

Report on the pathology and etiology of Asiatic cholera / as observed in Spain in the summer of 1885, by C.S. Roy, J. Graham Brown and C.S. Sherrington, and in Italy in 1886 by C.S. Sherrington.

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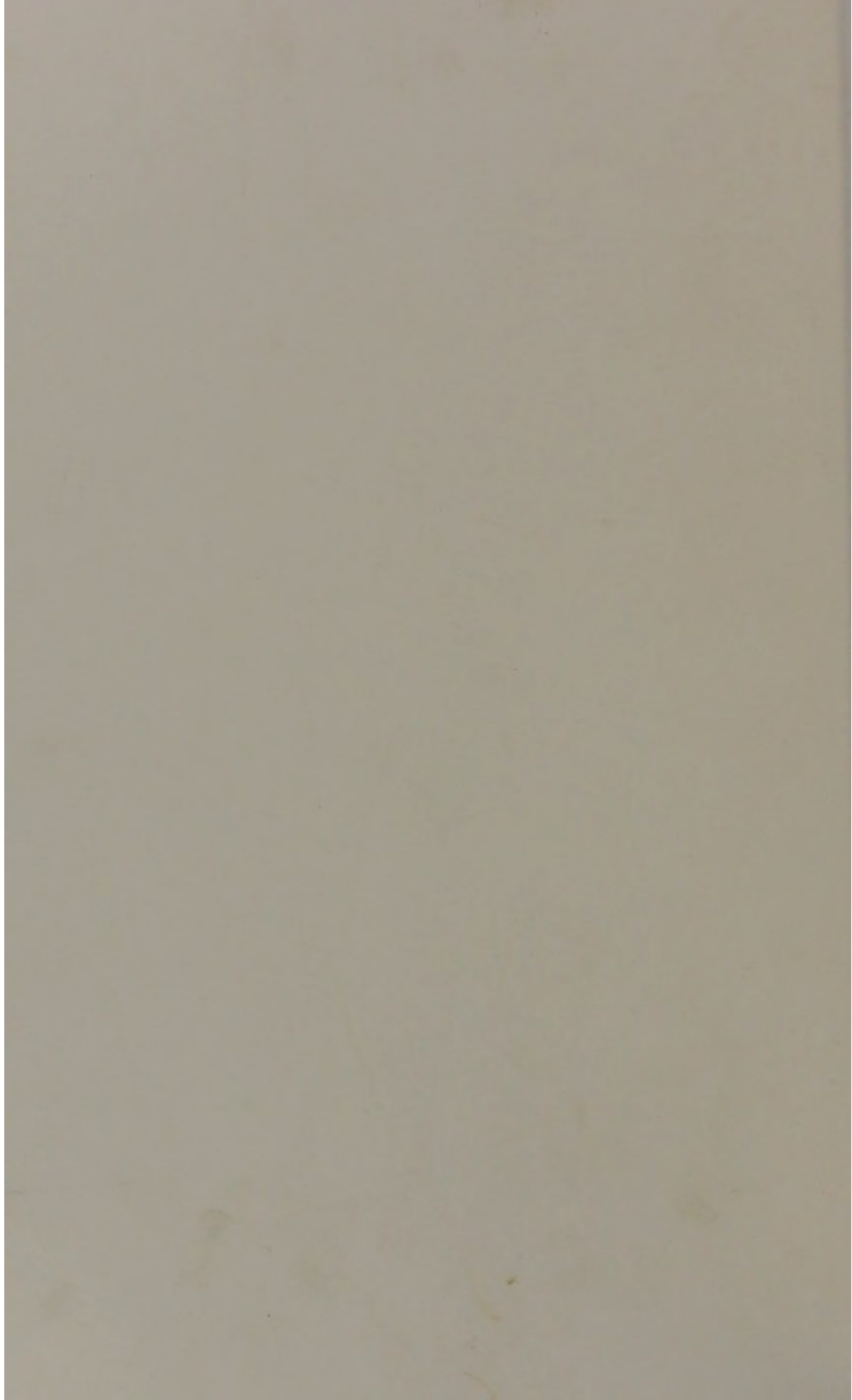
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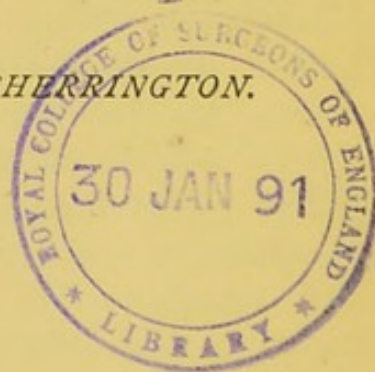
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REPORT
ON
THE PATHOLOGY AND ETIOLOGY
OF
ASIATIC CHOLERA

AS OBSERVED IN SPAIN IN THE SUMMER OF 1885.

