SOME RESEARCHES ON THE ETIOLOGY OF DYSENTERY IN CEYLON.

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DYSENTERY is, with the exception of Malaria, the disease most prevalent in Ceylon, and the mortality caused by it surpasses by far that due to any other cause. From the reports of Dr Perry, P.C.M.O. of the Colony, one sees that the total number of cases treated in all hospitals during the year 1902 was 3,017, with 999 deaths. In 1901 the number of cases was 4,177, with 1,543 deaths. The deaths due to Malaria were only 115 in 1902, and 89 in 1901 ; the deaths caused by Enteric Fever were 63 in 1902, and 74 in 1901.

At the suggestion of Dr Perry, to whom I am very much indebted for having facilitated in every way my researches, I have undertaken some investigation into the etiology of the disease in Ceylon. The researches have been carried out on cases treated in the Borella Convict Hospital or in the General Hospital of Colombo. I wish to express my thanks to Dr Johnson, Dr de Silva, and Dr Fernando, who were in charge of the patients. It is well known how different the opinions of authors are regarding the etiology of dysentery. At present the general tendency is to admit two forms of dysentery, an amoebic form, incorrectly called also tropical dysentery, and a bacterial form. Whether this last form is caused always by the same organism or not is not settled, though the most recent researches tend to show that the largest number of cases are due to the *Bacillus dysenteriae* described by Shiga and Kruse. Some authors recently have pointed out quite a different origin. I may mention as an example "La Dysenterie spirillaire" of Le Dantec. According to him in several cases of dysentery the mucus passed in the motions is practically a pure culture of Spirilla. He has never been able to cultivate them. A form of dysentery of children, or at any rate of a disease very nearly related to dysentery (Colitis

contagiosa of Escherich), common in some parts of Italy, is caused probably by the *Bact. coli dysentericum* of Celli.

The so-called "asylum dysentery" is due according to Durham to a very minute micrococcus; while Kruse believes it to be caused by bacilli very nearly related to the bacillus of dysentery.

Methods.

In my researches I adopted the following methods. Stools as soon as they were passed were collected in large sterile Petri dishes. The patients were instructed to pass their stools directly into these dishes, taking care that no admixture with urine took place. The stools were examined as soon as possible; very often as soon as passed. Fresh microscopical preparations for amoebae and other protozoa were constantly made. The warm stage was very seldom used, as in Ceylon there is no need for it. Stained preparations (using dilute fuchsine, and Gram's method) were made at once so as to afford some idea of the bacterial flora present. Agar and gelatine plates were made from flocculi of muco-pus. The best method is to pour the melted agar and gelatine into Petri dishes, let it set, and then smear the surface with a floccule of the pus. With the object of detecting *B. dysenteriae* any typhoid-like colony was examined according to the usual methods for the determination of bacteria. Inoculations in different media were made, using especially glucose-agar to see whether the germs isolated gave rise to gas production or not. I have examined 23 cases of dysentery by the above methods and with the following results.

Case I. K. 66, Borella Convict Hospital; acute case; death.

At autopsy the mucosa of the colon and the rectum was thickened, covered with muco-pus, and studded with a large number of very small superficial roundish ulcers.

The stools were twice examined during life; they contained practically no faecal matter and had no faecal odour. There was a large amount of pus and some blood. The microscopical examination showed a large number of leucocytes and red blood corpuscles. No amoebae or other protozoa could be seen. Some non-motile bacilli and some cocci arranged in pairs and chains were present. Several cover-slip preparations were stained with Löffler's methylene blue and by Gram's method. With methylene blue the cocci and bacilli were very well stained, and a few appeared to be inside the leucocytes. All the bacteria appeared not to stain by Gram, with exception of a few cocci and some rare bacilli. These last were large and rather thick, I failed to grow them. Several gelatine and agar plates were prepared from the stools. After 24 hours many colonies were present. In agar some of these were smaller, more delicate, somewhat translucent. In gelatine also some colonies were more delicate than the others and typhoid-like. Hanging

drop preparations from these colonies showed a non-motile, rather short, plump bacillus. From twelve of the more delicate colonies and twelve of the coarser and more opaque ones stab cultures were made in as many tubes of sugar agar. Eight tubes out of 12 of the first row did not show any evidence of gas production. The other four as well as the whole of the second row presented abundant gas bubbles. From the tubes without gas, inoculations were made in broth, gelatine agar, litmus milk, litmus agar, etc.

