THE ETIOLOGY OF ACUTE DYSENTERY IN THE UNITED STATES.

By E. B. Vedder and C. W. Duval,
Research Students in the Rockefeller Institute of Medical Research.

(From the Pathological Laboratory of the University of Pennsylvania.)

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The etiology of acute dysentery has been worked out in Japan by Shiga, in the Philippines by Flexner and by Strong, and in Germany by Kruse, but before the past summer no systematic attempt had been made to discover the cause of acute dysentery in this country. Our problem has been (1) to determine by comparative study whether the organisms described by these various observers are not really of the same species, though possessed, it may be, of individual differences and peculiarities, such as may readily exist within...

1 This investigation was conducted under a grant from the Rockefeller Institute of Medical Research.


5 Deutsche med. Wochenschrift, 1900, p. 637.
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the limits of a single species, and (2) to discover the cause of acute dysentery in this country, and if possible to identify it with the organisms of the observers mentioned. The problem is, to say the least, rather large. If we have succeeded, we have gone far towards proving that acute dysentery is the same the world over, and is due to a specific microorganism, Bacillus dysenteriae Shiga. How far we have succeeded must be left to the judgment of those who read the following report of our work. In so far as we have attained that success, the credit must be given mostly to Dr. Flexner, who suggested to us this line of investigation, was enabled to put at our disposal the means to carry it on, and gave us the results of his experience in a personal and almost daily supervision of the work.

In the following report we shall give a brief description of the technique, materials and results of our work.

TECHNIQUE.

The materials at our disposal consisted of the stools of persons supposed to have dysentery and the intestines of several fatal cases of the disease. The stools were examined in the majority of cases, and were collected either in a bed-pan, which was previously cleansed and partially sterilized by boiling water, or upon sterile gauze. In some cases a sterile spoon was introduced into the rectum and the cultures made from the material thus obtained. The stool was first examined microscopically for amebae, blood, bacteria, pus cells, etc. Three bouillon suspensions were then made, using three to ten platinum loopfuls of the dejecta for each tube of bouillon, according to the number of bacteria judged to be present as determined by microscopic examination. A series of four agar plates was prepared from each of these suspensions, so that in all cases at least twelve plates were made, and often this number was increased by making additional suspensions and plates. In inoculating the agar tubes, six loopfuls of the suspension were carried to the first tube, and so on to the fourth tube, always using six loops. With this method some of the plates would always have the right seeding, though whether the best plate would be the first or the last depended upon the number of bacteria originally present in the stool. When cultures were made at autopsies, the part of the intestine most affected, usually the sigmoid flexure, was examined, taking all the necessary precautions, such as burning the surface of the gut, to avoid...
contaminations. The suspensions were made, just as described above, by inoculating the tubes of bouillon with the material in the lumen of the intestine, and also with scrapings of the diseased mucosa.

The plates were incubated for 24 hours, and then all the colonies were marked, this step being described more in detail below (p. 195). The plates then were again incubated for another day, and from the new colonies all those which appeared to have the characteristics of B. dysenterie were inoculated by a simple stab in tubes of glucose agar. In order to select the right colonies, a very careful examination with both the naked eye and the microscope is necessary, and even then it is hard to exclude positively B. coli. Inasmuch, however, as the colon bacillus produces gas in glucose agar, while the dysentery bacillus does not, this step in the technique finally excludes all cultures of the colon bacillus.

As soon as inoculated, the glucose agar tubes were placed in the incubator, and remained there for 24 hours, when those that had not produced gas were subjected to further examination. At this stage we always looked for motility, but the morphology cannot be studied satisfactorily at this period on account of the early involution forms produced in glucose agar. Plain agar slants were now made from the glucose agar, and on the former the involution forms quickly disappear, so that at the end of 12 to 24 hours these cultures permit the study of the morphology and can also be used to make fresh suspensions of the suspected organism in order to test agglutinating properties with dysenteric sera. The technique of this test is exactly like that of the Gruber-Widal reaction in typhoid, so that it need not be here described. If the organism, as seen on the agar slant, presented the correct morphology, it was at once inoculated in all the common culture media.

Before the organism under consideration can be considered to be B. dysenterie, it must have fulfilled the following requirements:

a. It must give the proper cultural characteristics, as shown by standard cultures of Shiga, Flexner, Kruse, etc.

b. It must possess the right morphology, as shown by the same.

c. It must give a positive agglutinative reaction with some of the known dysenteric sera.

It is obvious that in many cases it will be impossible to obtain the organism in pure cultures on the plates, owing to the large numbers of bacteria present in the intestine under all conditions.
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Records of Cases Studied.

1. Philadelphia Cases.—These may be said to have been unfavorable cases for the purposes of our study. The stools and autopsies were obtained from various hospitals in the city, chiefly the Philadelphia Hospital, and there was in nearly every instance an unavoidable delay in the transportation of the specimen to the laboratory. It is our belief, judging from the results obtained in these cases, that Bacillus dysenteriae is present in overwhelming numbers in the intestines of patients suffering with dysentery, and that the colon bacillus is also present but in very small numbers; that the dysentery bacillus does not find the conditions in the stool after evacuation so favorable to its growth as does the colon bacillus, and that therefore in the stool the latter soon overwhelms the former, and if Bacillus dysenteriae is to be found, the fresh stool should be examined immediately.

Serapings from the intestinal mucosa are more favorable than the stool even when freshly collected.

Case 1. Kelly. The bacteriological examination in this case was made from the intestine after autopsy, the patient having died with the clinical diagnosis of acute dysentery. Unfortunately the patient had been dead for more than twelve hours before the autopsy could be performed.