BY
C. S. ROY, F.R.S., &c.; J. GRAHAM BROWN, M.D., &c.
AND C. S. SHERRINGTON, M.B.

AND IN ITALY IN 1886 BY C. S. SHERRINGTON.



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REPORT
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THE great advances which have been made of late years in our knowledge as to the relationship of micro-organism to disease, and the important advantages to medicine and to public health which have been derived therefrom, induced the German Government to send a scientific commission to Egypt in the summer of 1883 to investigate the pathology of cholera, which was then present in that country. The principal member of that commission was Prof. R. Koch, of Berlin. While still in Egypt, Koch made known that he had discovered a micro-organism belonging to the Schizomycetes, which he stated to be constantly present in the dejecta of cholera cases, as well as in the intestinal contents and mucosa of the intestine of those fatal cases which he examined after death. This parasite, now well known as the comma bacillus of Koch, grew, as he showed in a very characteristic manner, when cultivated on sterilized nutrient gelatine. This bacillus was claimed by Koch as the direct cause of Asiatic cholera, a view which was strengthened by the further investigations which were made by Koch in India, and by the results obtained by the administration of small quantities of artificially cultivated comma bacilli to certain of the lower animals. The number of comma bacilli, as compared with that of other organisms, present in the contents of the intestine, corresponded, he asserted, with the severity of the attack.

The Egyptian cholera epidemic was investigated by a Com-

mission sent by the French Government on the recommendation of the Academie Française. The members of this Commission were MM. Strauss, Roux, Nocard, and Thuiller. The report published by the French observers by no means confirmed the investigations of Koch; but M. Strauss (although while in Egypt the French Commission did not observe any special relation between the comma bacillus and cholera) was able to find comma bacilli in the intestinal contents of several fatal cholera cases at Toulon in the summer of 1884. He was not, however, prepared to accept Koch's conclusion as to the causal relation between the comma bacillus and cholera.

Van Ermengen and Watson Cheyne, who investigated the subject in France in 1884, confirmed the observations of Koch, although the latter investigator was unable to convince himself of the presence of the comma in the substance of the intestinal mucous membrane. Nicati and Rietsch obtained results which upon the whole accord with those of Koch.

The evidence adduced was, on the other hand, attacked by various members of the Indian Medical Service, especially by the late Dr. T. Lewis, who pointed out that an organism similar to that found by Koch in cholera was to be found in the mouth of healthy persons. Since then Finkler and Prior have found a comma bacillus in the dejecta from persons suffering from cholera nostras. These observers showed, moreover, that the bacillus found by them, when cultivated in nutrient gelatine, grows in a manner very similar to the comma bacillus of Koch. It has, however, since then been, we believe, admitted generally that there are certain differences, both in the size and mode of growth in gelatine of the comma bacilli described by Koch and by Finkler and Prior respectively.

Various other forms of curved bacilli have recently been found in decayed teeth, in cheese, &c. None, however, of these have been conclusively shown to be identical with Koch's bacillus. The artificial production of cholera, which Koch claims to result from the administration of pure cultivations of his comma bacillus in guinea-pigs, has been the subject of much discussion. The negative results at first obtained by Koch on administering comma bacilli by the mouth might be explained, he affirmed, by the destructive effect of the acid gastric juice upon the micro-organism in question. When the bacilli are introduced into the

duodenum directly by means of an abdominal section, diarrhœa and death result in a certain proportion of cases, which, when examined after death, show comma bacilli in enormous numbers in the contents of the intestine. That death in these cases is not the result of septicæmia has been, we think, conclusively proved by Koch. That the disease so produced is identical with cholera in man we see, however, no reason to believe; there being, as brought out by Klein and Gibbes, various important points of difference in the signs and symptoms of the two diseases. The negative result which followed swallowing pure cultures of comma bacilli in the experiments made by Klein on himself, and certain of the lower animals, do not appear to us conclusively opposed to Koch's theory on the subject, seeing that no sufficient precautions appear to have been taken to prevent the destruction of the micro-organism in the stomach by the gastric juice.

Klein and Gibbes, who proceeded to India in 1884, at the request of the India Office authorities, to investigate the pathology of cholera, obtained results strongly opposed to those of Koch. While admitting that the comma bacillus was present in the intestinal contents of the large majority of fatal cholera cases, they have not been able to find that micro-organism constantly present in the mucosa of the intestine. Where it was present in that tissue its situation and other considerations pointed in their minds to the probability that it had penetrated from the free surface after death. Klein's cultivations of the comma bacillus, and his experiments on the lower animals with such cultivations, led him to believe that the parasite in question is not pathogenic.

The somewhat embittered controversy as to the etiology of cholera between Koch and his supporters and those opposed to him, naturally left those who had had no opportunity of investigating the matter personally in some doubt as to the facts of the case, and it was felt desirable that further research should be made upon the subject.