The organism isolated showed all the cultural characters of the Kruse's bacillus, the description of which will be given later. It was well agglutinated (1 : 40) by the blood of the patient and also by the blood of several convalescents from dysentery. The blood of the patient likewise showed agglutinating power when tested upon a culture of *Bacillus dysenteriae* (Kruse), which I brought from Kruse's laboratory.

The bacillus isolated from the stools of the patient was inoculated subcutaneously (1 c.c. of broth culture) into two rabbits. One died after 3 days, the other after 5 days—neither of them presented any symptom of dysentery. At autopsy the spleen was a little enlarged, and the mucosa of the whole intestine was somewhat congested, but no ulcers were present. No lesions could be detected in the other organs.

Case II. K. 9110, Borella Convict Hospital; acute case; recovery.

The stools were examined 25. I. 1904, the patient having been ill three days. Much pus and very little blood. Kruse's bacillus was isolated by the usual method. The patient's blood did not agglutinate the bacillus until the 29. I., *i.e.*, on the seventh day of the disease. On the same date the faeces, which had become nearly normal in the meantime, were again examined: Kruse's bacillus could not be found. Agar and gelatine plates showed only abundant colonies of *B. coli* and some rare colonies of a coccus. 16. II. 1904, the stools were examined a third time: appearance completely normal. *B. dysenteriae* not found. *Amoeba coli* could not be found in this case though looked for on several occasions.

Case III. Sinhalese boy in General Hospital under care of Dr Fernando; subacute case; recovery.

Stools examined 12. II. 1904. Very little faecal matter, no faecal odour, mucus in large quantity, little pus and blood. Microscopically many leucocytes; rare red blood corpuscles. No *Amoebae coli* or any other protozoa. *B. dysenteriae* found. The bacillus easily agglutinated (dilution 1 : 40, time 2 hours) by the blood of the patient after the first 10 days of the disease and also by the blood of several people recovering from dysentery.

Case IV. Adult Sinhalese, No. 1419, Borella Convict Hospital; acute case; recovery.

Stools examined 29. I. 1904. Large amount of pus, very little blood, no faecal matter. Microscopically many leucocytes, few red blood corpuscles, no faecal detritus, *Amoeba coli* absent. Kruse's bacillus found.

Case V. No. 3383. Banda, Borella Convict Hospital; acute case; recovery.

Stools examined 31. I. 1904. No faecal matter, no faecal odour, large amount of muco-pus, and very little blood. Microscopical examination: large number of leucocytes, very few red blood corpuscles, no ova of worms, no amoebae or other protozoa. Some non-motile bacilli were seen. Stained coverslip preparations show

some plump, rather short bacilli, also cocci in pairs and short chains, both organisms staining by Gram. Agar and gelatine plates incubated for 24 hours at 37° C. showed colonies of *Bacillus coli* and of a *Streptococcus* which slowly liquefies gelatine (*Streptococcus coli gracilis* Escherich ?) besides a few colonies of *B. dysenteriae*.

Case VI. Mary, General Hospital ; chronic case ; died on 27. I. 1904.

At autopsy the mucosa of colon and rectum found thickened, showed a number of large irregular ulcerations with thickened edges. Several hepatic abscesses. Scrapings from the ulcers showed many *Amoebae*. Kruse's bacillus not found. Cultures from the intestinal contents yielded *B. coli* and *B. pyocyaneus*. The pus of abscesses did not show amoebae. Cultures therefrom showed no growth.

Case VII. K. 32, Borella Convict Hospital ; subacute case ; recovery.

Stools examined 2. II. 1904 contained no faecal matter, and possessed no faecal odour. Large amount of mucus, some pus and very little blood. Microscopical examination showed numerous leucocytes, some of which enclosed red blood corpuscles. Some free corpuscles, no crystals, no ova. *Amoebae coli* as well as other protozoa absent. Coverslip preparations treated with dilute Ziehl's stain (1 : 4) showed a large number of bacilli, some of which were rather long and others rather short and plump. Some very fine spirilla are also present ; cocci in pairs and short chains can be seen. Some of the cocci are contained in leucocytes. Preparations by Gram's method show the cocci and a few bacilli well stained. *B. dysenteriae* found, but many of the colonies found in the agar and gelatine plates were those of *B. coli*, of a streptococcus liquefying gelatine slowly (*Str. coli gracilis*) and of a large spore-forming motile bacillus also liquefying gelatine and probably belonging to the *B. subtilis* group. The spirilla could not be grown. *B. dysenteriae* isolated from the stools was readily agglutinated by the patient's serum after the fifth day of the disease.

