T H E S I S

For the Degree of M.D., EDINBURGH UNIVERSITY,

A STUDY of the NORMAL and PATHOLOGICAL INTESTINAL FLORA of INFANTS and very YOUNG CHILDREN.

by

W. R. LOGAN, M.B., Ch. B.,

March 1913.

I N D E X .

PART I.

PART II.

I N D E X (CONTINUED).

PART II. (CONTINUED).

PART III.

PART I.

INTRODUCTION.

The subject of this research was suggested to me by Dr James Ritchie, and the work has been done in the Research Laboratory of the Royal College of Physcians, Edinburgh.

The great majority of the specimens have been obtained from children in Dr Fowler's Ward in the Royal Hospital for Sick Children, Edinburgh, and I have to thank Dr Fowler for much kindness and help in this way.

A few other specimens have been obtained from other Wards and from the Medical Out-Patient Department of the Sick Childrens Hospital, and also from the Simpson Memorial Maternity Hospital, and for these I have to thank various doctors and nurses.

For the use of notes of cases I wish to thank Drs Shafto and Meyer, and for sera, Drs McGowan and Reynolds.

I wish to acknowledge with much gratitude the guidance and advice of Dr Ritchie at various periods during the year occupied by the work.

SCOPE/

SCOPE OF THE WORK.

The aim has been to ascertain the bacteriological conditions obtaining in the intestine of infants and young children, in health, and when suffering from diarrhoea.

The healthy children have been divided into classes according to the nature of the feeding -- breast fed children, bottle fed children, and children on a mixed diet.

The flora of the children suffering from diarrhoea has been studied and by a comparison with normal cases, an attempt was made to arrive at conclusions as to the cause of diarrhoea.

The cases of diarrhoea studied were not cases of Epidemic Summer Diarrhoea. They occurred during the very cold and wet summer of 1912, and during the winter following.

I hesitate, however, to call them cases of Sporadic Diarrhoea, as there was some evidence that some cases had derived infection from others, or that some common factor was at work.

All through, as was suggested to me by Dr Ritchie/

Ritchie, I have endeavoured to study the flora as a whole, and tried not to be seduced into the particular study of one group of organisms to the exclusion of others. This has involved the formulation and practice of a somewhat lengthy scheme of routine examination.

A total of 56 specimens has been examined. Of these, 11 are not included in the present thesis as they were used while studying means of isolation in order to formulate a satisfactory routine examination.

3 others are not included as they were older children suffering from intestinal indigestion.

21 cases free from diarrhoea, and which always had been free from diarrhoea were put through the routine examination. These cases throughout the thesis I have called normal. I wish to say that by this I mean completely free from diarrhoea. Many of the children were not healthy.

14 cases suffering from diarrhoea have been studied, and in addition 4 specimens from cases convalescent, or recovered, from diarrhoea have been obtained, as well as the specimens obtained from the same/

same cases while the diarrhoea was at its worst.

A specimen of faeces and a catheter specimen of urine were also obtained from 3 cases of Coli Pyelitis with the object of comparing the colon bacilli of urine and faeces.

THE ARRANGEMENT OF THE THESIS.

The Thesis has been divided into 3 parts.

In PART I. is a description of the intestinal flora of normal infants under different conditions of feeding; a description of the flora of diarrhoea cases; and a discussion of the deductions to be drawn from the differences between those two groups of cases, finishing up with a summary of the results of the whole work. This part of the thesis has been kept as free as possible from unnecessary detail.

PART II. consists of a study of various groups of organisms isolated, and also includes different series of experiments conducted with these organisms. I have divided the isolated organisms for convenience into groups and have discussed previous literature on the subject in reference to each of these/

these groups. These groups are:-

I. The Gram-positive Bacilli of the Acid-tolerant group.

II. The Gram-negative Lactose-fermenting Bacilli.

III. The Gram-negative Non-lactose-fermenting Bacilli.

IV. The Coccal Forms.

V. The Spore-bearing Bacilli.

Then follow some Experiments with these organisms.

The Bibliography is also contained in this Part.

PART III. contains a full description of the Scheme of Examination, and also the Results of the detailed Examination of each of the Specimens.

In a separate Album is a Series of Coloured Drawings of Microscopical Fields, executed by myself.

PREVIOUS/

PREVIOUS WORK.

The greater portion of the previous work done on this subject has been concentrated on different groups of organisms. I have, therefore, found it convenient to discuss previous literature under the different groups of organisms described in PART II. of the Thesis.

In this part I would merely refer to the work of Escherich in Germany and Tissier in France on the intestinal organism of infants.

Tissier was the first to give a complete description of the flora of nurslings.

Since then the work in different countries has been, as I say, largely concentrated on different branches of the subject.

In Germany much more attention has been paid of late years to the chemical than to the bacteriological side of the question.

In France recent work has been done on older cases, especially in studying putrefaction.

In America research on intestinal organisms in/

in children has resolved itself into a hunt for the Dysentery Bacillus.

In England almost all the work has been on organisms of the Non-Lactose-Fermenting group and especially Morgan's No.I Bacillus.

THE/

THE FLORA OF NORMAL CHILDREN ON THE BREAST ONLY.

Six specimens from six normal breast-fed children have been examined, the ages of the children varying from 2 to 10 days. In 1, derived from a 2 days' old child, the films showed no organisms and cultures remained sterile. This was of course meconium, and it is doubtful whether the child had had the breast before the passage of the specimen.

In four of the others the results were nearly identical.

The films showed a nearly pure culture of gram-positive bacilli of the acid-tolerant group. These were of the type that takes up the stain in a "punctate" manner and which is regarded by Tissier, (1) as being a form of the anaerobic Bacillus Bifidus discovered by him in 1899. From the difficulty of isolating these organisms, it is evident that the great majority of these organisms seen in films are dead, as indeed their very degenerate appearance would lead one to suppose.

The small, rather lanceolate diplococcus first discovered by Thiercelin, (25) in 1899, was present/

present in small numbers in all these cases.

Members of the coli group were also obtained in all these cases in very small numbers relatively to the bacilli of the acid-tolerant group. Various members were studied and their pathogenicity tested. Their reactions are given in Table A; Bacillus Lactis aerogenes was obtained from one case.

The sixth case was somewhat different.

In it, the bacilli of the coli group were present in fair numbers and there were also spore-bearing organisms present of the type of the bacillus enteritidis sporogenes. The bacilli of the acid-tolerant group were nevertheless dominant in this case also.

No members of the non-lactose-fermenting group of organisms were obtained in any of these cases.

SUMMARY

In the specimens from normal breast-fed infants,bacilli of the acid-tolerant group dominated all others: the enterococcus and indol-producing B.coli were also present in small numbers. In one case a spore-bearing organism resembling the bacillus enteritidis/

enteritidis sporeogenes was present.