The Spanish cholera epidemic of last year gave an opportunity of obtaining material without its being necessary to proceed to India, and we were aided by the Association for the Promotion of Medicine by Research, acting in conjunction with the Royal Society and the University of Cambridge, to investigate the question in Spain.

After losing some time in an unsuccessful attempt to obtain material in Aranjuez, we were able to make autopsies of fatal cholera cases in Madrid itself, where we were able to pursue our inquiry under fairly satisfactory conditions. Owing to the efficient support which we received from the staff of the British Legation in Madrid, and especially from Mr. William Macpherson, the British Consul, we were able to obtain free access to the cholera hospital, and to make satisfactory post-mortem examinations of twenty-five fatal cases of Asiatic cholera.*

At every post-mortem examination we had with us a large number of freshly drawn capillary pipettes, with which we invariably took from different parts of the unopened intestine specimens of the contents, to be employed in making plate and test-tube cultivations on our returning to our temporary laboratory. We also ligatured off small loops of the intestine at different parts, which we took with us in sterilized glass bottles, and the contents of which, when opened at our place of work, were employed in making cultures and microscopic specimens.

In order to obtain specimens of the different tissues and organs as free as possible from any post-mortem putrefactive changes, we always carried to the hospital a large number of wide-necked phials containing absolute alcohol, into which portions of the

* The autopsies were in nearly all cases made between 5 and 6 A.M., at an hour therefore when the temperature even in Madrid in midsummer is not high; while most of the cases we examined were of persons who had died the preceding night. Some of our autopsies were made on persons who had died while we were within the precincts of the hospital, and whose bodies we were able to examine *immediately* after death. The majority of our post-mortem examinations were made within a few hours of death. It should be mentioned that the treatment employed in most of the cases examined after death by us was either by opiates given by the mouth or hypodermically, or by intravenous injections of aqueous solutions similar to those employed at Marseilles in the previous year. We found that these latter introduced a source of possible error which it is necessary to refer to. In some of these cases we found red patches upon the skin, small areas of coagulation necrosis in various organs, and micrococci or bacilli present in large numbers in the blood; showing that these patients had suffered from septicæmia, presumably owing to the fluids injected into the veins not having been boiled before use. These were, however, cases of septicæmia supervening upon cholera, the characteristic post-mortem appearances of the latter being practically as well marked in the majority of our cases in which no septicæmic infection had been produced.

large intestine, kidney, liver, spleen, &c., were placed while the autopsy was being made.

Regarding the microscopic changes found after death we have nothing to add to what is already known on the subject. In all of our cases there was thick dark blood in the vessels, the dryness of the tissues, the very characteristic sticky change in the serous surfaces, and the ill-defined, patchy, livid colour of the small intestine, which at once struck the eye on opening the abdomen. The intestines contained either the rice-water contents so characteristic of cholera, or contents equally thin, showing comparatively few flocculi. The solitary glands and the Peyer's patches were in most cases swollen and of a greyish-white opalescent appearance, but none of our cases showed any ulceration in the small intestine. In one or two cases, however, there were congestion and scattered ulceration in the large intestine.

With regard to the morbid histology of cholera, as well as the microscopic appearances of the disease, our observations are fully in accord with the classical description given by Strauss, Roux, Nocard, and Thuiller.*

Our cover-glass preparations of the intestine—*i.e.*, of the fluid part as well as the mucous flakes—were stained in various ways; Gramme's method, methylene blue, Spiller's purple, and fuchsin being the principal dyes employed. Much time was occupied in making these preparations, but the results obtained may be stated very briefly. In four or five of our twenty-five cases, dried and stained films, prepared from the contents of the lower part of the small intestine, showed little besides comma bacilli. In the majority of our cases, however, commas were found only in comparatively small numbers; while in six or seven of our cases we were unable to find any comma bacilli in the preparations made. In these latter cases we failed to find any comma bacilli in films prepared from different parts of the small intestine.

We cannot, of course, say that had our search for the commas in the contents of the intestines in these cases been still more extensive, commas would not have been found. We can only say we spent much time in examining the films from these cases, and have no hesitation in saying that comma bacilli, if present, must have been in very small number in proportion to other micro-

* *Archives de la Physiol. Normale et Pathol.* 1884.

organisms—certainly much less than one comma to a thousand of other forms. It may be added that when we went to Spain we expected to find comma bacilli discoverable in the intestinal contents of all fatal cholera cases, and also that these bacilli would be present in larger number than that of other micro-organisms. This, in spite of careful and conscientious work, we distinctly did not find. With regard to the other micro-organisms present in the intestinal contents of our cases, we were at first led to think that a small diplococcus was especially frequently present, and indeed throughout all our cases this diplococcus was sufficiently often met with to lead us to think that it might have some possible bearing on the etiology of cholera; some of our films, indeed, prepared from the contents of the intestine, show little besides the diplococci referred to. Our further researches, inoculations, &c., are, however, opposed to the view that there is any more than an accidental connection between this diplococcus and cholera asiatica.

