	Slight fol. colitis
1692	11 mos.	13 days	" "	Yes	45 min.	Membranous colitis.
1765	10 mos.	10 days	" "	Yes	15 min.	Membranous colitis
1731	2 yrs.	16 days	" "	Yes	25 min.	Follicular colitis
1775	4 mos.	9 days	" "	No	30 min.	Membranous colitis
1788	8 mos.	9 days	" "	Yes	40 min.	Follicular ulcerative colitis
1738	21 mos.	16 days	" "	Yes	60 min.	Follicular ulcerative colitis
1699	3 mos.	2 days	" "	Yes	105 min.	Follicular ulcerative colitis
1793	7 mos.	20 days	" "	No.	75 min.	Ulcerative follicular colitis
1777	8 mos.	19 days	" "	Yes	15 min.	Memb. ulc. follicular colitis
1850	10 mos.	14 days	" "	Yes	10 min.	Slight ulcerative colitis
1842	4½ mos.	Not recorded	" "	Yes	2 hrs.	Ulcerative follicular colitis
1895	3 mos.	7 days	" "	Yes	15 min.	Membranous ulcerative colitis
1908	20 mos.	8 days	Intoxication	No.	95 min.	Membranous ulcerative colitis