The intestinal mucosa was greatly injected and studded with minute ulcers, together with some of large size (1 cm. in diameter). The lumen of the intestine contained bloody mucus and flakes of grayish membrane were adherent in places to the walls. The intestine was opened with the usual precautions, and bouillon suspensions were made both from the bloody mucus in the intestine, and also from scrapings of the mucosa. These were carried to the laboratory and agar plates were immediately made from them.

Out of 18 glucose agar tubes inoculated from the plates, 14 consisted of B. coli, 3 of unknown organisms, and 1 was a pure culture of a bacillus, giving the typical cultural characteristics, to be described later, of the bacillus of dysentery, and also positive agglutination reactions with blood from cases of dysentery (vide infra).

This case is one of the examples of the rapid growth of B. coli and of the equally rapid disappearance of B. dysenteriae, when conditions
cease to be favorable to its growth. Fifteen hours elapsed between the death of the patient and the time of making the plates.

Case 2. Smith. In this case the stool was the subject of examination. It was very small, liquid, and bloody, and also contained much mucus. Fecal odor was entirely absent. Microscopic examination showed a field of almost pure blood. Bacteria were not numerous and were entirely bacilli of colon-like morphology, many of which were motile; pus cells were also found. The stool was absolutely fresh when the bacteriological examination was begun. Ten glucose agar tubes were made from the plates with the following results: No colon bacilli were found; two tubes contained organisms that were easily excluded, and eight were pure cultures of the bacillus believed to be B. dysenteriae.

Case 3. Davis. Specimen obtained at autopsy. The appearance of the large intestine resembled that of the other Philadelphia cases. The lower portion of the colon was the part chiefly affected. A false membrane was present, and the mucosa showed areas of necrosis. The intestine contained little fecal material, but the liquid present was not particularly bloody. The walls of the intestine were greatly swollen. Plate cultures were made from the scrapings of the mucosa, and all colonies appearing after 24 hours were marked. The later colonies which seemed to resemble those of the dysentery bacillus, were transplanted to glucose agar. Twenty-four tubes were inoculated, and of these 18 did not produce gas. On examination of the latter, 4 cultures gave the serum reaction with several positive bloods, and also characteristic growths on the various culture media, corresponding with B. dysenteriae of the authors mentioned in every particular. The other cultures were negative. Unfortunately we were unable to test the organisms with the patient's own blood, as we tried to do in all our cases, because nothing was sent but the specimen of the colon.

Case 4. Vincent. The specimen that we examined consisted of a portion of the large intestine obtained at autopsy, the patient having died with the clinical diagnosis of acute dysentery. The greater part of the large intestine was found to be affected, the lesions extending from the splenic flexure to the rectum. The walls of the intestine were thickened, the mucosa dotted with small ulcers, and also exhibiting in many places small scars, probably the result of a previous attack. The ulcers were of a greenish-brown color, and a pseudo-membrane was everywhere present. Agar plates were made from scrapings of the
mucosa in the usual way, and promising colonies were transplanted to glucose agar. Out of 41 tubes, all but 8 proved to be B. coli or some other gas-producing organism, and of these eight tubes all were finally excluded except one tube which contained a pure culture of B. dysenteriae, giving a positive agglutination reaction with Shiga's anti-dysenteric serum, and corresponding entirely in morphology and manner of growth with the cultures of Shiga, Flexner, etc.

Case 5. Examined at autopsy. The whole of the large intestine was affected, and the morbid process extended several centimetres even into the small intestine, but the chief seat of the lesions was in the sigmoid flexure. The mucosa was ulcerated, swollen, congested, and covered with a fine granular exudate. Cultures were taken in the usual manner by scraping the mucosa, including that of the appendix, and from the mesenteric glands. All the organisms isolated from the appendix and glands proved to be either B. coli or B. proteus, but from the cultures made from the scrapings of the mucosa, 18 out of 24 glucose agar tubes proved to be pure cultures of B. dysenteriae, giving the agglutination reaction with Shiga's anti-dysenteric serum, etc., and agreeing with the standard cultures in all particulars.

2. Lancaster Cases.—These were from the Lancaster County Almshouse and Insane Asylum. We are greatly indebted to Dr. Samuel Miller, the Medical Director of that institution, for notifying us of the epidemic, and for that continual courtesy which enabled us to pursue the work to a successful termination. The epidemic had been very severe, having led to a number of deaths; but at the time of our studies it was on the decline, and only three cases were obtainable.

Of these, two had been acute but they had greatly improved under treatment, while the third (Benson) began with symptoms of acute dysentery, which, however, soon subsided, and the attack seemed to have been aborted. These cases, therefore, were not so favorable as many of the others which we studied.

Case 6. Benson. The specimen examined was a very small, grayish-brown, liquid stool, which seemed to the naked eye to contain pus, but no blood and very little mucus. Its odor was strong and rather characteristic, but not at all fecal. Microscopic examination showed many pus cells, some blood corpuscles, and many bacteria—mostly
bacilli of colon-like morphology, together with some of a larger size. Many were motile.

The attempt to isolate B. dysenteriae by means of the regular technique resulted as follows: Out of ten glucose agar cultures made from plate colonies, three proved to be of gas-forming organisms, three were of other organisms, probably of the proteus group, and four were pure cultures of B. dysenteriae, giving the positive agglutination reaction and cultural characteristics described below.