With regard to the straight bacilli which present themselves in the contents of the intestine in fatal cholera cases, these we found to present numberless varieties in form and dimensions; but it need hardly be said that these it is impossible to identify with forms already known by the characters under the microscope alone. Our films may therefore contain the straight bacilli described by Strauss, Emmerich, Klein, and other observers. The micrococci met with in films prepared from the intestinal contents of fatal cholera cases show great variety in size and grouping, but, like the bacilli, they cannot safely be identified by these characters alone, as seen under the microscope.

These films we found presented structures stained with the dye employed, and which present certain resemblances to a micro-parasite which we have found in the substance of the mucous membrane of the intestine, as described below.

The appearances given, however, are not well marked enough to enable us to satisfy ourselves that the contents of the intestine contain the same parasite as we have found in the mucosa of fatal cholera cases.

Our plate cultures from the intestinal contents were made in the usual way, by mixing a drop of the contents of the intestine with about an ounce of sterilized $\frac{3}{4}$ per cent. saline solution, or with sterilized nutrient chicken broth, from which mixture, after

shaking, a drop was transferred in the usual way by a sterilized pipette to another quantity of sterile salt solution, and so on until sufficient dilution had been produced. The quantity of such a mixture requisite was then taken to mix with some fluid nutrient gelatine, which after resolidifying in a sterilized glass vessel, was protected from the access of germs from the air of the room. The temperature of Madrid in midsummer is, we need hardly say, such that gelatine will not remain solid in ordinary rooms during the day. This difficulty we met, however, by keeping the gelatine plate cultivations in a cellar that was comparatively cool; there the temperature never rose so high as to liquefy the gelatine. Our cultivations in agar-agar jelly we kept for the most part in the incubator, the temperature of which was always between 37° and 40° Centigrade. The plate cultivations we made gave results fully in accord with the results observed in the films prepared for the microscope as above described. In our plate cultivations also we found an unexpected scarcity of Koch's comma bacilli. Speaking roughly, it may be said that the proportion of comma bacilli to other forms was the same as the relative proportion found under the microscope in our cover-glass films. We may well add here, that great care was taken to make our gelatine and agar-agar culture media slightly alkaline, and that in other respects also we followed implicitly the processes described by Koch.

The results obtained from microscopic examination and cultivation of the intestinal contents of the fatal cholera cases examined by us are strikingly opposed to those found by Koch. Even Klein and Gibbes found the comma-bacillus more constantly present in the intestinal contents of cholera cases than we have been able to do. Our observations on the micro-organisms to be found in the tissue of the intestinal mucous membrane of fatal cholera cases are also at variance with those of Koch. In other words our results confirm fully those of Strauss and Klein as to the absence of any comma bacilli in the mucosa of the lower or other parts of the small intestine in the great majority of cholera cases. In most specimens of the wall of the small intestine from cases of cholera asiatica, the epithelial covering of the villi becomes detached during the course of preparation, so that we cannot speak as to the frequency with which the comma bacillus is present in the epithelium. In the comparatively few cases in

which we found these bacilli in the tissue of the mucosa, the situation was always either close to the free surface, or else in or close to the tubules of Lieberkühn's glands; that is, in parts to which they might readily have entered either after death or during the few hours preceding death, when the patient was lying in a moribund condition. We cannot confirm Koch's statement of their number present in the mucous membrane bearing any definite relation to the acuteness or severity of the attack. We need not say more on this subject. The small numbers of the comma bacilli to be found in the contents of the intestine in fatal cholera cases, and their apparently complete absence in many cases of cholera, together with the fact that we very rarely found them in the substance of the mucous membrane, make it impossible for us to accept absolutely the conclusions arrived at by Koch as to the causal relation between his comma-shaped bacilli and Asiatic cholera. Koch's theory of the relation of the comma bacilli and cholera was based—at first at least—entirely on the supposed constant occurrence of the parasite in the contents of the intestine and in the mucous membrane.

If his observations on these points are mistaken, as our own researches on the subject oblige us to conclude, we cannot see on what sure basis his theory rests.* Koch, by experiments on the effects of cultures of the comma bacillus when introduced into the small intestine of certain lower animals, has obtained facts which have had considerable weight in bringing about the general acceptance of his theory. We need not enter in detail into these experiments, seeing that, even accepting, as we are quite prepared to do, the accuracy of the facts observed by him, and admitting that the fatal results which he obtained in a proportion of his cases were not due to septicæmia, we cannot satisfy ourselves that the animals died from a disease identical with Asiatic cholera.