Case VIII. I. 4589, Borella Convict Hospital ; chronic case.

Stools contained no blood ; only a little pus and mucus. Large amount of faecal matter. Microscopical examination showed much faecal detritus, many ova of *Trichocephalus dispar*, some leucocytes and rare blood R. C. No *Amoebae coli*. *B. dysenteriae* not found. The plates showed only colonies of gas-producing organisms. The patient's serum readily agglutinated the Kruse bacillus.

Case IX. Karuppen. General Hospital.

The bacteriological examination was made from the intestine after autopsy 2. II. 1904. Mucosa of caecum, colon and rectum thickened, covered with muco-pus and presenting many small superficial roundish ulcers. Spleen slightly enlarged. Nothing to be noted as regards other organs. *Amoebae coli* absent. *B. dysenteriae* found. From the spleen and blood of the heart no germs could be grown.

Case X. No. 9601, unconvicted, Borella Convict Hospital ; subacute case.

Stools examined 8. II. 1904, the patient having been ill for 6 weeks. Stools consist practically of muco-pus and blood only. *Amoebae coli* absent. *B. dysenteriae* found.

Case XI. Menatshe. General Hospital ; chronic case.

The bacteriological examination was made from intestine after autopsy 9. II. 1904. Mucosa of colon and rectum thickened and studded with many small roundish ulcers. *Amoebae coli* absent. *B. dysenteriae* found.

Case XII. Patient admitted into Borella Convict Hospital 20. ɪɪ. 1904. Pulse 112. Temperature 102° F. Complained of griping pains. Frequent motions with blood and pus. Recovery after a few weeks.

Stools examined 20. ɪɪ. 1904. No faecal detritus; great number of red blood corpuscles and leucocytes; no protozoa; no ova; a few non-motile bacilli and some rare cocci. A bacillus very similar to Kruse's was isolated from the stool, this bacillus showed some slight differences which will be described presently.

Case XIII. Prisoner 125 (Daniel), Borella Convict Hospital; acute case; recovery.

Stools composed of a very large quantity of mucus, some pus, and very little blood; faecal odour entirely absent. Microscopical examination; many leucocytes, some red blood corpuscles, *Amoebae coli* absent. Coverslip preparations stained with dilute fuchsine (1:4) show a large number of short, plump bacilli, some of which are grouped in clumps. *B. dysenteriae* found. It is remarkable that in this case the colonies found in the plates contained a large majority of Kruse's bacillus, while colonies of *B. coli* were extremely rare.

Case XIV. Borella Convict Hospital; acute case; recovery.

Stools examined 6. ɪv. 1904, were passed by the patient directly into a Petri dish and examined at once. They consisted of muco-pus and a very little blood. *Amoebae coli* absent. Coverslip preparations stained with dilute carbol-fuchsine show some plump short bacilli and some spirilla. By Gram's method bacilli and spirilla are decolorized. Kruse's bacillus found. Plates showed some colonies of *B. coli* and other gas-producing organisms. The spirilla could not be grown.

Case XV. T. 6185, Borella Convict Hospital; acute case.

Stools passed by the patient directly into a sterile Petri dish and examined at once. Very little faecal matter; faecal odour absent; large amount of mucus; very little pus or blood; a few ova of *Trichocephalus dispar*; *Trichomonas intestinalis* present; *Amoebae coli* absent. Kruse's bacillus present. The blood of the patient taken on the 3rd day of illness did not agglutinate Kruse's bacillus, neither the original one nor any strain isolated by me from other cases; agglutination positive after 7 days of illness.

Case XVI. Kelavanti. General Hospital.

Bacteriological examination made from intestine after autopsy. Mucosa of small intestine greatly injected, that of colon swollen and studded with many small superficial ulcers. A large amount of muco-pus covered the mucosa. The examination was made from flakes of the muco-pus. No protozoa were found. *B. dysenteriae* present.

Case XVII. Dr H....... Colombo General Hospital, Paying Ward; chronic case. Suffered from his first attack of dysentery some years ago. After that an abscess of the liver developed, which was successfully operated on. A few months ago another attack set in; again an abscess of the liver developed. The patient died two days after abscess operation.