THE INTESTINAL FLORA OF ARTIFICIALLY-FED INFANTS.

15 specimens have been examined from cases which had never suffered from diarrhoea.

Of these 5 were on the bottle and breast as well, 7 were on the bottle only, while 3 were getting cornflour, porridge, &c.,

The 5 who were on the breast and bottle presented a flora very similar to that of breast-fed cases. Gram-positive bacilli of the acid-tolerant group were the dominant form. They, however, consisted mainly of the facultatively aerobic and easily cultivated B.acidophilus of Moro, instead of the strictly anaerobic Bacillus Bifidus of Tissier.

In 4 of these 5 cases the gram-negative bacilli were somewhat more numerous in the films than in the cases fed on the breast only, but on isolation and study were found to be of much the same type, (see table B), being coli-like bacilli. The enterococcus formed a small but constant part of their flora.

A spore-bearing bacillus resembling morphologically/

morphologically the bacillus enteritidis sporogenes was present in fairly large numbers in one of them.

In one of these infants fed on breast and bottle, non-lactose-fermenting organisms were found to have largely replaced the ordinary intestinal B.coli. On isolation these were found to be Morgan's No.I. Bacillus. I was able to obtain a serum from a rabbit by immunising it with repeated doses of vaccines of a Morgan's No.I. Bacillus isolated from a case of acute diarrhoea, which serum agglutinated its own strain up to a titer of 1-5000. It also agglutinated the Morgan's No.I. obtained from this normal case up to titer.

It was noteworthy in this case that it was the colon bacilli which had been replaced by Morgan's No.I. That is to say, the gram-positive bacilli of the acid-tolerant group were not at all diminished relatively to the gram-negative forms.

The 7 cases who were on the bottle only, showed a somewhat more varied flora. The main groups of organisms present were the same, but the proportions were altered, and there were also differences in the characters of individual organisms present.

In/

In 4 out of the 7 cases there was only one main difference from the breast-fed cases, and that was merely an exaggeration of the difference noticed in children on the bottle and breast.

This difference was that gram-negative bacilli of the lactose-fermenting group were somewhat more numerous, as were also coccal forms. The gram-positive bacilli of the acid-tolerant group were still the dominant form and were now the facultatively aerobic type called the B.Acidophilus. The coccal forms also showed more variety and in addition to the enterococcus of Thiercelin, a somewhat larger oval gram-positive coccus made its appearance.

The members of the coli group studied showed no marked difference from those isolated from children otherwise fed. Their reactions are given in Table C.

The flora of the three other bottle-fed cases showed a difference. In one, specimen XXXI, a non-lactose-fermenting bacillus was isolated (XXXI. 1,) whose cultural characteristics are fully gone into elsewhere. This bacillus corresponded culturally to the Paratyphoid-gaertner group, and particularly/

particularly to B.Paratyphosus A, but its agglutination reactions proved it to be quite distinct from Para A. Further agglutination tests are being carried out.

This organism was only present in extremely small numbers in the faeces, in which bacilli of the acid-tolerant group were vastly predominant. This case later developed acute diarrhoea with Morgan's Bacillus in the stools. In one other case an identical bacillus was discovered in fairly large numbers; identical, that is to say in cultural characteristics. This organism (XLIX.1,) tested in various dilutions against highly agglutinating sera of Bacillus Paratyphosus A, Bacillus Paratyphosus B, and B.Gaertner gave completely negative results. This case was suffering from pyloric stenosis, and had never had any diarrhoea, but rather a tendency to constipation.

The last case was one in which Morgan's No.I. Bacillus was present in the motions in fairly large numbers. This specimen (L) was obtained from a child suffering from rickets who had never suffered from diarrhoea.

The point on which I wish to lay stress is that/

that all these cases the gram-positive bacilli of the acid-tolerant group, were predominant. The only case in which gram-negative forms were as numerous as the gram-positive was the case of pyloric stenosis from which specimen XLIX. was obtained.

In the children who were getting a diet including other articles besides cow's milk, the acid-tolerant group was still present in large numbers, and were of the Bacillus Acidophilus type. Coliform bacilli were also numerous, and the characters of the various lactose-fermenting bacilli isolated are given in Table D.

The coccal forms in these cases were very varied but consisted mainly of diplococci of the enterococcus type, and of the large oval type of cocco-bacillus. Streptococci in long chains were never seen.

Spore-bearing organisms were present in small numbers in one of these cases, but played a very inconspicuous part.

In one of the cases a non-lactose-fermenting bacillus was isolated, which did not correspond culturally to any known variety. Its reactions (XXXII. 1.)/

(XXXII. 1,) are given elsewhere.

In another, bacilli of the same cultural characters (XLVIII. 1,) as those isolated from two previous cases (XXXI. 1, and XLIX. 1), that is to say with the cultural characteristics of the Para-typhoid-gaertner group, were isolated. These were tested against highly agglutinating sera of B.Para-tyosus B, B.Paratyplosus A, and B.Gaertner with completely negative results even in low dilutions.

XLVIII. 1, and XLIX. 1, were rapidly pathogenic to guinea-pigs, while XXXI. 1, did not produce a fatal result.

These three organisms have not yet been tested against aertryck serum, but this will shortly be done.

In addition to these, 4 specimens have been examined from cases convalescent or recovered from diarrhoea. These are discussed later.

SUMMARY OF THE RESULTS OBTAINED IN "NORMAL" CASES.

In all of the 21 cases examined bacilli of the acid-tolerant group were predominant. The method of feeding had a marked effect on the proportions of different groups of organisms.

In/

In the breast-fed cases which we may regard as the ideal, the bacilli of the acid-producing and acid-tolerant group were usually present in nearly pure culture.

The coli group and coccal forms were very scanty in those cases, and no members of the non-lactose-fermenting group were present.

A noteworthy point is that the colon bacilli from breast-fed cases were as virulent as, or more virulent than, colon bacilli from cases of acute diarrhoea.

In the children on breast and bottle the chief difference was a numerical increase of colon bacilli, and in one Morgan's No.I. Bacillus was isolated.

In the bottle-fed children a still further step was taken from the ideal and colon bacilli and coccal forms were still more numerous. In 3 of these cases non-lactose-fermenting bacilli were isolated, one being Morgan's No.I. Bacillus, the other 2 being bacilli resembling the Paratyphoid-gaertner group, which agglutination tests proved to be distinct.

In children on mixed feeding, the colon group/

group and coccal froms were still more numerous and varied, and a Morgan's No.I. Bacillus, and another Paratyphoid-like bacillus were isolated.

THE INTESTINAL FLORA IN CASES OF DIARRHOEA.

14 specimens were obtained from cases suffering from diarrhoea and I propose to divide these into two classes.

The first class, into which 7 of the cases fall, includes cases in which the diarrhoea was moderate or severe but in which no blood or mucus was ever seen.