Case 7. Franklin. The very small liquid stool consisted mostly of pus with a little bloody mucus. Microscopic examination showed blood and large quantities of pus cells. Bacteria were not very numerous, and were nearly all bacilli of variable morphology, many being motile. The stool was immediately plated, and of fifteen glucose agar tubes inoculated with colonies from the plates, thirteen contained gas-producing organisms, and two were pure cultures of an organism giving all the cultural characteristics of B. dysenteriae, and also good agglutination reactions with the patient's own blood serum and with several other dysenteric sera. It may be mentioned here that Franklin was an insane patient.

Case 8. Hoffman. The stool was large in quantity, liquid and almost entirely fecal; no blood or mucus was found. Microscopic examination failed to show blood, but demonstrated the presence of large numbers of bacteria, most of them of colon-like morphology and actively motile. The stool was at once plated, and of the twenty glucose agar tubes inoculated from the colonies, fourteen were cultures of a gas-producing bacillus, four were of other organisms not identified, and two were pure cultures of B. dysenteriae.

These last two cases were convalescent, and Hoffman was practically well of his dysentery. The bacteriological examination of the stools showed that the dysentery bacillus had begun to disappear, and that the colon bacillus was resuming its place as the dominant microorganism of the intestinal tract. The conditions here were in marked contrast to those in the New Haven cases, which were very acute, and gave in the glucose agar tubes nearly pure cultures of Bacillus dysenteriae.

3. New Haven Cases.—These cases were obtained at the Springside Home, New Haven, Conn. The epidemic was very severe, fifty or more cases occurring within three weeks among the 350 in-
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mates, all being of the very acute form, with fatalities. The pa-
tients were stricken suddenly, and were very ill for a few days, their
evacuations being extremely bloody and mucoid, containing little or
no fecal material, and being odorless during the height of the dis-
ease. Thanks to Dr. Gompertz, Visiting Physician to the Home,
and to the hospital assistants, every opportunity was afforded for
thorough investigation.

Case 9. Alyvard. The bacteriological examination was made from
a fresh stool, which was large in quantity, liquid, very bloody and
mucoid. Microscopic examination showed numerous pus cells, and
bacteria which were almost all bacilli. From favorable colonies on the
agar plates, seventeen glucose agar tubes were inoculated, out of which
twelve tubes afterwards proved to be cultures of B. dysenteriae.

Case 10. Tedymus. Bacteriological examination was made at the
autopsy. The colon from the splenic flexure to the rectum was the
seat of ulceration, intense hyperemia and pseudo-membranous forma-
tion. Cultures were made from scrapings of the mucosa and also from
the contents of the intestine in several localities, with the result that
out of twenty-seven glucose agar tubes, eighteen contained cultures of
B. dysenteriae, the majority being obtained from the scrapings of the
mucosa rather than from the intestinal contents.

Case 11. Seward. The stool from which the cultures were taken,
was liquid, very bloody, mucoid, and devoid of odor, with no fecal ma-
terial present. It was obtained fresh and plated in the usual manner.
Out of twenty-four glucose agar tubes, twenty-one proved to be pure
cultures of B. dysenteriae. The patient subsequently died and an au-
topsy was performed. On opening the intestine, the lower two-thirds
of the large gut was thickened, ulcerated and closely dotted with small
hemorrhagic areas. No considerable pseudo-membrane was found.
Plate cultures were made from scrapings of the mucous coat, and ten
glucose agar tubes were finally inoculated from colonies, all of which
contained B. dysenteriae. These results obtained from the same case,
which was particularly favorable for our study, both from the stool
during life and from the intestine at autopsy, indicate that the latter
method when applicable offers the best chances of success.

Case 12. Cook. The specimen was a stool, typical of the acute
malady. The patient was in a comatose state from which she could
not be aroused. The movements of the bowels were continuous and
contained no fecal matter, consisting of blood and mucus. Microscopic
examination showed large numbers of pus cells and red corpuscles, and many bacteria. In this case cocci were rather numerous. Owing to the state of the patient, the evacuations had to be collected in a bed pan under unfavorable conditions, which may account for the small number of successful cultures from a case apparently so favorable. Suspensions were made from the material thus collected, which was by no means fresh, and the regular technique was followed, but out of ten glucose agar tubes but two finally proved to contain B. dysenteriae.

Case 13. Kenney. Stool liquid and bloody, with a little fecal matter. Microscopic examination showed quantities of pus cells, and many bacteria, which were mostly bacilli. Out of thirteen glucose agar transplantations from colonies, seven did not produce gas, and further examination showed these to contain a non-motile bacillus which responded to all the tests for B. dysenteriae.

Case 14. Higgins. From a typical liquid and bloody stool, suspensions and plates were made in the usual manner, and eight out of ten glucose agar inoculations did not produce gas. One of these cultures proved to be of a very motile bacillus; the other seven were of the same organism isolated and described in the foregoing cases, giving the serum reaction with the patient’s blood as well as with other positive sera, as shown in the tables on pages 201-2.

Case 15. Perkins. This was another case of dysentery in the insane. The stool was extremely bloody, and did not differ from the others in this series. Twelve out of fourteen glucose agar cultures were non-gaseous, and of these four were excluded, since they contained a short, thick, motile bacillus, not corresponding also in other respects to the bacillus of dysentery. The other eight cultures were of non-motile organisms, giving the agglutination reaction with other positive bloods, but not with the patient’s serum. The cultures of bacilli from Shiga, Kruse, Strong, and Flexner also failed to react with the patient’s serum. The patient died after a few days’ illness, and it is possible that he was not ill long enough for the agglutinating properties to develop in his blood. We wish to lay especial stress upon the fact that the organisms isolated did give positive reaction with other known dysenteric sera.