* As very strong views have been and are held by many pathologists both for and against Koch's theory of the etiology of cholera, we feel it possible that we may be suspected of having been biassed against his views from the first. This was not, however, the case, each of us being, we believe, in doubt as to the conclusions that might be drawn from the observations of Koch, v. Ermengen, Watson Cheyne, Nicati, and Rietsch, &c., on the one hand; and those of Strauss Klein, Emmerich, and others, upon the other hand. We felt and feel strongly that the observations of so brilliant an investigator as Koch, whose previous discoveries have successfully withstood the test of time and virulent attacks, deserve greater respect than has sometimes been accorded them.

Fatal diarrhœa, which is all that he describes, is a different thing from the very characteristic phenomena which constitute Asiatic cholera. Moreover, until comma bacilli are proved to be a cause of Asiatic cholera, their presence in vast numbers in the intestinal contents of the fatal cases in these experiments cannot, we imagine, be accepted as proof that the disease of which the animal dies is identical with cholera.

One of our number (S.) was deputed by the University of Cambridge to investigate the method of protective inoculation against cholera which was devised by Dr. Ferran of Valencia. We had not an opportunity of meeting that gentleman, nor did we think it advisable, considering the repulse received by Dr. Brouardel, to expend precious time in following the movements of Dr. Ferran, who was travelling from place to place; all the more so as we had plenty of opportunity of observing the manner in which his inoculations were carried out, and were also able to examine with the microscope some of his cultivations. At Aranjuez especially—whither a large quantity of his culture-fluids was sent under charge of a duly authorized agent of his, at the request of the Spanish Government, to inoculate the soldiers quartered in that town—we had opportunity of examining for ourselves the micro-organisms which these cultures contained. We saw none of the large peronospora-like bodies described by Dr. Ferran in the article by him in the *Archiv für Klinische Medicin*, nor indeed did we find any comma bacilli. The cultures which we saw were impure, containing bacilli of several sizes, as well as more than one kind of micrococcus. These cultures were employed with the object of protecting the troops at Aranjuez against cholera.

The fact that such impure cultures could be employed by Dr. Ferran's agents make in our opinion the conclusion which he has drawn from his statistics utterly untrustworthy. As we did not meet Dr. Ferran, we have no right to say that the cultivations of the comma bacillus made by him did not show the modifications which he has described in the paper above referred to, written by him. The cultures employed for inoculating purposes may have been pure when they left his hands; but those which we saw, and which were employed, had suffered contamination, and the vessels, moreover, in which they were contained were such as rendered contamination during transit practically unavoidable.

From various sources we have reason to believe that some of the changes in form described by Dr. Ferran to occur in the comma bacillus—namely, the small rounded swellings in the course of a spirillum—may present themselves under certain conditions. That the larger peronospora-like bodies are ever developed from the comma bacillus receives no support from any of the facts which have come to our knowledge. We are inclined to think that the small rounded swellings are the result of degenerative changes, analogous to those which occur in certain conditions in anthrax and some other bacilli. We need not say more than that the facts which came to our knowledge, both as to the accuracy of Ferran's observations and the value of his method of inoculation, are entirely opposed to the statements made by Ferran and his supporters. That inoculation made with impure cultures, such as we saw, must expose the recipients to serious risk of septic infection, is self-evident; and we heard of persons suffering from what we believe to be septicæmia after inoculation by Ferran's method. We had, however, no opportunity of observing such a case.

With regard to the straight bacilli found by Emmerich and Klein in their investigations into cholera, we may say that Dr. Shakespeare of Philadelphia has informed us that their difference in size makes it practically certain that they are different forms of micro-organisms. We have not been able to confirm the observations of Emmerich as to the relation of the straight bacillus found by him with Asiatic cholera. He was unable to detect the bacillus in question in microscopic preparations of the tissues from cholera cases, and could only discover it by placing portions of kidney, &c., or blood taken from cases of cholera, in nutrient cultivating media. We can only say that the cultivations we attempted to make from the blood, and kidney, and liver of cholera cases gave no results. A similar negative result has been obtained by others; and we see therefore no reason for supposing Emmerich's bacillus bears any constant relation to Asiatic cholera. It must, we imagine, be looked upon as a septicæmic form of pathogenic micro-organism accidentally present in the tissues in certain fatal cholera cases. This view, we think, the effects obtained by Emmerich by inoculations on the lower animals do not exclude.

A small straight bacillus, found by Klein so frequently present in the contents of the intestine and in the mucosa, we have not been able to identify with certainty in our own preparations.