The second class, including the other 7, consists of cases of severe diarrhoea with blood and mucus in the stools, which may be called cases of ileocolitis.

All these cases were being artificially fed prior to the onset of the diarrhoea.

In cases of the first class there was one in which examination showed no difference from normal cases. Bacilli of the acid-tolerant group were numerous in this case, and non-lactose-fermenting bacilli were absent. It may be noted that one had to/

to depend on the mother's statement in this case as to the severity of the diarrhoea, as the child was an out-patient, and as he was not brought up a second time it may be taken that the diarrhoea was very slight.

In another case departure from the normal was slight. The gram-positive bacilli were possibly slightly reduced in numbers in this case, and the gram-negative bacilli increased, but the difference was doubtful, and non-lactose-fermenting bacilli were not isolated.

In the other 5 specimens of this first group, there was one marked difference from the normal which was common to all - the gram-positive bacilli of the acid-tolerant group were markedly diminished in numbers, in one case being practically absent. Their place in the films was taken by gram-negative coliform bacilli, with coccal forms. On further examination it was found that in 4 of these 5 cases the usual colon bacilli had been to some extent replaced by non-lactose-fermenting organisms. In 2, Morgan's No.I. Bacillus was present, in one an organism resembling Shiga's Dysentery bacillus/

bacillus but motile, and in the fourth, an atypical organism with a distant resemblance to the Para-typhoid group but quite distinct (XIX. 3,) was found. In the fifth case Morgan's No.I. Bacillus was found in very small numbers. It is to be noted that this case had another attack of diarrhoea later on, and an examination of a later specimen yielded Morgan's No.I. Bacillus in large numbers.

In the other seven cases a very constant result was obtained.

In all, the gram-positive bacilli of the acid-tolerant group were extremely scanty and in 2 cases none could be found. In all, gram-negative bacilli of the coli-typhoid type were present in nearly pure culture. In all but one, many of these gram-negative bacilli were found to be organisms of the non-lactose-fermenting group. In addition to these gram-negative bacilli, coccal forms were present in all cases and showed a much greater tendency to occur in long chains than they did in normal cases.

The one exceptional case in which

non/

non-lactose-fermenters were not present was one in which gelatin-liquefying members of the proteus group were found in large numbers. This case was examined again after recovery from the diarrhoea and the flora was found to be transformed. Gram-positive of the acid-tolerant group had reappeared in large numbers, gram-negative bacilli had markedly diminished, and liquefying organisms had disappeared.

Of the other cases, one was a child of four years and therefore it was not easy to guess how far its very varied flora differed from the normal. In this case, however, gram-positive bacilli of the acid-tolerant type were very scanty, gram-negative bacilli were numerous, and non-lactose-fermenting bacilli studied in this case did not correspond to any known varieties. They resembled the Para-typhoid-Gaertner group except that they did not form gas in sugar media, and agglutination tests with Para B. and Gaertner serum proved them to be quite different.

Of the other cases 4 were interesting as occurring in the ward at the same time, all starting after admission to the ward. It was thought that this/

this must be due to infection by one organism, but on examination of the cases the non-lactose-fermenting organisms isolated were found to be different in all the cases.

In two of these, Morgan's No.I. Bacillus was obtained, but on testing the strain from one case against a serum produced by vaccines from the strain isolated from the other case, they were found to be completely distinct. Yet this serum agglutinated highly, strains of Morgan's No.I. Bacillus from a normal case and from diarrhoea cases, though it did not agglutinate two other strains from diarrhoea cases. This is discussed later under Morgan's No.I. Bacillus.

From the third case, a bacillus having a resemblance to Morgan's No.I. Bacillus, but differing in being a rapid liquefier of gelatin was obtained, and this also was not agglutinated by Morgan's No.I. Bacillus Serum of a Titer of 1-5000.

From the fourth case a Bacillus having all the cultural characteristics of B.Dysenterial type "Y" of Hiss and Russell was obtained. On testing this against a Dysentery "Y" serum of a supposed Titer of 1-5000, it was completely agglutinated at 1-20, but no agglutination took place at 1-2000 nor 1/

1-5000. I think one is justified in regarding this as a member of the Dysentery group, but further agglutinating tests will be made to confirm its identity.

In these 4 cases, occurring at the same time in a ward, and supposed to be infected by the same cause or from one other, the non-lactose-fermenting bacillus present in such large numbers in each, was found to be different in each case.

In the seventh case the same condition was observed - a practical absence of bacilli of the acid-tolerant group, a large increase of gram-negative bacilli many of which turned out on isolation to be non-lactose-fermenters, and a fair number of coccal forms. In this case again a bacillus was obtained indistinguishable from Bacillus Dysenterial "Y". This bacillus was also tested against the same Dysentery serum and was also agglutinated at 1-20. This case was in the ward long after the previous case had gone out, but there was a possible connecting link to which I shall now refer.

This was a case referred to under the first group of diarrhoea cases. This case was admitted to/

to the ward on October the 9th, suffering from acute diarrhoea. On examination of the specimen next day, I was able to isolate Morgan's No.I.Bacillus in very small numbers. Three weeks later, the case being still in the ward, though much improved, I again examines a specimen of the motion and this time found Morgan's No.I. Bacillus in fairly large numbers, along with another bacillus which differed slightly from Morgan's No.I.Bacillus, (XXXIX.2). A month later the case, still in the ward, had another attack of diarrhoea and 3 months later, I examined its stools once more, and this time found in its motions large numbers of Bacilli culturally identical with B.Dysenteriae "Y", which also were agglutinated at upwards of 1 in 20 by dysentery serum.

The three cases in which Dysentery Bacilli were found, were thus connected to a certain extent, as both the other cases had been in the ward at the same time as this case.

Another case of the first group of diarrhoea cases was examined, after recovery from diarrhoea when it was found that gram-positive bacilli had reappeared/

reappeared in large numbers in its stools while the non-lactose-fermenting bacilli (XIX. 3) had disappeared.

A large number of strains of lactose-fermenting bacilli have been studied, and their reactions and identification are given in Table E.

Except in one case (X.) there was no reason to suppose that the strains present were abnormal varieties. Four were tested as to their pathogenicity on guinea-pigs, and were found to be less pathogenic than several isolated from healthy breast-fed infants.

A COMPARISON OF THE "NORMAL" AND DIARRHOEA CASES.

Two great differences between the diarrhoea cases and those not suffering from diarrhoea were observed.

The first was that in the diarrhoea cases the normally dominant gram-positive acid-tolerant bacilli were largely replaced by gram-negative bacilli of the colon-typhoid type.

The second was that several members of the non-lactose-fermenting group of organisms were present/

present in large numbers in diarrhoea cases, notably Morgan's No.I. Bacillus and Dysentery Bacilli.

It was also noticed that especially in the cases of ileo-colitis, streptococci replaced the usual intestinal diplococci.