Case 16. Parmlee, also an insane patient. The stool was streaked with blood, but also contained considerable fecal matter. Nine out of sixteen glucose agar transplantations, contained pure cultures of B. dysenteriae. These gave the agglutination reaction with the patient’s own blood serum, as well as with the other positive sera in our posses-
The patient's serum had been previously tested with Shiga, Flexner, Kruse, and Strong cultures, and gave positive reactions with all these, thus proving that it was a positive serum.

The results obtained in these three cases of dysentery in the insane were therefore identical with the results furnished by the other cases of acute dysentery that we had the opportunity of studying.

Case 17. Dobell. The examinations were of a stool that appeared in every way typical. Out of twenty-four glucose agar tubes inoculated with apparently favorable colonies, seven contained a non-motile organism with a variable morphology, and failed to give the agglutination reaction. The other fifteen gave this reaction and corresponded in morphology and cultural characteristics with the standard cultures.

Case 18. Kittler. This stool was not very favorable as it contained considerable fecal material and was only streaked with blood in places. Four out of twelve glucose agar transplantations gave the agglutinating reaction, and agreed in other respects with the cultures of Shiga, Flexner, etc.

Case 19. Prescott. The stool was large, liquid, and contained finely divided fecal particles, together with some blood. Out of eighteen glucose agar tubes five contained the same organism isolated from all the foregoing cases.

Case 20. Cunningham. The stool contained a few pus cells, and was semi-solid in consistency. It was fecal in character and had a bad odor, as did the other stools containing feces. We were successful in isolating B. dysenteriae in nine out of fifteen glucose agar tubes.

Case 21. Howard. The stools throughout the whole course of this case were large, and, while containing considerable blood, were at no time free from fecal matter. Out of eight glucose agar inoculations, only two gave the characteristic agglutination reaction and cultural properties shown by the standard cultures. The clinical history of the case and the character of the stools show that this was by no means so acute a case as most of the others at New Haven, and in this respect as well as in the smaller percentage of successful glucose agar tubes it resembles those of the Lancaster epidemic.

Case 22. Wells. Stool semi-solid and fecal, but streaked with blood and mucus. Three out of eight glucose agar transplantations contained B. dysenteriae. The epidemic was by this time on the decline, as is shown by the character of the stools of the last two or three patients, and after this, no other typical cases appeared at New Haven.
CULTURAL CHARACTERISTICS.

With the view of determining how close the relationship between the various bacilli described by Shiga, Flexner, Kruse, and Strong, really is, a series of parallel cultures of all of these was made, beginning with agar plates, and afterwards carrying them through all the common culture media. Later the bacilli from our own cases narrated above were also included and made a part of the series.

In the course of this study we observed a few slight differences between the varieties of the several observers. For example, the colonies of one variety might be a trifle darker in color than those of another; or one variety might, at a given age, have slightly larger colonies than another; or one variety might produce a greater amount of acid in litmus milk than another. But these differences were nowhere greater than might be expected of individuals of the same species, and moreover they were by no means constant, but might be noticed on a first trial and be absent on a second or third.

We endeavored to rule out the personal equation in the following way: One of us would make a series of cultures or plates, keeping the names hidden, and the other would try to identify them. We varied the experiment by requesting other laboratory workers, not interested in the problem, to distinguish between the various cultures, but such attempts always failed.

We are therefore forced to the conclusion that the cultural characteristics of the various forms studied are essentially alike, for, however they might vary when we knew them by name, these variations were so inconstant that it was impossible to distinguish one culture from the other when the names were hidden. In fact these slight variations must be considered as one of the peculiarities of the organism, just as a rather wide variation in morphology is also characteristic. Since we have been unable to discover any real or constant differences between the varieties, it would be a vain and tiresome repetition to give the cultural characteristics of them all seriatim, as we had at first intended, and we shall therefore be contented with giving a careful description of the characteristics as we observed them, with the understanding that this description fits any one of the
varieties studied, including those which we isolated the past summer.

Surface Colonies.—The appearance of the colonies of course changes gradually, but it is convenient to describe several stages.

Twelve hour stage.—The colonies are circular in outline, and about one millimetre in diameter; to the naked eye they are whitish, but very translucent, and resemble ground glass in color. The margin of the slightly raised colony is perfectly smooth and regular. The colonies are smooth in texture, shining, and resemble the icing on a cake. Under the microscope, they are finely granular throughout, and so translucent that it is often difficult to focus them. The central part by transmitted light is pale yellow, gradually fading to a lighter shade towards the margin, while the outer third is absolutely colorless, thus giving the impression of two zones, which fade one into the other so gradually that there is absolutely no line of demarcation. Even under the microscope, the margin is almost mathematically regular and accurate.

Twenty-four hour stage.—The appearance now is also quite characteristic. The colonies have reached a size of from two to four millimetres, and are more nearly creamy in color and general appearance. Their texture is not so smooth, nor are they so shiny and refractive; they are also more elevated above the surface, though this is not a marked feature at any stage. They are still somewhat translucent, and their outline is circular and regular as before. Under the microscope, however, the margin is by no means so regular as it was at twelve hours, but presents a slightly ragged appearance, and the most striking change of all is, that there are three zones instead of two. In the centre a circular nucleus is seen of a light yellowish-brown color, very distinctly darker than the surrounding area. The nucleus is granular like the rest of the colony, but the granules are a little coarser, while the granulation of the rest of the colony is as fine as it was in the twelve hour stage. Around the nucleus is a pale greenish-yellow area, deepest in color towards the centre, and fading gradually away towards the periphery until it merges into the last or outer zone, which is absolutely colorless like that of the twelve hour stage, but has become more limited in extent.
Thirty-six hour stage.—The macroscopic appearance is very similar to that of the twenty-four hour stage, except that the colony is now larger, but the microscopic characteristics are much changed. The outline of the colony varies considerably in different colonies. Often it is still fairly regular, but in many instances it is decidedly ragged; always, however, it is approximately circular. The most apparent change is in the nucleus. This has become irregular and ragged in outline, of a deeper brown color, and is surrounded by a number of small lumps of irregular shape, size and arrangement, which have apparently been broken off from the nucleus. The color and granulation of these globules is the same as of the nucleus, in which the granules have, by this time, become so dense and thickly packed, that individual ones are no longer readily visible. It is also very noticeable that the granulations throughout the remainder of the colony are much coarser than before, and the area of the clear colorless outer zone is much diminished. The general color of the colony is a deeper yellow than before, and the translucency is greatly diminished.