Stools examined 17. ɪv. 1904. There was some faecal matter with muco-pus and blood. Microscopically a large number of *Amoebae* were found. These amoebae were of large dimensions (50—80 μ) and slowly emitted blunt pseudopodia. Many *Trichomonas* were also present and also some other protozoa to be described

afterwards. Kruse's bacillus not found. The agar and gelatine plates showed only gas-producing organisms. Serum reaction with Kruse's bacillus was always negative with any dilution of the blood (1 : 2, 1 : 20, 1 : 40) using the original strain as well as any other Ceylon strain isolated by me.

Case XVIII. T. 6124, Borella Convict Hospital ; acute case ; recovery.

Stools examined 23. IV. 1904, consisted practically of pus, mucus and blood. Faecal odour completely absent. The microscopical examination showed numerous leucocytes, red blood corpuscles and a few non-motile bacilli. *Amoebae coli* and other protozoa absent. *B. dysenteriae* was grown easily, the greater majority of the colonies in the agar and gelatine plates being colonies of this bacillus while the colonies of *B. coli* were exceptionally rare. Agglutination positive with any strain of Kruse's bacillus.

Case XIX. K. 6285, Borella Convict Hospital ; acute case ; recovery.

Stools examined 8. IV. 1904. A very large amount of mucus, little pus and blood ; no faecal matter ; faecal odour completely absent. *Amoebae coli* absent. *B. dysenteriae* found.

Case XX. Prisoner No. 7592, Borella Convict Hospital ; acute case.

Stools passed 4. V. 1904, showed large amount of mucus, some blood and pus. Microscopically many leucocytes, some red blood corpuscles and a few non-motile bacilli were observed ; but no amoebae or other protozoa. *B. dysenteriae* was found alone in gelatine and agar plate cultures made from the stools.

Case XXI. H. 8949, Borella Convict Hospital ; acute case.

Stools consist of mucus and a little pus and blood. No faecal odour whatever. Microscopically some leucocytes and a few red blood corpuscles were seen. Stained preparations show a few cocci and some rare bacilli. *Amoebae coli* absent. *B. dysenteriae* present.

Case XXII. K. 3866, Borella Convict Hospital ; acute case.

Stools examined 10. V. 1904. Large amount of blood and pus ; no amoebae. *B. dysenteriae* present.

Case XXIII. K. 6262, Borella Convict Hospital ; acute case.

Stools examined 10. V. 1904. No faecal matter ; no faecal odour ; much muco-pus and a little blood. Microscopically many leucocytes and a very few red blood corpuscles found. Some rare short non-motile bacilli were seen. Amoebae and other protozoa absent. *B. dysenteriae* present.

To sum up, I have examined 23 cases of dysentery occurring in Ceylon. In 19 of these cases the presence of *B. dysenteriae* Shiga-Kruse was demonstrated in the intestinal contents or stools. In one case the bacillus could not be grown, but the blood of the patient agglutinated the bacillus. In one instance (Case XII.) a bacillus closely resembling Kruse's bacillus was grown.

In two instances (Cases VI., XVII.) a large number of *Amoebae* were found in the stools, whilst the presence of *B. dysenteriae* Shiga-Kruse could not be demonstrated.

Morphology and biology of the Shiga-Kruse Bacillus as found in Ceylon Dysentery.

The discoverers of the *B. dysenteriae*, Shiga in Japan, and Kruse in Germany, differ slightly in their descriptions of the organism. The most important difference refers to its motility. Kruse described the germ as non-motile. Shiga on the other hand described it as motile. Flexner also admitted some degree of motility, at least in young cultures. Vedder and Duval state that they never came across any motile strain, but nevertheless have been able to detect flagella. I can say at once that as regards my cases in Ceylon all the strains I have grown were absolutely non-motile, and although using different staining methods, I could never detect flagella. As regards the morphology of the Shiga-Kruse bacillus, it is a rather short, plump bacillus, easily stained by the usual aniline dyes. It is not stained by Gram's method. In cultures all the strains isolated by me in Ceylon behaved in the same way as the original strain from Germany. In broth the bacillus gives rise to a general uniform turbidity without any formation of a pellicle on the surface, or of gas bubbles. The indol reaction is always negative. It does not produce gas in glucose-agar, nor does it coagulate milk. In gelatine plates, in Piorkowski media, in Elsner potato-gelatine, the colonies always show a great resemblance to those of *B. typhosus*. In agar plates the colonies are often more delicate and smaller than those of *B. coli*, and they are somewhat translucent. Still, some strains of *B. coli* form colonies in agar absolutely identical with those of the Shiga-Kruse bacillus. According to my experience litmus-milk cultures after 15 hours show very little acidity—not enough to distinctly alter the colour of the medium, and after 3 to 5 days the milk becomes alkaline. I have never seen any coagulation. It is evident from this description that a great resemblance exists between the dysentery and the typhoid bacilli. Practically they cannot be distinguished by their cultural characters—though they can be easily distinguished by the fact that Kruse's bacillus is certainly non-motile. Further, the serum of an animal immunized against typhoid has no action on Kruse's bacillus, and *vice versâ*. Another point of resemblance, in addition to many others, between the Kruse and the typhoid bacillus is, according to my experience, in the production of haemolysins (see *Lancet*, 1902, February 15th). Both organisms are capable of forming a haemolysin which produces complete solution of dog's erythrocytes. The maximum amount of haemolysins is found in filtrates of 2 weeks' old cultures. The