Out of 21 normal cases, non-lactose-fermenters were obtained in 6. Two of these were Morgan's No.I. Bacillus, 3 were organisms resembling, but distinct from, the paratyphoid group, while one slightly resembled but was distinct from the dysentery group. One of these cases had later an attack of diarrhoea; 3 of these organisms were pathogenic to guineapigs, while 2 were not.

Of 14 cases of diarrhoea examined, 2 cases of the first group showed no members of the non-lactose-fermenting group. 3 cases from the first group showed Morgan's No.I. Bacillus in the stools, while, 2 showed unnamed non-lactose-fermenting bacilli.

Of the second group of cases, or cases of ileo-colitis, one showed members of the Proteus group which disappeared after recovery from the diarrhoea. The other six cases all showed members of the non-lactose-fermenting group of organisms.

These/

These were in one case a non-gas-producing bacillus resembling, but distinct from, the para-typhoid group, in 2 cases a dysentery bacillus, in 2 cases Morgan's No.I. Bacillus, and in 1 case an atypical organism which resembled Morgan's No.I. Bacillus, but liquefied gelatin.

The following table shows the cultured characteristics of these non-lactose-fermenting bacilli isolated from normal and diarrhoea cases. The tests are those advised by Lewis.

FROM/

FROM NORMAL CASES.

	Lactose.	Glucose.	Mannit.	Dulcit.	Saccharose.	Salicin.	Motility.	Indol.	Lit.Milk.	Gelatin.	Identity.
XXXI.1.	–	AG	AG	AG	–	–	+	–	A	–	Unnamed.
XXXII.1.	–	AG	AG	–	–	–	+	–	A	–	Unnamed.
XV.4.	–	AG	–	–	–	–	+	+	Alk	–	Morgan's No.I.
XLIX.1.	–	AG	AG	AG	–	–	~~+~~ –	+	A	–	Unnamed.
L.1.	–	AG	–	–	–	–	+	+	Alk	–	Morgan's No.I.
XLVIII.1.	–	AG	AG	AG	–	–	~~+~~ –	+	A	–	Unnamed.

The following table gives the reactions of the non-lactose-fermenting bacilli isolated from the diarrhoea cases, both during the attack & while convalescent.

All these organisms were put through these tests a second time some months later and all gave identical reactions except XXXIII. 4, (change in dulcit).

XIX./

	Lactose.	Glucose.	Mannit.	Dulcit.	Saccharose.	Salicin.	Motility.	Indol.	Gelatin.	Lit.Milk.	Identity.
XIX.3.	–	AG	AG	AG	–	AG	–	+	–	–	Unnamed.
XX.3.	–	A	–	–	–	–	+	–	–	Alk	Unnamed.
XXI.1.	–	AG	–	–	–	–	+	+	–	Alk	Morgan's No.I.
XXIV.6.	–	AG	–	–	–	–	+	+	–	Alk	Morgan's No.I.
XXIX.22.	–	AG	–	–	–	–	+	+	–	Alk	Morgan's No.I.
XXXIII.4.	–	A	A	A	–	–	+	–	–	Alk	Unnamed.
(XXXIX.1.	–	AG	–	–	–	–	+	+	–	ALk	Morgan's No.I.
(XXXIX.2.	–	AG	AG	–	–	–	+	+	–	Alk	Unnamed
(XLI.11.	–	AG	–	–	AG	–	+	+	+	A	Unnamed
(XLI.10.	–	AG	–	–	–	–	+	+	+	A	Unnamed
XLII.5.	–	AG	–	–	–	–	+	+	–	Alk	Morgan's No.I.
XLIII.6.	–	AG	–	–	–	–	+	+	–	Alk	Morgan's No.I.
XLIV.1.	–	A	A	–	–	–	–	–	–	Alk	Dysentery Bacillus
XLV.2.	–	A	A	–	–	–	–	–	–	Alk	Dysentery Bacillus
XLVI.1.	–	A	A	–	–	–	–	+	–	Alk	Dysentery Bacillus

These/

These results, so far as the non-lactose-fermenting bacilli are concerned, tally with those obtained by Morgan, and Lewis, working at his group of organisms in England during times of epidemic summer diarrhoea.

It will be noted that the cases from which my specimens were obtained occurred in Edinburgh, some being obtained during a very cold and wet summer (1912) others during the winter following.

We are thus justified in making the following broad statement: -

In the cases of diarrhoea examined bacilli of the acid-tolerant group have been much diminished.

Bacilli of the non-lactose fermenting group have been much increased, and of these the group of organisms known as Morgan's No.I. Bacillus has been most frequent.

A dysentery bacillus has been obtained in 2 cases of severe ileo-colitis (both of which died) and from a case of severe recurrent diarrhoea.

DISCUSSION/

DISCUSSION of the RESULTS.

It is one thing to prove the presence of an organism in a pathological condition. It is another to prove that the organism is the cause of the condition. The matter becomes still more complicated, when one is dealing, not with one organism but a group.

The first question that arises is this. Is the non-lactose-fermenting group of organisms capable of producing diarrhoea? The question can be answered in reference to, at least two of the organisms isolated from cases of diarrhoea, - the Dysentery Bacillus and Morgan's No.1 Bacillus.

The Dysentery Bacillus in subcutaneous or intravenous inoculation, produces severe inflammation of the intestine in rabbits, due, it is believed, to the action of its toxins. This has been especially studied by Flexner. Morgan's No.1 Bacillus was shown by Morgan to be capable of producing diarrhoea and death in monkeys, when administered by feeding.

On the other hand, Morgan's No.1 Bacillus has been found by both Morgan and Lewis in the stools of perfectly normal children, who had never suffered from/

from diarrhoea. This they thought was probably due to a variation in virulence between different strains. Further, it has been known for a short time that the name Morgan's No.1 Bacillus, includes more than one variety of organism, as can be shown by agglutination tests.

But I have been able to isolate Morgan's No.1 Bacilli from a case of acute diarrhoea, and from a perfectly normal case, which the agglutination tests proved to be identical.

Again Duval and Schorer have isolated the Flexner-Harris dysentery bacillus from the stools of two children in perfect health.

The fact that these organisms may be found in small numbers in children not suffering from diarrhoea, does not prove that they are not capable of causing diarrhoea. But I think it does prove one thing: and that is, that some intestines offer a much stronger defence against the attack of these organisms, than others do.

As a result of the study of my series of cases, I have come to be very strongly of opinion that part of the intestinal flora of infants, has a strong retarding influence on the growth of other organisms.

I wish, first of all, to lay very strong emphasis/

emphasis on the fact, that breast feeding is unanimously agreed to be the ideal form of feeding for infants, and it has been proved over and over again, that bottle-fed children are infinitely more susceptible to diarrhoea of the epidemic type, than are breast fed children. This has always been taken to be due to infection of cow's milk by organisms capable of producing diarrhoea.