The results recorded above do not permit us to accept of the views either of Koch or of Emmerich as to the cause of Asiatic cholera; they are therefore purely negative. Feeling as we did, however, that there are many facts which point to the etiology of cholera being best explained by the action of some form of pathogenic micro-organism, we employed all the most recent methods with which we are acquainted to discover such parasites in the blood, tissues, and intestinal contents. Examination of the films prepared from the intestinal contents did not appear to us likely to give satisfactory information on the subject, seeing that so many forms of micro-organisms are present in them, and also because the evidence that the poison of cholera is constantly present in the dejecta is by no means convincing. The pathology of cholera makes it, we think, in the highest degree improbable that it is due to the action of an irritant contained in the intestine. Were such the case we should expect to find either catarrh or ulceration of the mucous membrane. If, on the other hand, the phenomena of cholera were due to some poisonous alkaloid formed in the intestine, we should expect that its absorption would lead to other organs being affected as well as the intestine. That other organs besides the intestinal tract are affected in cholera is of course well known; but the dominant feature in the disease is certainly the hyper-secretion by the mucous membrane of the digestive tract, and the diminution or arrest of absorption by that mucous membrane—two conditions which are quite fitted to cause the cerebral symptoms, the fall of temperature, cramps, &c. The pathology of the disease is therefore centred in the change in function of the digestive tract. The chemical characters of the dejecta from cases of Asiatic cholera, which were investigated by Kühne and others, are entirely opposed to the view that the hyper-secretion from the mucosa is of a catarrhal nature. Fatal cholera cases are, we are well aware, met with in which the signs of local irritation and catarrh of the intestinal mucous membrane are well marked. In some cases indeed deep patches of necrosis and ulceration are found. Such necrotic and catarrhal changes of the mucous membrane are by no means constantly met with, although they appear to be more frequently present in some epidemics than in others. For example, in our Spanish cases ulceration and catarrh were very rarely met with, while in the sixteen cases from Italy which we have since examined ulceration

and sloughing were very much more frequently found. The absence of signs of catarrh in many of our cases is in our minds a proof that it is not a constant and essential part of the choleraic process. On the other hand, the hyper-secretion from the mucous membrane of the intestine can be produced artificially with chemical characters practically identical with those of choleraic evacuations. The artificial hyper-secretion referred to is produced by Moreau's experiment of section of the nerves accompanying the mesenteric vessels supplying a loop of the intestines, the contents of the intestine having been previously pressed out, and the loop isolated by ligatures from the rest of the intestinal canal. In a few hours after this operation, which we have repeated, the loop of intestine is found distended with a watery fluid containing mucous flocculi, and which in appearance is identical with the rice-water stools from cases of Asiatic cholera. The intestinal wall in these cases is somewhat congested, but does not show inflammatory changes. If this paralytic hyper-secretion, which occurs after section of the nerves in Moreau's experiment, can, as we believe it may, be considered almost, if not absolutely, identical with the hyper-secretion which takes place in Asiatic cholera, we should expect that the cause of the latter, if of the nature of an organized ferment, would be found in the mucosa itself, or in the nerves supplying the mucosa, and not in the contents of the intestine.

From reasons such as those above stated, we thought that the wall of the intestine was more likely to contain the as yet unknown micro-parasite which causes cholera, if such exist, than the contents of the intestine. Examination of films prepared from the intestinal contents we thought less likely to be satisfactory, partly because so many forms of micro-organisms are present in the intestinal contents, and partly because the evidence that the virus of cholera is constantly present in the dejecta, is by no means conclusive. At first, and indeed for a considerable period, the results which we obtained from examination of the tissues stained in various ways were purely negative. The absence of the comma bacillus in the mucous membrane of the intestine in the great majority of our cases we have already referred to.

Besides this we observed one characteristic of the sections taken from our Spanish cases which attracted our attention—viz., that in many of our microscopic preparations of intes-

tine, which had been stained by fuchsin after the method described by Weigert, there were to be seen granules stained red as deeply as the nuclei of the cells of the tissue, but which differed in appearance from the granules which have been produced by the excessive division or breaking up of the nuclei in some specific inflammations. These granules, moreover, were characterized by having one or two processes from them which were unstained or faintly stained, and which could not therefore be followed amongst the tissue elements. These granules did not arrest our serious attention until, in a section of the kidney from our fifteenth case, we saw them in a situation which enabled us to trace the course taken by the processes referred to. Lying in the lymph space outside the membrana propria of one of the tubuli contorti were five or six granules of the kind above mentioned, and we were able to see against the clear background the arrangement of the processes attached to them. Some of the granules appeared as swellings either in the course or at the end of a single filament, while others formed nodes from which the filaments branched off. This characteristic structure we were unable to recognize as resembling in appearance either the nerves or connective tissue found in the normal or diseased kidney. On trying carefully the various staining methods known to us in which methylene blue is employed, we found that Löffler's solution, followed by weak acids, gave results which were at least fairly satisfactory. With this method of preparation both granules and filaments of the mycelium-like structure can with care be stained sufficiently deeply to enable them to be seen among the tissue elements. When satisfactorily stained with methylene blue there can be no doubt as to the structure referred to being a vegetable parasite of some kind. Having found a staining method fitted to show the existence of this parasite when present in the tissues, we proceeded to search for it in tissues taken from all of our cases *seriatim*. In all of our twenty-five cases we were able in the mucous membrane of the small intestine to find micro-organisms resembling that above referred to. We found them to differ somewhat in appearance in different specimens, but they were all of the character to be presently described, resembling one another sufficiently to give us no reason for suspecting that we had to do with more than one parasite. Even in those of our