bacillus does not produce spores. It possesses but little resistance to heat and to the usual fluid disinfectants.

Pathogenicity. My experiments were carried on with guinea-pigs and rabbits only, using strains of *B. dysenteriae* isolated in Ceylon as well as cultures brought by me directly from Prof. Kruse's laboratory, in Bonn. I have not observed any appreciable differences as regards the pathogenicity of the various strains. Intraperitoneal inoculations of 1 c.c. of broth culture kill guinea-pigs in 15 to 20 hours, a much smaller dose is sufficient to kill the animal by sub-dural inoculation. Rabbits inoculated hypodermically waste very rapidly and seldom live longer than 6 to 8 days. Sometimes experimental animals may suffer from violent diarrhoea, but in my experience the stools never contain blood or pus. At autopsy the intestinal mucosa is generally congested; I have never seen any ulcerations. These results do not correspond with the experiments of Vaillard and Dopter (*Presse Médicale*, No. 39, 1903), who claim to have reproduced dysentery in rabbits by means of sub-cutaneous injections of the Shiga-Kruse bacillus. On the other hand my experiments perfectly tally with those of Kruse himself, and with the experiments I carried on in his laboratory when he discovered the organism.

Agglutination. The technique was the same as that usually employed. Generally dilutions of 1 : 40 were employed and the preparations examined after two hours. All the strains from Ceylon and Germany behave practically in the same way, with the exception of the strain isolated from case XII (see p. 505). The blood shows presence of specific agglutinins only after the first 5–8 days of the disease. The agglutination is generally well marked in convalescents. I have not data enough to justify a statement as to how many months the blood of the patient retains the agglutinating power. In a case 4 months after the disease had been cured agglutination was still very marked. The agglutination in several cases is not regular, the reaction seems to be sometimes almost intermittent. One day with the dilution 1 : 40 there is a very good agglutination; the day after with the same dilution there is no agglutination at all, though there may be perhaps with a dilution of 1 : 10 or 1 : 20. The reaction is rarely positive using a dilution of more than 1 : 150.

The intensity of the agglutination does not proceed parallel to the severity of the disease; the agglutinating power of the blood may drop very low, *sub finem vitae*. The blood of healthy persons never agglutinated any of the strains even in dilutions of 1 : 10. The blood of patients

suffering from diseases other than dysentery generally did not show any agglutinating power as regards Kruse's bacillus, with the exception of one case of typhoid fever and one case of malaria. In the former the reaction was positive up to a dilution of 1 : 30, in the latter up to a dilution of 1 : 20. Both patients denied having ever suffered from dysentery. In dysentery then, as in typhoid fever, it is advisable to use a dilution of not less than 1 : 40.

Immunization and Vaccination. So far I have conducted few experiments in this connection. I have tried to immunise rabbits by sub-cutaneous inoculations of live cultures, or of cultures killed by exposure for one hour at 75° C. Using live cultures the rabbits constantly died after a few days. Treated with dead cultures two rabbits lived for about one month; but their sera showed only slight agglutinative power. Two weeks after inoculation the serum when diluted to 1 : 200 agglutinated the Shiga-Kruse bacillus, but the protective power of the serum was practically *nil*. Even 2 c.c. of the serum added to the minimal lethal dose of Kruse's bacillus was not able to save a guinea-pig of 250 grms. No dissolution of the bacilli could be noticed in the peritoneal liquid extracted in the usual way by means of fine capillary tubes. The results are quite like those I obtained in Germany, probably better ones would follow if I could use donkeys and horses. Kruse has succeeded in immunizing horses and in getting a powerful serum, which has given apparently fairly good results in the treatment of several cases. Shiga also and others have succeeded in preparing sera of high protective power by different methods. As regards the preparation of an anti-dysenteric vaccine this I think might be worth a trial in localities where severe epidemics of dysentery are frequent. Kruse inoculated himself and two of his assistants subcutaneously with 1 c.c. of a broth culture, previously kept for an hour at a temperature of 65° C. The inoculation caused some slight elevation of temperature and a certain degree of malaise. These symptoms disappeared soon. There was also some local reaction at the seat of inoculation. After a few days the serum agglutinated the dysentery bacillus. It was therefore to be surmised that a certain degree of immunity had been acquired.