There is, however, another side to the question. Morgan was not able to isolate his bacillus from cow's milk in infected houses, and, more striking still, he was unable to cultivate Morgan's No.1 Bacillus in unsterilised cow's milk, though he was able to cultivate it, in sterilised cow's milk. The reason of this was, that other organisms growing in the unsterilised cow's milk, are able to produce an acidity sufficient to inhibit the growth of Morgan's No.1 Bacillus.

This vulnerability to acid of Morgan's No.1 Bacillus, I wish to lay particular stress on.

I now wish to draw attention to the gram-positive-acid-tolerant group of bacilli. In the "ideal" intestines, namely those of breast-fed infants/

infants, these organisms are present in nearly pure culture. This group of organisms has several peculiarities which distinguish them from other intestinal organisms; one is, that they grow poorly or not at all in media from which sugar is absent, and the other is, that they are able to resist a greater strength of acid in culture, than any other variety of organism, intestinal or otherwise. They are, also, able to ferment sugar strongly, notably lactose and glucose, and thus are able to produce acid in considerable amount.

Mereshkowski and his pupils, from a prolonged study of this group of organisms, came to the conclusion, that they might have strong powers of inhibiting the growth of other intestinal organisms, and that they probably had as their role "the regulation of the intestinal flora".

In PART II. of this thesis, which contains the studies of organisms isolated by means of the technique described in PART III., will be found the description of a series of Experiments on the growth in sugar tubes of intestinal b.coli, Morgan's No.1 Bacillus, and the facultatively aerobic member of the acid-tolerant group, namely the Bacillus Acidophilus.

The/

The main results of these experiments are, that b.coli in a lactose solution, and even more quickly in a glucose solution, produce by fermenting the sugar, a sufficient acidity to kill themselves off within a certain number of days.

If b.coli and Morgan's No.1 Bacillus are grown in the tubes, the Morgan's No.1 Bacillus succumbs first.

If B.Acidophilus and the other two are grown in the tubes, Morgan's No.1 Bacillus succumbs, and then the b.coli leaving the Bacillus Acidophilus in pure culture in the strongly acid medium.

The next series of experiments are these. If faeces containing Morgan's No.1 Bacillus in extremely small numbers are emulsified in ordinary, comparatively sugar-free broth, McConkey Plates spread direct from this emulsion will show a pure growth of lactose fermenting colonies of colon bacilli. If a sufficient number of plates are spread, a relatively very small number of Morgan's No.1 will grow. If this sugar-free emulsion of faeces be incubated at 37°c. for 3 weeks, its reaction at the end of that time will be found to be slightly alkaline, and McConkey plates spread from the emulsion, will show/

show Morgan's No.1 Bacillus in pure culture or vastly dominant.

Therefore, in the absence of sugar, Bacillus acidophilus is not able to inhibit the growth of Morgan's No.1, which is to be expected, as in the absence of sugar, it can only grow with great difficulty. But, in the absence of sugar even bacillus coli is overridden by Morgan's No.1 Bacillus.

What would happen to a breast fed infant supposing he were to swallow some Morgan's No.1 Bacillus? Considering the nature of his intestinal flora, and the presence of milk sugar in his intestine, it is reasonable to suppose that the Morgan's No.1 Bacillus, would be quickly killed off in the slight acid intestinal contents of the breast fed child. But if, from some cause, the breast milk contained no sugar, the inhibiting flora would grow with difficulty and would not be able to produce acid. If the child now swallowed Morgan's No.1 Bacillus, this organism would be able to increase in large numbers and produce diarrhoea.

A bottle-fed child, in whose intestines the acid-tolerant flora is relatively less numerous than in those of a breast-fed infant, would offer a still/

still weaker resistance to the invasion of bacilli of the Morgan's No.1 Type.

I, therefore, put forward the following theory as to the etiology of infantile diarrhoea, based on the examination of these Specimens and on the study of isolated organisms.

That organisms of the Non-lactose-fermenting group, notably Morgan's No.1 Bacillus and the Dysentery Bacillus, may be present in the intestines of children without producing diarrhoea, provided the chemical condition in these intestines is such, as will permit the growth of the inhibiting flora of the intestine; that is, chiefly the gram-positive bacilli of the acid-tolerant group, and to a lesser extent, the coli group. But, if from some cause an alteration in intestinal conditions is produced, so that the inhibiting flora is reduced in numbers, or is not able to exercise its inhibiting action, then Morgan's Bacillus, or other members of the non-lactose-fermenting group, are able to increase in large numbers.

The effect of this increase in large numbers of this group of organisms, and of the substitution by them of the normal flora, will vary according to the virulence of the particular organism which has become dominant. If it is the Dysentery Bacillus/

Bacillus, a severe ileocolitis will probably be produced: if Morgan's No.1 Bacillus, acute diarrhoea may or may not be produced, according to the virulence of the strain. Other members of the non-lactose fermenting group (note especially the Specimens XLVIII & XLIX.) seem to be able to become very prevalent in the intestine without producing any bad results.

I submit, that it is only on a theory such as this, that are explainable my results in the four cases of ileo-colitis in Charteris Ward (XLI-XLIV) undoubtedly all due to one cause, where non-lactose fermenters were present in large numbers in all, but where the organism was different in each case. In the same way the presence of large numbers of different members of the group in epidemics of summer diarrhoea, as studied by Morgan and Lewis: the frequent isolation of two different members of the group from one case: the frequency of cases of "Double infection" in America, where two different types of dysentery bacilli were found, all are explainable by organisms of this group finding a suitable pabulum for their growth, with a lack of inhibiting influence at certain times in the infantile intestine.

The next question is, what is the nature of/

of the change, which destroys the inhibiting action of the protective flora? From a study of the acid-tolerant organisms and Morgan's Bacillus, it is evident that there is one very important factor in the production of this inhibiting influence, and that is the presence of sugar.

During hot summer weather, when diarrhoea is prevalent, milk is liable to contain a much larger number of organisms than during cold weather, and it is a matter of common knowledge, that many of these organisms are capable of "souring" milk.

This is interesting from 2 points of view. Firstly, milk during hot weather provides a very much less suitable pabulum for Morgan's No.1 Bacillus and other non-lactose fermenters, than does milk during cold weather. Therefore, if Morgan's No.1 Bacillus is communicated to bottle-fed babies in their milk, we ought to have outbreaks of epidemic winter diarrhoea, not of epidemic summer diarrhoea. Secondly, milk in hot weather may have a strongly acid reaction, when far short of being "curdled", and this acidity is produced by means of the sugar splitting organisms, mainly b.coli and streptococcus lacticus.

There is, as a rule, then, less sugar in milk/

milk during the hot weather by the time the baby gets it, than there is, during cold weather.