Forty-eight hour stage.—The colonies now average from five to six millimetres in size, although there is much variation in this, many being smaller. They are quite white, often rather rough in texture, are no longer translucent, but are still circular in outline. The changes seen under the microscope are the same in character as those noticed before, but they are much more pronounced. The changes in shape may be summed up by saying that the tendency is for the granulations to become much coarser, so that at this stage, the colony is lumpy rather than granular. The nucleus is no longer circular in outline, but is usually oval, often lobulated, and of a deep brown color. The entire colony, with the exception of a small area around the periphery, is closely packed with irregularly shaped globules of all sizes, but the larger ones are mostly grouped around the nucleus and these are of the same color and structure as the nucleus, but of a lighter shade. Toward the margin of the colony the globules become more scattered, and less closely packed, until, as already noted, there is a narrow band around the periphery where there are hardly
any globules or lumps, but which is filled with very coarse granulations. Even in the old colonies, the outer rim is nearly colorless, a gradual transition taking place between the deep brown of the centre and the gradually fading brownish-yellow of the outer portion. Often colonies are found in which the nucleus is entirely broken up, and the only difference between the central portion and the remainder is that the lumps are rather larger.

Beyond this stage, the changes are neither particularly characteristic nor interesting, and consist merely of a further disintegration of the structure of the colony, which reaches its point of highest development in the twenty-four hour stage.

The deep colonies are of either a lenticular or irregularly spherical shape. To the naked eye, they are dirty yellow in color, but are yellowish-green under the microscope by transmitted light. They are smaller than the surface colonies, and are finely but thickly granulated. Their margins are at first quite smooth, but as they grow older, they become lined with protrusions and excrescences, and finally become lobulated to such an extent as to lose their original shape. Coincidentally with this change they become deeper in color, until finally they are of a rather deep brown. They reach their typical appearance at about the twenty-four hour stage, and the lobulation and deepened color are quite characteristic after forty-eight hours' growth.

It is seen from this description that the colonies are very similar to those of the colon and typhoid bacilli. In our work of isolating Bacillus dysenteriae from the stools, we were never troubled by having to distinguish between that organism and the typhoid bacillus, because of the ease of distinguishing clinically between dysentery and typhoid fever, but B. coli was a continual thorn in the flesh. Not only is it present in practically all stools except those of the very acute dysenteries, but when it is plated out, it is almost impossible to distinguish it from B. dysenteriae. Of course, all cultures of the colon bacillus unintentionally made from plate colonies are easily excluded in the glucose agar stage of the technique, but the real difficulty in all but the very acute cases, is to succeed in getting any cul-
tures of *B. dysenteriae* at all, since the colon bacillus is present in vastly greater numbers, and, without any sure method of distinguishing the two species, most of the cultures would according to the law of chances prove to be *B. coli*. Therefore we have been continually on the lookout for a ready method of distinguishing the two species, and we think we have been successful to a considerable extent.

The colonies of *B. dysenteriae* do not grow so luxuriantly as those of *B. coli*, and are always paler, smaller, and less white, and can usually be distinguished in this way. After experimenting along several lines, we have added to our regular technique a modification that we believe to be of great assistance in separating the dysentery bacillus from the colon bacillus. This is based upon the more rapid growth of the latter. The plates are made in the usual way, and are set in the incubator for twenty-four hours, when they are taken out and every surface colony on the plate is marked by scratching on the glass directly over it with a blue wax pencil. The plates are now put in the incubator again for from 12 to 24 hours, and when they are examined at the end of this period, it will be found that many new colonies have come out. It has been our experience that, although among the colonies that have thus developed after the first twenty-four hours, there will be a few colonies of *B. coli*, by far the greater proportion will be of the dysentery bacillus, and that this method therefore renders very valuable assistance in isolating the latter.

Ordinary Culture Media.—Here, as in the case of the plates, the cultures from the different sources mentioned presented essentially similar characters. Shiga's and Kruse's bacilli sometimes produced in litmus milk a little more acid at first than either Flexner's or Strong's bacillus, but after the subsequent change to the alkaline reaction one culture could not be distinguished from another. The organisms isolated in this country also coincide in all particulars with those of the observers mentioned.

Plain agar.—At twelve hours a thin but well-marked, dull grayish-white, translucent growth is observed all along the line of inoculation, having a perfectly regular margin.
At twenty-four hours there is an increased growth, with a uniform lateral spreading, but otherwise not much change.

After thirty-six hours the edges of the growth become rather uneven, and the lower part of the streak is somewhat wider than the upper portion. It is raised somewhat above the surface, more especially in the centre of the streak, and the translucency is now about gone, the color being more nearly creamy white.

At forty-eight hours the margin of the streak has become crenated, especially at the lower portion, while the whole of the growth is denser and more elevated.

After seventy-two hours often a slight depression may be noticed in the centre of the streak, and the color is a decided cream white.