cases in which septicæmia had been produced by intravenous injection, we were able to find this micro-organism in the substance of the intestinal mucous membrane, in addition to the septic micro-organisms present in the blood-vessels.

As to the characters of the micro-organism it may be said to consist of rounded or triangular granules, situated either at the extremity or on the course of filaments of varying thickness. There is no appearance of division into component cells, nor do any of our preparations show zoosporangia. Some of our preparations show fine filaments forming at one part a close network between the cells of the tissue. They are sometimes so fine that they appear, when examined with $\frac{1}{18}$ Zeiss' immersion, as simple lines. On the other hand, the mycelium-like threads which connect the granules vary considerably in thickness in different specimens. They usually show irregular moniliform thickenings at different parts of their course. The distance which separates the granules belonging to an individual micro-organism (the length of the mycelium-like threads) varies considerably; in some specimens the granules are placed quite close one to another, whilst in others the mycelial part is the more prominent feature. The granules vary in size very much, even in the same micro-organism, the largest being rather above the size of a human coloured blood corpuscle; others (smaller) appear simply as little knobs, causing a local thickening of the mycelium, and between these all varieties of size are met with. When deeply stained by methylene blue, small, round, darkly stained bodies may be seen in the filaments and swellings of many of the structures, and also occasionally small, round, unstained parts, suggesting a possibility of spores being contained in them. With Löffler's method, both mycelium and swellings are coloured to an equal extent; but when stained with fuchsin, followed by dilute nitric acid, the mycelium loses the dye sooner than the swellings.

One of the sources of possible error to which we naturally turned our attention was the possibility of accidental contamination after death. That this parasite could not have penetrated from the free surface of the mucous membrane may safely be concluded from, firstly, their frequent position quite deep in the mucosa—often, indeed, close to the muscularis; and secondly, because some of our autopsies were made *immediately* after death. Regarding the precautions taken to avoid contamination after

removal of the specimens and while they were being prepared for examination, we may mention that the tissues were placed in absolute alcohol while the autopsy was being made, which alcohol was afterwards changed at sufficiently frequent intervals; the mucilage in which they were placed before being cut was boiled, and had dissolved in it as much thymol as it would take up; the other fluids—*e.g.*, methylene blue solution, eosin solution, through which the sections were passed—were also boiled, and mixed with thymol solution. The precautions above referred to seem to us sufficient to exclude the possibility of the micro-organism found by us being due to accidental contamination. This being the case, the question arises as to the relation of this micro-organism to cholera asiatica.

The constancy with which these parasites was met with in the wall of the small intestine from our Spanish cases naturally led us to suppose that it might have some casual connection with Asiatic cholera, as will be found stated in our preliminary report to the Royal Society (Proceedings, 1886). Before publishing our final decision on the subject, however, we delayed the printing of this report until we had examined the material obtained by one of us (S.) in Italy during the summer of 1886. This latter material, which was treated in exactly the same way as that obtained in Spain, does not contain the micro-organism above described. We are therefore forced to conclude that it is not to be found in all cases of Asiatic cholera.

NOTE.—*Dr. E. Klein has recently, in letters to the LANCET, NATURE, BRITISH MEDICAL JOURNAL, PRACTITIONER, &c., asserted that the parasite described by us is nothing more than common mould, the appearance of which in our specimens he states to be due to imperfect preservation.*

We need not answer seriously an attack of this kind, in which assertion supplies the place of evidence. We may say, however, that we took proper precautions to prevent contamination of our material as detailed above, and also that we are perfectly familiar with the appearance of common mould in the tissues, and that the reactions to staining fluids of the micro-organism described by us, and its appearance as seen under the microscope, make it impossible for any one who has gone over our material to confound for one moment this parasite with common mould.

Mr. Gardner, who at first led us to suppose that the parasite in question belonged to the chytridiaceæ, has since modified his views on the subject believing that it is an involution form of some bacillus, a view which seems to be accepted by Mr. Crookshank.

As to what conclusions may be drawn from the constancy of its occurrence in our Spanish cases, we prefer in the meantime to withhold our opinion.