I do not wish to stretch this point too far, as it is very probable other factors are at work, but it is very certain that a deficiency of sugar in milk, would produce ideal conditions within the intestine, for an overgrowth of organisms of the Morgan's No.1 Bacillus type.

This would explain the very small incidence of diarrhoea among children on breast milk, in which the amount of sugar is very constant; and would explain the liability to diarrhoea of bottle-fed children, where the regulation of the amount of the sugar in milk is largely in the hands of the mother.

PREVENTION of DIARRHOEA.

The method of feeding which offers the best chances against attacks of diarrhoea is breast-feeding, in which the intestine shows a flora very strongly inhibiting the growth of pathogenic organisms.

In feeding with cows' mild, it seems to be extremely important that a sufficiency of sugar should be given. I am not able to say which sugars are/

are the best, but either lactose or glucose fulfils the requirements as shown by laboratory experiments.

In hot weather, cows' milk should be chilled as soon as obtained from the cow and kept in cans on the system of the Thermos flask at a low temperature. As far as diarrhoea is concerned, if this were done, no pasteurisation or sterilisation would be necessary.

TREATMENT of DIARRHOEA.

In addition to the usual methods of treatment, I would suggest, as Tissier did as long ago as 1906, that the infant should be given large amounts of lactose, and should also be fed daily on living cultures of the acid-tolerant group.

SUMMARY/

S U M M A R Y

SPECIMENS of faeces from 21 normal infants and young children on different methods of feeding have been obtained and their flora examined.

SPECIMENS have been obtained from 14 children duffering from diarrhoea, none being cases of epidemic summer diarrhoea.

4 SPECIMENS of faeces from 3 of these 14 children have been examined at a later date.

3 SPECIMENS of urine and faeces from cases of coli-pyelitis have also been examined.

Bacilli of the acid-tolerant group have been numerous in all the normal specimens, being present in almost pure culture in breast-fed cases. They have been very scanty and in some cases absent in cases of diarrhoea. After recovery from diarrhoea they have reappeared in the faeces.

Bacilli of the coli group have been present in very small numbers in breast-fed cases: in considerably larger numbers in bottle-fed, and in diarrhoea cases. There has been no difference in the type of the bacilli present in breast-fed children and in bottle-fed children: no difference in type between those/

those present in normal cases and those present in diarrhoea cases: and no difference in pathogenicity between those present in normal cases and those present in diarrhoea cases. A study of their reactions to McConkey's Tests has been made, and the conclusion arrived at that they are sufficiently stable.

Members of the non-lactose fermenting group have been isolated from 6 of the young cases examined, that is 6 out of 21. They have also been isolated from 2 out of 3 of the coli-pyelitis cases, who were older children. Two of these 6 showed Morgan's No.1 Bacillus. The other 4 showed unnamed types with a resemblance to the Paratyphoid-Gaertner group, but distinct from the members of this group.

Members of the non-lactose fermenting group have been isolated from 11 out of 14 cases of diarrhoea, and Bacillus Proteus in large numbers from a twelfth. Five of these 11 were Morgan's No.1 Bacillus 2 were a Dysentery Bacillus, while 4 were other unnamed forms. A dysentery Bacillus was also isolated from a case of diarrhoea, which had previously contained Morgan's No.1 Bacillus. Studies of their virulence have been made, and a study of the agglutination reactions of Morgan's No.1 Bacillus was also made.

Coccal/

Coccal forms were present in all the cases. In breast-fed cases, they occurred in small numbers as diplococci of moderately uniform appearance. In bottle-fed and older cases, their morphology was more varied and larger forms made their appearance. In the diarrhoea cases, coccal forms were always present and showed a tendency to grow in chains.

Spore-bearers have been present in a few normal cases, but were never seen except in extremely small numbers in diarrhoea cases.

Studies have been made of the influence upon each other, as regards growth of various groups of intestinal organisms under different conditions.

C O N C L U S I O N S .

(1). The flora of breast-fed infants consists mainly of bacilli of the acid-producing and acid-tolerant group, which have an inhibiting action on the growth of other organisms. Colon bacilli and enterococci are present in small numbers.

(2). The flora of bottle-fed infants contains moderate numbers of these inhibiting organisms, along with colon bacilli, coccal forms, and sometimes members of the non-lactose fermenting group, including Morgan's No.1 Bacillus.

(3). The flora of children suffering from diarrhoea contains large numbers of organisms of the non-lactose fermenting group, including Morgan's No.1 Bacillus and Dysentery bacilli. Colon bacilli and coccal forms, many in chains, are also present. The inhibiting flora is reduced greatly in numbers, sometimes absent.

(4). In cases of diarrhoea, the increase in numbers of non-lactose fermenting bacilli is secondary to a decrease in numbers and in inhibiting powers/

powers of the protective flora.

(5). The persistence of coccal forms and their occurrence in chains, is also secondary to the altered conditions within the intestine.

(6). It is possible that the decrease in numbers of the inhibiting flora is due to a deficiency of sugar in the diet.

(7). It is possible that a deficiency of sugar in cows' milk, during the hot weather, may be due to the sugar-splitting action of various organisms.

(8). Chilling of the milk and preserving it at a low temperature, would prevent this destruction of sugar.

(9). Cows' milk during the hot weather is a much less suitable pabulum for Morgan's Bacillus than it is during cold weather.

(10). The colon bacilli of breast-fed cases, bottle-fed cases, and diarrhoea cases, are all of the same/

same type, and there is no exaltation of virulence in diarrhoea.

(11). The reactions of this group toward McConkey's tests, are moderately stable.

(12). "Morgan's No.1 Bacillus" is a group, containing more than one organism.

(13). Spore-bearers played no part in any of the cases of diarrhoea.

PART II.

THE ACID-TOLERANT GROUP OF ORGANISMS.

The existence of a specially acid-resistant group of intestinal organisms has been known since 1900.

The first member of this group isolated was obtained by Tissier, (1) in 1899, and called by him later the Bacillus Bifidus. This bacillus was described by him as being a strict anaerobic, and he considered that the intestinal flora of breast-fed infants consisted of this organism is almost pure culture (3). Tissier was, however, not aware of the acid-tolerant properties of his organism, and, indeed at first denied strongly this characteristic attributed to the B. Bifidus by other workers.

Moro (2) was the first worker to discover the acid-tolerant properties of this group. In 1900 he isolated a gram-positive bacillus, which differed from the B. Bifidus in being a facultative aerobe, and which was able to resist an acidity in artificial culture sufficient to kill off B. coli and other intestinal organisms. This bacillus he called the Bacillus Acidophilus, which is a misnomer as the organism/

organism is acid-tolerant but not acidophil. Moro was of opinion that the gram-positive bacilli seen in such large numbers in the stools of nurslings were the B. Acidophilus, and not the B. Bifidus.

Since that time a large amount of work has been done on this group of organisms, and an extraordinary variety of opinions arrived at.