From now on, the principle change of interest takes place along the margin of the growth, and this may be said to be characteristic. Especially if the agar is still fairly moist, the margin tends to become feathery, slender processes budding out from the sides, and branching dichotomously. Inasmuch as this process is more marked towards the bottom of the slant, there is a gradual tapering off towards the top, with the result that the growth now reminds one strongly of a northern fir.

Gelatin.—After twelve hours of incubation, there is a fine film-like growth along the path of the needle. The growth on gelatin is neither so rapid nor so profuse as on agar.

At twenty-four hours there is no perceptible change, except a very slight increase in the amount of the growth, and even this is often dubious.

At thirty-six hours, however, the growth is more clearly visible, and if examined closely, the individual colonies can be made out. There also now begins to be some little growth on the surface, immediately encircling the entrance of the needle. There are no further changes. There is little or no further growth, absolutely no tendency to spread out over the surface of the gelatin, and at no period is there liquefaction or clouding of the medium.

Potato.—At twelve hours there is a slight growth visible, which is rather translucent, so that the tube must be held in a proper light to
E. B. Vedder and C. W. Duval

permit the growth to be seen readily, the margin is irregular, and at first there is no coloration.

At twenty-four hours the growth is fairly profuse, of a faint yellow color, with a shiny surface, spreading rather freely, with irregular edges.

At thirty-six hours the growth still continues to spread beyond the line of inoculation, and is raised above the surface of the medium.

At forty-eight hours the surface of the growth has become roughened, with a yellow color centrally that fades to a gray towards the periphery. The potato is usually somewhat discolored around the growth.

At seventy-two hours the growth seems to cease, and becomes depressed in the middle portion. Beyond this stage there seems to be very little change, until the time when the potato becomes dry and shrivelled.

Blood serum.—The growth is about as rapid and profuse as upon agar. At twelve hours it is very easily discernible, though in color it can hardly be distinguished from the medium, and is shiny and translucent. The margin is regular.

At twenty-four hours the streak is larger in extent, and tends to sink below the surface of the medium, probably on account of some slight liquefaction, so that it gives the impression of being inlaid. The margin has become slightly corrugated.

After thirty-six hours there is very little change. The streak may increase slightly in size, but there is nothing further characteristic.

Glucose agar.—At no period is gas produced.

At twelve hours there is a distinct granulated growth along the path of the needle, of a dirty grayish-white color.

At twenty-four hours the quantity is increased, and there is a small irregular circle of growth surrounding the point of entrance of the needle on the surface. This tendency toward surface growth seems to be strictly limited, for it only spreads a few millimetres, and then no further change is observed. After some days, the medium becomes slightly and uniformly clouded.

Bouillon.—A cloud is produced in several hours, which becomes
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gradually heavier, until at the end of twenty-four hours, it is very dense and a sediment fine and silt-like in character has begun to fall to the bottom of the tube. This sedimentation continues for several days while the supernatant liquid becomes gradually clearer and clearer, until finally the sediment is several millimetres in depth, and the supernatant fluid is nearly as clear as before inoculation. At no time is there a pellicle formed.

Bouillon containing one per cent of saccharose, lactose, and glucose was prepared and inoculated. The growth was similar in character to that of the plain bouillon, and no gas was produced at any stage, with any of the sugars.

Litmus milk.—After twelve hours, there is a varying amount of acidity, but at no period is there any tendency toward coagulation.

At thirty-six hours the milk is still acid, but the reddish shade is not so pronounced, and at forty-eight hours it is very evident that a gradual change back to the original color of the medium has set in. For a varying period of time thereafter, the milk remains alkaline. This state of affairs usually lasts several weeks, when there may be a second production of acid, but one very much less noticeable than the first stage of acid production. If the culture is kept for several months, it may become perfectly white. This condition, brought about by the abstraction of oxygen from the litmus, may be readily overcome by shaking the tube and thus bringing the liquid into a more intimate association with the oxygen of the air, when the litmus assumes a bluish tint.

MORPHOLOGY.

The bacillus is a slender rod with rounded ends, about 1 to 3 μ long. It has no tendency to form groups, and is usually found singly, but sometimes in pairs. It stains readily with the ordinary aniline dyes, is not stained by Gram's stain, possesses flagella, and a capsule is sometimes present. Involution forms develop on glucose agar in a very short time, often in twelve hours. Under these conditions the bacilli are very much larger in every way and of strikingly irregular form.
Flagella.—After many unsuccessful attempts we succeeded in demonstrating the presence of flagella. They are numerous, entirely surround the body of the bacillus, and are eight to ten times its length. They are very delicate, and are arranged in a wavy manner that recalls the delicate hair-like fibrils of floating seaweed. In other specimens in which the flagella were more broken up by the manipulations, they simply form a net work radiating in all directions, without creating the beautiful picture referred to above. To stain the flagella is a very difficult task, inasmuch as no directions can be given that will ensure success without a good many preliminary trials. Even when we felt that we had the method perfected, we did not succeed to our satisfaction oftener than once out of ten or fifteen trials. The following method is the one with which we were most successful, and is a modification of van Ermengem’s method. On an absolutely clean cover slip place a drop of sterile distilled water, and inoculate this with bacteria obtained from a plain agar culture not more than twelve hours old, being careful not to make too heavy a suspension. Cover the slip carefully to prevent dust from falling on it, and allow it to stand for 15 to 20 minutes. This is to allow the bacteria to become scattered over the surface, without the necessity of spreading them with the platinum needles, which breaks up the flagella. We have never obtained a specimen with the flagella unbroken that has been spread with a needle. Dry in the air, without heating at all, and then pass the slip once through the flame for fixation. Now cover the slip with van Ermengem’s solution no. 1, and allow it to remain for two or three hours, without heating it, adding enough of the solution from time to time to prevent its drying on the slip. The mordant is to be washed off very gently with distilled water, until it is all removed, and without drying the slip, the second solution, silver nitrate 0.6% is dropped on. This is to remain for five minutes, when it is poured off, and the slip is placed in van Ermengem’s solution no. 3. This is allowed to remain until the color is a light brown (not dark), when it is poured off and the film is washed again in distilled water, and cleared in 1 to 1000 acetic acid, and again washed in water, dried and mounted. It will be seen from this account, that
our principle modifications consisted in not heating the mordant, but in allowing it to remain on for a much longer time in the cold, in not using any alcohol after the mordant, and in using a somewhat stronger silver nitrate solution than van Ermengem gives in his formula.