Prevention of dysentery. Though one cannot deny that drinking may convey the germ, it must be noted that here in Colombo the water is from a bacteriological point of view a very good one. This also holds for the water used in the gaols—where cases of dysentery are so common. I think the best way to diminish the number of cases of dysentery is to improve the sanitation—especially in regard to the removal of excreta,

drainage, etc. In Germany it has been observed that outbreaks of dysentery occur often in places which have a good supply of water but a bad system of drains. Patients with dysentery should be isolated and their stools disinfected at once. Another possible means of prevention (I am speaking of course of the bacterial form of dysentery) would be an anti-dysentery vaccination. This is worthy of trial, but does not appear to me promising.

Observations on the Intestinal Bacterial Flora of Dysentery Cases.

B. dysenteriae is usually found associated with several germs in cases of dysentery. In fact it is exceptional—personally I have met only with one such case—to find the dysentery bacillus alone, *B. coli* is, in my experience, almost constantly present; generally, even when very fresh stools are examined, the majority of colonies grown on gelatine and agar plates are those of *B. coli*. If the stools are not examined very soon after they have been passed the *B. coli* multiplies to such an extent that it becomes very difficult to isolate the dysentery bacillus. I have grown various strains of *B. coli*—all alike in their more important cultural characters (coagulation of milk, etc.). Most of these strains were non-motile; some sluggishly motile; a very few actively motile. The blood of rabbits immunized with one strain did not show agglutinative power for the other strains. Some of the strains were not agglutinated at all by the blood of the patients, a few were agglutinated up to a dilution of $1:20$ and one $1:30$. But they were agglutinated to the same extent or very little less by the blood of perfectly healthy persons. The blood of typhoid patients agglutinates them in some cases more than the blood of dysentery cases. The possibility of normal sera, typhoid sera, etc., agglutinating certain strains of the colon bacillus has been experimentally demonstrated by Iatta and other authors including myself. Iatta has shown that the serum of an animal immunized with the typhoid bacillus besides agglutinating this bacillus agglutinates also—though to a very much less extent—some strains of coli; moreover he has demonstrated that a certain parallelism may be observed between the two agglutinations, viz. if the agglutinative power of the blood for the typhoid bacillus increases, it increases also for those strains of the colon bacillus. No importance then can be attached to the fact that the sera of some dysentery patients may agglutinate some strains of *B. coli*.

In two cases a bacillus producing a green pigment with all the characters of *B. fluorescens liquefaciens* was grown. In two cases

organisms of the *subtilis* group were present. In three cases anaerobic motile bacilli with characters similar to *B. enteritidis sporogenes* Klein were isolated. In several instances coverslip preparations made directly from the pus in the stools and stained with dilute carbol-fuchsin or methylene blue showed Spirilla of two types: some were finer, longer and rather faintly staining, others were thicker, shorter and appeared deeply stained; both types were decolorised by ·Gram's method. I never succeeded in growing them. Cocci not decolorised by Gram's method were present very often in coverslip preparations made from the muco-pus; though in the agar and gelatine plates I could seldom obtain colonies of cocci. As a rule these cocci—in broth cultures— appeared arranged in chains. Some strains of these streptococci liquefied gelatine. Among the liquefying strains some in broth cultures possessed chains composed of very small individuals ($0\cdot2—0\cdot4\,\mu$): *Streptococcus coli gracilis* of Escherich. In two cases diplococci were grown having practically all the characters of *Streptococcus lanceolatus*. Blastomycetes were very rarely observed.

None of these different germs had anything to do with the etiology of the disease; their inconstant presence in dysentery, and their frequent occurrence in other diseases, as well as occasional presence in normal individuals prove this, though it is possible that in some cases they may modify the course of the disease by acting as secondary infectious agents.

Paradysentery.