Tissier (3) in 1900 described another variety, a facultative aerobe, which he claimed to be distinct from the B. Acidophilus of Moro, and which is named the Bacillus Exilis.

Rodella (4) studied this group of organism in 1901. He described one variety which he thought might be distinct, as it constantly grew in gelatin, while the other varieties did not. He was able to isolate acid-tolerant bacilli from both breast and bottle-fed children, but he did not isolate the anaerobic Bacillus Bifidus.

Cahn (5) also in 1901, always found the Bacillus Acidophilus in the stools of children fed on the breast, though not in such large numbers as in children fed on cow's milk. He was of opinion that the B. Exilis of Tissier was not a distinct variety./

variety. He was able to isolate the Bacillus Bifidus from breast-fed children, and to a lesser extent from bottle-fed. He, however, said he was never able to get it in pure culture as it was always mixed with the B. Acidophilus, so I think we may take it that Cahn was unable to isolate the strictly anaerobic Bacillus Bifidus.

In 1905 a long and careful study of the acid-tolerant group of organisms was published by Mereshkowsky(6 - 9). This worker, with his pupils Bjeloussow, Obraszow, Podgaetzky, Trussow and Lukin, made in St. Petersburg an extensive study of acid-tolerant organisms isolated from the intestines of a wide variety of animals. They did not in any case isolate the anaerobic Bacillus Bifidus described by Tissier. Those which they isolated they divided into two classes, according to the appearance of colonies on plate culture (6). Type 1, was a smooth edged variety, and this Mereshkowsky considered to be the same as the Bacillus Bifidus of Tissier; but it was neither bifid nor anaerobic.

Type 2, showed colonies with a border, and this variety Mereshkowsky considered to be the B. Acidophilus of Moro. Type 2, grew better than/

than Type 1, on gelatin.

Obraszow (7) examined the faeces of a very large number of animals ranging from molenses to man and including a great number of varieties, cold and warm blooded, and was able to find either one type or the other in all.

Podgaetzky (7) examined the faeces of 8 human beings from one month old to 34 years, and found acid-tolerant bacilli in all.

They fed puppies on these organisms for long periods (8) and were able to transform the intestinal flora so that the faeces contained these organisms in almost pure culture. The motions became more loose, and their reaction strongly acid, but the puppies remained well.

These authors therefore, came to the conclusion that organisms of this group were innocuous, and that they probably had as their rôle the regulation of the intestinal flora.

Tissier (10) found the Bacillus Bifidus in breast-fed infants and in smaller numbers in bottle-fed children, in different parts of the intestine from the stomach to the arms. The Bacillus acidophilus/

acidophilus he also found in large numbers in breast-fed children and in small numbers in bottle-fed.

In 1906 Tissier (11) published a paper on the use of organisms of this group in cases where a harmful flora had replaced the normal one.

He referred to the action of bacilli of this group upon sugars, whereby acid was produced and harmful forms killed. In cases of infantile diarrhoea treated by means of cultures of Bacillus acidiparalactici alone or mixed with Bacillus Bifidus, and large amounts of lactose, he obtained good results. He also applied this method of treatment to older children and adults, keeping them on a strictly vegetarian diet, with good results.

Cohendy (12 - 16) published in 1906 a series of papers dealing with the transformation of the flora by a lactic ferment, and the treatment of colitis by this means. This research he had carried out as a result of Metchnikoff's publications on intestinal putrefaction.

In 1908 Jacobson (17) studied 5 cases; 4 breast-fed children and one bottle-fed.

He stated that one way to isolate the Bacillus Bifidus was by the use of acid media, and considered/

considered that the Bacillus Bifidus when perfectly pure needed a complete absence of oxygen for growth. He was, I believe, the first to point out that the B. Acidophilus grows with great difficulty on acidified solid media unless it is first grown in acidified fluid media.

Noguchi (18) in 1910 studied the growth characteristics of Bacillus Bifidus, and was able to transform it in the laboratory from the strictly anaerobic type (B. Bifidus) to the facultatively aerobic type (B. Acidophilus) and back again.

In 1911 Distaso (19) studied the acid-tolerant group, which he was the first to call by this name. He divided the group into 3 classes according to the kind of acid produced by the fermentation of sugars by these orgnisms i. acetic acid, ii, lactic acid, iii. bubyric acid.

The acetic-acid-producing group to which both the Bacillus Acidophilus and the Bacillus Bifidus belong, he called the Acetogenes group, and recognised two varieties only, the B. Acetogenes A. or B. Acidophilus of Moro, and the B. Acetogenes B. or B. Bifidus of Tissier.

He/

He considered that B. Bifidus was distinct from the B. Acidophilus in being a strict anaerobic, in showing true bifurcation, in never producing formic acid, and because it is agglutinable by a serum produced by vaccination with B. Bifidus whereas B. Acidophilus is not.

Distaso gave no details of his agglutination experiments.

The acetogenes group, he found, produced acetic acid, but also small amounts of lactic acid. The B. Acidophilus also produced formic acid.

To the third group of acid-tolerant organisms namely the butyric-acid-producers belong the Bacillus Welchü and one or two others.

Distaso found that the Bacillus Bifidus out grew the Bacillus Welchü and others in an acid medium.

He concluded that the acetogenes group by the production of acid inhibit the growth of other organisms.

Other varieties of the acid-tolerant group have been described by other workers. These different varieties were mainly founded on differences in morphology./

morphology. As these bacilli are extremely polymorph a classification founded on morphology is of no value, and, therefore, I do not more than allude to these so called varieties.

Thus one may say that in 1900 two forms were recognised, the B. Bifidus of Tissier which is a strict anaerobe, and the B. Acidophilus of Moro which is a facultative aerobe. That in the next few years several new varieties were described which were in turn discarded, and that 10 years later it was still a matter of opinion whether even the two original varieties were distinct.

In the present research members of the acid-tolerant group have been isolated with ease in practically all the bottle-fed normal children, and in normal children on mixed feeding. Between various strains there have been numerous differences.

In morphology it has been rare to find strains from different cases which closely resembled each other.

All the morphological variations which have been described under different names have been isolated, and, in addition, many of the morphological variations/

variations have been observed in the same bacillus when grown on different media and under different conditions.

Strictly anaerobic types and facultatively aerobic types have been isolated from both breast-fed and bottle-fed cases. The strict anaerobes have all grown in the presence of oxygen after some weeks culture on artificial media.

Several strains have been studied as to their action on carbohydrates, and differences have been observed in the fermenting power of different strains.

Differences have also been observed in (1) growth in glucose agar stab, (2) growth in gelatin at room temperature, (3) growth on potato, and (4) appearance of colonies on glucose agar plates.