Motility.—We can only say with regard to this point, that in none of our cultures was motility observed, and this applies to the organisms of Shiga, Flexner, Strong, Kruse, and those that we isolated. After having demonstrated the presence of flagella, it is at any rate possible, and even probable, that at some period of their existence, or under favorable conditions as yet undetermined, the bacilli are motile; but we have looked for motility at almost all ages, and under varying conditions, and have never succeeded in finding it.

Pathogenic Properties.—The pathogenicity of the bacillus has up to this time been little studied by us by experimentation upon animals. Guinea-pigs succumb to intraperitoneal inoculation in less than twenty-four hours, the lesions consisting in those of a sero-fibrinous peritonitis. Bacilli are abundant in the exudate, and may also be cultivated from the heart's blood.

AGGLUTINATION REACTIONS.

Much time was devoted to a study of the agglutination reactions of the bacilli isolated by us and, at the same time, of those cultures with which we first worked, namely, Shiga's, Flexner's, Kruse's and Strong's. The blood for the tests came from a wide series of cases, including the majority of those from which stools were studied, and some in which stools or autopsies were not obtainable. In addition to the human blood we were supplied with the sample of anti-dysenteryc serum sent by Dr. Shiga to Dr. Flexner. Some interesting results were also obtained with several specimens of blood sent by Dr. Ross from Morven, North Carolina, where a short time previously acute dysentery had prevailed.

In all cases, control tests of the blood sera were made with suspensions of B. coli and B. typhosus, and normal blood was frequently used as a control for the specific sera. We never obtained an agglutination in any of these control tests, no matter what dilution was used.
The tests consisted (1) of the reactions of the patient's own blood with the cultures of Shiga, Flexner, Strong, Kruse; (2) of the reactions of the bacilli isolated by us with the patient's own blood and with other sera that had been previously tested and proved to possess agglutinating properties toward B. dysenteriae; and (3) the reactions towards Shiga's anti-dysenteric serum.

In order to avoid repetition and the use of unnecessary space only two tables will be given. They represent the results of tests made with the sera obtained from cases of dysentery and with the anti-serum of Shiga. The bacilli and the blood are designated by the name of the patient from whom they were obtained. The first column gives the source of the serum, the second of the culture, the third the dilution employed, and the fourth the result. The final result may represent a reading as late as several hours after mixture of the culture and diluted serum.

**TABLE I.**

**AGGLUTINATION REACTIONS WITH BLOOD OF PATIENTS.**

<table>
<thead>
<tr>
<th>Blood</th>
<th>Suspension</th>
<th>Dilution</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shiga</td>
<td>1:30</td>
<td>+</td>
</tr>
<tr>
<td>Cook</td>
<td>Strong</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>Flexner G</td>
<td>&quot;</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot; H</td>
<td>&quot;</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>Kruse</td>
<td>&quot;</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>Vincent</td>
<td>&quot;</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>Davis</td>
<td>&quot;</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>Vincent</td>
<td>1:50</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>Strong</td>
<td>&quot;</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>Flexner G</td>
<td>&quot;</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot; H</td>
<td>&quot;</td>
<td>+</td>
</tr>
</tbody>
</table>

The uniformly positive results in the tables given serve as examples of the numerous additional tests made.

Certain variations in the appearance of the clumping were observed. The forms taken by the masses of bacilli may be divided into two groups: in the one, the bacteria were tangled in tight bunches, as is
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commonly seen in the Gruber-Widal reaction; in the other, the bacteria were united end to end and thus formed long threads which were interlaced so as to give rise to a loose skein. This latter appearance has been noted both by Kruse and by Flexner, but it seems to be the exception.

TABLE II.

Agglutination Reactions with Shiga's Anti-Dysenteric Serum (designated A. D. S. in the Table).

<table>
<thead>
<tr>
<th>Suspension</th>
<th>Dilution</th>
<th>1 hr.</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. D. S.</td>
<td>Flexner G</td>
<td>1-200</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot; H</td>
<td>&quot;</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>Shiga</td>
<td>&quot;</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>Strong</td>
<td>&quot;</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>Kruse</td>
<td>1-300</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot; G</td>
<td>&quot;</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>Shiga</td>
<td>&quot;</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>Strong</td>
<td>&quot;</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>Shiga</td>
<td>1-500</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>Kruse</td>
<td>1-300</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>Flexner</td>
<td>1-500</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>B. typh. as control</td>
<td>1-25</td>
<td>-</td>
</tr>
<tr>
<td>&quot;</td>
<td>Davis</td>
<td>1-200</td>
<td>-</td>
</tr>
<tr>
<td>&quot;</td>
<td>Vincent</td>
<td>1-100</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>Tedymus</td>
<td>1-500</td>
<td>-</td>
</tr>
<tr>
<td>&quot;</td>
<td>Strong</td>
<td>1-500</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>Sykes</td>
<td>1-50</td>
<td>-</td>
</tr>
<tr>
<td>&quot;</td>
<td>Alyward</td>
<td>1-100</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>Seward</td>
<td>1-100</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>Cook</td>
<td>1-100</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>Kelly</td>
<td>1-25</td>
<td>+</td>
</tr>
<tr>
<td>&quot;</td>
<td>Smith</td>
<td>1-20</td>
<td>+</td>
</tr>
</tbody>
</table>

The agglutinating properties do not appear in the blood immediately upon the appearance of clinical symptoms. This is shown by the fact that we have had some patients suffering from undoubted dysentery, from whose stools we secured B. dysenteriae, and yet their blood serum was negative or nearly so.