Paradysentery.—From case XII, which clinically did not differ from an ordinary acute case of the disease, I grew a bacillus which culturally can scarcely be distinguished from the Shiga-Kruse bacillus. The only cultural peculiarities—to which very little importance can be given— are its more abundant growth on agar, its production of indol, and its more marked production of acids. The typical Shiga-Kruse bacillus grown in litmus-milk forms very little acid, so that the colour of the medium is only very faintly changed and the medium becomes alkaline shortly after. With the strain grown from case XII the colour of the litmus-milk was changed to red in 12 hours and has remained so for over two months. There was no difference otherwise between this germ and Kruse's bacillus: the milk was never coagulated, and no formation of gas took place in sugar agar. The behaviour of this strain as regards agglutination was very interesting. It was very well agglutinated by the blood of the patient, but it was never agglutinated by the blood of

any of the other cases of dysentery I tested with it. The blood of case XII never agglutinated any other strain of dysentery bacillus to any appreciable degree, as will be seen by reference to the following table.

TABLE I. *Blood from Case XII. Agglutination with different strains of dysentery bacillus.*

Date from onset of illness	Strain isolated from stools of patient XII.	Strain from Germany	Strain from Case I.	Strain from Case III.	Strain from Case VII.
3rd day	1 : 10	–	–	1 : 10	–
7th day	1 : 30	–	–	–	–
10th day	1 : 100	–	–	1 : 10	–
18th day	1 : 100	–	–	1 : 10	–

Time of observation 2 hours.

1 : 10, 1 : 20, etc., means that the agglutination reaction is positive up to a dilution of the blood of $\frac{1}{10}$, $\frac{1}{20}$, etc. The sign "–" means that with a dilution $\frac{1}{10}$ the reaction was negative. A less dilution than $\frac{1}{10}$ was not tried.

Bacilli similar or possibly identical to that of case XII have been isolated by Kruse in cases of a peculiar form of dysentery which is endemic in many asylums in Germany. Kruse has called such organisms *Bacilli pseudodysenterici*: the existence of these bacilli has been denied recently by Vedder and Duval, though they have been found again by other authors. These bacilli, according to my experience in Germany, are non-pathogenic to rabbits, but in my case here in Ceylon the strain was pathogenic to rabbits in the same way as the true dysentery bacillus. The subcutaneous inoculation of 0·5 c.c. bouillon culture of the strain from case XII was sufficient to kill rabbits in 4 to 7 days. At autopsy the intestinal mucosa showed marked congestion but no ulcers. I think that as there are cases of paratyphoid —clinically indistinguishable from typhoid (though some authors have pointed out a few slight differences), but due to organisms very nearly related to the *Bacillus typhosus*—so there are cases of dysentery, practically indistinguishable from the ordinary ones, but caused by bacilli very nearly related to the *Bacillus dysenteriae*. Such cases might be termed in analogy Paradysentery and the germ *B. paradysentericus*, of which most probably there are different strains.

Amoebic Dysentery in Ceylon.

From cases VI and XVII neither *B. dysenteriae* nor any organism resembling it could be grown. The intestinal flora was represented by *B. coli* and some cocci. No spirilla were present. In case VI, which was

studied by me only at autopsy, the heart blood was found not to agglutinate any strain of dysentery or paradysentery bacilli. In case XVII the blood was tested several times during life with several strains of dysentery and with the strain of paradysentery always with negative results. The faeces of both cases contained a very large number of amoebae. Both cases presented abscesses of the liver. The pus of the liver abscess of case VI was sterile. Amoebae could not be detected. The pus of the liver abscess from case XVII could not be examined. It is well known how many discussions have taken place on the question of the existence of an amoebic form of dysentery. If authorities like Kartulis, Kruse, Councilman, Manson, Koch, etc., admit it, other authorities like Grassi and Celli deny it. The authors who do not admit the existence of an amoebic form of dysentery base their opinion especially on the fact that amoebae may be found in the stools of perfectly normal persons or in people suffering from other diseases. This is certainly correct, though here in Ceylon out of more than 150 examinations of faeces I have been able to find amoebae only in cases VI and XVII, which I consider cases of amoebic dysentery. In all the cases of bacterial dysentery so far I have never found amoebae.