While in Spain, within reach of cases of cholera, we found it absolutely impossible, both from the time involved in making cultivations of the enormous number of micro-organisms which we found in the intestines of cholera cases, and from the limited appliances at our disposal, to follow out the life-history and investigate the physiological characteristics of all the micro-parasites met with.

We thought it on the whole wisest to cultivate only the Schizomycetes, assuming, perhaps somewhat hastily, that it was *à priori* improbable that the unknown parasitic cause of cholera could belong to any of the other forms of micro-parasites. When, therefore, in any of our plate cultures we found colonies of growths which were not bacteria, bacilli, or micrococci, we did not seek to make test-tube cultivations from them; and indeed, unfortunately, save in a few cases, we omitted even to make very minute microscopic examinations of them.

As to the question whether this parasite is usually or constantly present in the intestinal contents and dejecta of cholera patients, that is a matter with regard to which we have not been able to satisfy ourselves. Some of the films taken from the contents of the intestine show structures presenting a certain resemblance to the micro-organism found by us in the substance of the mucosa, but none of our films have been found to show structures unmistakably identical with those in the tissue itself.

We have only to add that care is required to stain this parasite in the tissue successfully by Löffler's method, but that when properly stained it is easily found with a good $\frac{1}{12}$ immersion lens (Powell and Lealand's $\frac{1}{12}$ oil is the lens we have chiefly employed). In the vast majority of our cases every section shows three or four specimens of the micro-organism, the number present appearing to us to go hand in hand with the extent of the histological changes in the structure of the mucous membrane. In some cases the parasites are fewer in number, but it is rare to find a section from the lower part of the small intestine which, when satisfactorily stained, does not show one of the parasites, or a portion of one.

CONCLUSION.

We may here state some of the conclusions regarding the pathology of cholera to which we think our investigations point.

The chief object of our inquiry was the relation that exists between Koch's comma bacillus and Asiatic cholera. That this bacillus is not constantly discoverable either in the contents of the intestine or in the mucous membrane of cholera cases, the results of our inquiry make it impossible for us to doubt. We feel, however, that the frequency with which the comma bacillus is to be found in the dejecta of cholera cases, together with the evidence as to its pathogenic nature which has been recorded by Koch, v. Ermengen, and others, as well as the facts showing it to be distinct from other forms of comma bacilli, make it probable that it has some relation to cholera. That it is the direct cause of that disease is proved, we believe, by the facts recorded above, and by those obtained by Strauss and Klein. We are not, however, prepared to look upon Koch's comma bacillus as either harmless or accidental in its bearing upon the etiology of cholera asiatica. It must not be forgotten that in all, or nearly all, epidemics of cholera, many persons suffer from more or less severe attacks of diarrhœa—the so-called premonitory diarrhœa—the relation of which to true Asiatic cholera is a matter of considerable interest. While the epidemic of cholera was present in Madrid last year many of our friends as well as ourselves suffered from this form of diarrhœa, which to us seemed unmistakably different from true cholera, appearing to resemble more the diarrhœa caused by errors in diet. There are frequently watery stools, griping, lassitude and weakness, sometimes cramps in muscles, but there is none of the nervous prostration or fall of temperature, both of which occur so early in true cholera. Other facts which came to our notice seem to us to point strongly to this so-called premonitory diarrhœa being different in nature from true cholera.

On the other hand, its frequent occurrence in persons resident in places where cholera is present, and the impossibility which we experienced in finding any of the ordinary causes of diarrhœa for it either in errors or changes of diet, or in fear, or indeed any other known cause of diarrhœa, seem to us to indicate that it

must have some relation to cholera asiatica. Whatever premonitory diarrhœa indicates, it frequently precedes and apparently predisposes to an attack of true cholera. If the premonitory diarrhœa be a predisposing cause to true cholera, its frequent, perhaps constant, appearance at the same time and place as the latter disease, obliges us to conclude that it must be one at least of the influences which assist the propagation of cholera. The many points of similarity between Koch's comma bacillus and that found by Kühler and Prior in cholera nostras, has naturally led us to consider the possibility of the comma bacillus of Koch being the cause of the premonitory diarrhœa, and therefore perhaps an indirect cause of Asiatic cholera. This would explain why a pathogenic micro-organism, which cannot be looked upon as the direct cause of the disease, is so frequently present in cholera asiatica. Unfortunately, while in Spain our inquiries had not advanced sufficiently far to convince us that Koch's comma bacillus was not the direct and only cause of cholera; so that we did not pay the attention to the micro-organisms present in the dejecta from cases of premonitory diarrhœa which we believe they merit. We trust this question will receive attention at the hands of other observers.

The absence of the micro-parasite described above in our Italian material shows that it is not to be found in all cholera epidemics. We hope after further research on the subject to make clear the meaning of its presence in our Spanish cases.

June 1, 1886.