The reaction is, moreover, capable of disappearing from the blood in a rather sudden manner. The McShara case of the New Haven epidemic indicates this. When we reached New Haven the patient was convalescent, but her blood reacted with the bacilli of Shiga, Flexner, etc., and also with the cultures from the Philadelphia cases. The dilutions employed were 1 to 200. Two weeks later, fresh
blood being taken, reaction even in 1 to 10 dilutions could not be obtained with any of the bacilli in our possession.

While the agglutinating properties of the bacilli from different sources are very much the same, still there are differences in degree. Certain strains of bacilli react better with a given serum than do others as is shown by the fact that if high dilutions are used, some forms will always drop out sooner than others. We have never made a test with high dilutions in which all the varieties would be positive to a certain point and then all drop out together. Again, with certain weak sera, while certain of the varieties of bacilli were positive, occasionally we have found one or two that absolutely refused to react. It must also be noted that certain sera are very powerful agglutinators, while others, taken from patients with just as severe a dysentery, which has existed quite as long, are comparatively very weak. In general it may be said that the sera obtained from the New Haven patients were very strong, while those obtained from Lancaster, Philadelphia and Morven, N. C., were in almost all cases comparatively weak.

DISCUSSION OF THE RESULTS.

To sum up the results of this study would be to state that the observations of Shiga made upon the dysenteries in Japan, of Flexner upon the same disease in the Philippine Islands and in Porto Rico, and of Kruse in Germany, can be applied to the acute dysenteries of this country. So far as the results of modern bacteriological study can be trusted, all the criteria have been successfully fulfilled in establishing the bacillus obtained from the wide range of cases here reported to be the cause of the dysentery from which the patients suffered—that is, all the criteria which have been set up as the result of the study of the disease in the places mentioned, for in all one condition has been lacking, namely, the production of the disease by inoculation. Of the pathogenicity of the organism, there is abundant proof; and the bacillus isolated by us is likewise pathogenic for laboratory animals. But in no case, unless the effects of the subcutaneous
injections carried out by Flexner have specific intestinal lesions been produced in animals inoculated with the organism. In two instances related by Flexner circumscribed lesions consisting of swelling and necrosis of the intestinal mucous membrane in rabbits followed the injections. The character of the lesions, only briefly described, agrees with similar appearances which occasionally result from the inoculation of these animals with virulent cultures of B. typhosus and B. coli. In two instances the symptoms of dysentery have followed ingestion of cultures of B. dysenteriae by human beings. The first is reported by Flexner and was the result of accidental inoculation; the second by Strong, who fed a culture to a Filipino prisoner. Both men developed characteristic symptoms, and Strong recovered the bacillus from the dejecta of his patient.

The present study also bears upon two other important phases of the problem: the cause of sporadic dysentery, and that of the dysentery of institutions, such as those for the insane. The cases arising in Philadelphia were not a part of an epidemic; they were scattered cases, most of them being among the inmates of the Philadelphia Hospital. But others arose in widely removed portions of the city, and were encountered in the Pennsylvania Hospital. Neither did these cases become centres of infection, for at no time in the summer, while this work was being prosecuted, did a considerable number of them occur.

It is also significant that several of the sporadic cases were “terminal” dysenteries. The patients were chronic invalids, and had suffered long from chronic Bright’s disease, the autopsy establishing the existence of the small contracted kidney.

The Lancaster and New Haven outbreaks were typical institutional epidemics. Since the appearance of Kruse’s second paper it has become of much interest to decide upon the exact nature of the so-called institutional dysentery. Kruse considers that the cause of
this is different from that of the epidemic dysenterics in general, and he has been led to choose the unfortunate term of "pseudo-dysentery" to designate the disease as it appears in asylums for the insane. Our studies, which were mainly upon institutional epidemics, show this position to be untenable. For not only are the cultural properties of the bacillus obtained by us from institutional outbreaks identical with those of bacilli obtained from ordinary epidemics, but they agree with the standard cultures used throughout this investigation, and exhibit similar agglutinating reactions with them to positive blood sera.

The question of motility has been somewhat mooted, in that Shiga, Flexner, and Strong all described some motility, while Kruse has never been able to detect it. Our observations are in this regard in agreement with those of Kruse, although the demonstration of flagella has an important bearing upon the ultimate solution of this question.

CONCLUSIONS.

1. The several standard cultures used in this study are indistinguishable—a conclusion previously reached and stated by Flexner.

2. The acute dysentery of the United States is due to a bacillus indistinguishable from that obtained from the epidemics of dysentery in several other parts of the world.

3. The sporadic and the institutional outbreaks of acute dysentery are caused by the same microorganism, and this organism is identical with that causing epidemic acute dysentery.

4. The cause of acute dysentery, whether sporadic, institutional, or epidemic, is *Bacillus dysenteriae* Shiga.