Does the so-called *Amoeba coli* represent one species only or several species? Celli distinguishes several species of amoebae inhabiting the human intestine; Kruse and Pasquale admit two different species— a harmless one living in the intestine of healthy persons, and a pathogenic one which is the cause of a form of dysentery. Schaudinn recently, after some very interesting experiments, came to the conclusion that under the denomination of *Amoeba coli* two species of amoeba, different morphologically and biologically, are included. The two amoebae, according to this author, present such important differential characters that they might almost constitute two different genera. He has called the one species, which is harmless, *Entamoeba coli* Lösch; the other, which is pathogenic, *Entamoeba histolitica*. Schaudinn states that a distinction between ectoplasm and endoplasm is very difficult in *E. coli*—impossible in the resting amoebae. The nucleus is clearly defined, very distinct and contains several nucleoli. Reproduction takes place by fission and by formation of cysts, each containing eight nuclei. On the other hand in *E. histolitica* the nucleus is very indistinct, and often absolutely invisible. Reproduction takes place always by fission—never by formation of cysts presenting eight nuclei, so characteristic of the *E. coli*.

In my two cases (VI and XVII) I noticed that the amoebae

presented a very indistinct nucleus—often quite invisible—and I never observed cysts with eight nuclei. These two characters would point to its being the *Entamoeba histolitica* of Schaudinn.

Presence of other Protozoa in the Intestine of cases of Amoebic Dysentery.

In my cases VI and XVII—besides many amoebae—a large number of *Trichomonas intestinalis* were present. The most of them were of large dimensions, with a pear-shaped or roundish body and very actively motile.

In case XVII, in addition to many Trichomonas, I observed several examples of another form of protozoon (see Figure). This was of some-
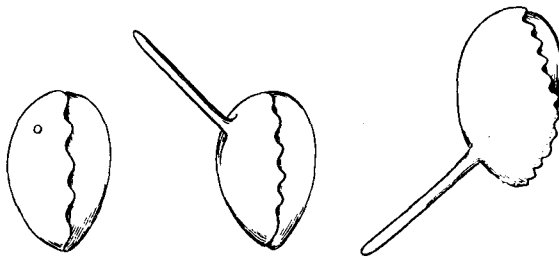
Appearance of protozoon (*Entamoeba undulans* Cast.) at different intervals—(semi-diagrammatic) stools of Case XVII.

what larger dimensions than *T. intestinalis*, the maximum diameter reaching from 18 to 30 μ. The usual shape was oval. There was an absolute absence of flagella. The organism presented a continuous rapid undulating movement from one to the other extremity of its body, and always in the same direction, this pointing to the presence of an undulating membrane. Now and then at an interval of 15–20 seconds a very narrow long pseudopodium was shot out from the body. Only one pseudopodium was emitted at a time. The pseudopodium was emitted very quickly, and very quickly retracted. The pseudopodium was sometimes protruded from one part of the body and sometimes from another part. The organism had a finely granulated protoplasm; a differentiation between ectoplasm and endoplasm apparently did not exist, the protoplasm being practically of the same character throughout. In a few individuals something like a very indistinct nucleus could be observed, but in most of the specimens no nucleus at all could be seen.

One small vacuole was often present—seldom more than one. The vacuole never possessed the characters of a contractile vacuole. The position of the vacuole varied. The protoplasm never contained red blood corpuscles, but often some bacteria and granules.

Preparations stained by different methods were far from being satisfactory. The sudden death of the patient prevented a more thorough investigation of this protozoon. Apparently it was not a Cercomonas or a Trichomonas, as flagella were invariably absent. Nor was it one of the amoebae usually met with in the human intestine, seeing that the organism possessed an undulating membrane, and the shape and the mode of emission of the pseudopodia were different from what one sees in *Amoeba coli*, in which the pseudopodia are of large dimensions, blunt, and emitted and retracted rather slowly. In case XVII both organisms and also a large number of Trichomonas were present, rendering comparison between all of these parasites easy.

CONCLUSIONS.

From my investigations it would appear that in Ceylon there are several forms of dysentery:

(1st.) By far the most frequent one is the bacterial form due to the *Bacillus dysenteriae*, Shiga and Kruse.

(2nd.) A rare form is due to bacilli very nearly related to the typical Shiga-Kruse bacillus. Such a form might be called para-dysentery in analogy to paratyphoid—leaving the term pseudodysentery to denote forms of disease absolutely different from dysentery, as for instance, pseudodysentery from Bilharzia (see Low and Castellani, *Zeitschr. f. Schiffs- u. Tropen-Hygiene,* 1904, Band VIII).

(3rd.) A third form of dysentery is represented by amoebic dysentery; and the species of Amoeba which causes it in Ceylon is probably *Amoeba histolitica* Schaudinn. This form of dysentery is apparently rare in the island.

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