I have not felt justified, however, in making a classification upon the strength of these differences, and indeed it is difficult to see what good purpose could be served by so doing. From the point of view of practical utility it is sufficient that these organisms are non-pathogenic, acid-producing, and acid-tolerant, and prefer a medium containing sugar and little oxygen.

A/

A point of some importance, however, is that in some cases on the breast only the faeces showed organisms of this type in practically pure culture. Yet when an attempt was made to isolate these organisms it was found to be extremely difficult as compared with bottle-fed cases in which these organisms were much less numerous; indeed in three of the breast-fed cases no organisms of the acid-tolerant group were isolated.

It is noteworthy that in these films the bacilli showed the irregularity of staining reaction, giving the so-called "punctate form of Bifidus", and were extremely degenerate looking.

This is, I think, explained by Tissier's opinion that practically all the bacilli of the acid-tolerant group which form such a large proportion of the flora of normal breast-fed children, are strictly anaerobic. In the lower part of the intestine they meet with oxygen, and, after passage of the motion, are exposed entirely to the air; as they have not the advantage of living in symbrosis with facultatively aerobic forms as is the case in children where the flora is more mixed, they die, and I believe that the so-called "punctate" forms of B. Bifidus/

B. Bifidus are in reality dead bacilli.

It is for this reason that I conclude that the strictly anaerobic type is the form met with in nearly pure culture in breast-fed cases.

With these reservations I describe the growth characteristics of those bacilli isolated and studied, under one group.

GROWTH CHARACTERISTICS.

In common they are gram-positive bacilli, are very polymorph, grow well at an acidity which kills off other organisms and ferment lactose and glucose with the production of acid.

MORPHOLOGY AND STRAINING.

All are gram-positive in young culture. In old forms bacilli may be seen which take up the counter stain, while others stain in a "punctate" manner, parts of the bacillus taking up the stain while parts remain unstained.

In morphology they vary extremely. Forms may be met with which resemble the diphtheria bacillus, while others are not far short of the B. aerogenes capsulatus in size.

In/

In bottle-fed children the type most commonly met with is a moderately regular, moderately slender straight or slightly curved bacillus which tends to lie in clusters side by side. This is a facultatively aerobic organism and is typical of the form described by Moro as the B. Acidophilus.

Another type is extremely irregular in shape and size. In pure culture spirilla, comma forms, long threads, coccal forms, straight and curved bacilli, may be seen. This type grows very poorly as a rule in the presence of oxygen, and is the type described by Tissier as the Bacillus Bifidus.

Another very common form is a straight slender delicate bacillus which was described by Tissier as the Bacillus Exilis.

Other forms are numerous, long slender threads may be seen, usually in old cultures. Rather plump squat bacilli were fairly frequently isolated from bottle-fed cases and cocco-bacillary forms were also met with.

If a regular bacillus (type acidophilus) is grown on media unfavourable to its growth, such as glycerin agar, or ordinary broth, very irregular forms such as are supposed to be characteristic of Bacillus/

Bacillus Bifidus will grow. This supports Distaso's theory that the forms of Bacillus Bifidus seen on artificial media are "formes de souffrance."

In no case, either in films or culture, have the bifid forms been seen from which the Bacillus Bifidus gets its name.

Bodies resembling spores may be sometimes seen in these bacilli, and occasionally they resisted pasteurisation at 80°c. before isolation from the faeces, but in pure culture these were killed by pasteurisation.

All forms examined for motility were non-motile

ON GLUCOSE AGAR,

All members grew well in the absence of oxygen, and the great majority grew well in its presence.

The colonies are minute, and are first visible between the second and third day as a rule. About the 4th day they attain the size of a streptococcus colony.

Three types of colonies were seen,

i. A smooth edged rather dense white type.

ii. A smooth edged very delicate type, and

iii/

iii. A peculiar type that resembled a little collection of tangled threads under the low power of the microscope.

Types i. and ii. under the low power are brownish and delicately granular.

IN GLUCOSE AGAR STAB.

The growth as a rule was visible before 48 hours. In most cases it grew right up to the surface though frequently heavier below than above, but in a few, a space of $\frac{1}{2}$ an inch with little or no growth was left at the top of the tube. On repeated subculture, however, all these organisms grew to the top of the tube.

Three main types of growth were observed,

i. A massive growth with heavy leaf-like out growths

ii. A delicate straight growth with some minute out growing colonies to be observed, some of them on stalks, and

iii. A vague ill-defined pale growth.

There is another type sometimes observed, where all the colonies near the top are very small, while those below are much larger. This always has the appearance of being a mixed growth, and is, I think/

think what Cahn was describing when he had what he described as mixed growths of B. Bifidus and B. Acidophilus. If, however, one breaks the tube and makes subcultures from the upper and lower parts respectively, replates these and picks out fresh colonies, and again puts these into glucose agar stabs the appearance is repeated. It is a facultative aerobe.

GLUCOSE BROTH.

Luxuriant growth takes place in this medium under anaerobic or aerobic conditions. A marked cloudiness is produced within 2 days with a white tenacious deposit. After about 10 days the supernatant fluid is fairly clear with a heavy deposit.

GLUCOSE BROTH + ·4% ACETIC ACID.

Growth in this is similar to growth in glucose broth. Some were tested in glucose broth + ·6% acetic acid in which all grew well, and in glucose broth + 1% acetic acid in which some grew while others did not.

ORDINARY AGAR SLOPES AND PLATES.

Some grew as well on this medium as on glucose agar, both aerobically and anaerobically, but as a rule growth was very poor or absent.

In most cases instead of growing as colonies with/

with a marked tendency to remain discrete as they do on glucose agar, a faint film of coalesced colonies was seen. It was usually difficult even under the low power to decide whether growth was present.

IN ORDINARY BROTH.

Growth in all cases was extremely poor or absent, aerobically and anaerobically.

ON GLYCERIN AGAR.

A growth of the same nature as on ordinary agar took place. Two grew strongly with discrete colonies.

ON POTATO.

Some grew luxuriantly with a raised white growth. Others grew invisibly but stained films showed growth to be present. Others did not grow at all.

IN GELATIN STAB. (At room temperature).

With some strains a delicate growth took place; with others no growth occurred. No liquefaction ever took place.

IN PEPTONE WATER.

No growth took place.

IN ORDINARY AGAR STAB.

A heavy growth occurred in some; in most a weak/

weak growth took place; in some none.

IN LITMUS MILK.

Of 8 strains studied in this medium, 6 produced acid and clot within a fortnight, 2 produced acid only.

On sugars in peptone water as a rule no fermentation or growth of any kind takes place. This, I take to be, because the medium is too alkaline for growth. If, however, a strain is introduced to a sugar peptone water tube along with another sugar-fermenting organism such as B. coli, a good growth takes place in the quickly acidified medium, and at the end of a certain time the acid-tolerant organism is left in pure culture.

The following were the reactions of 6 strains tested on sugars in broth.

	LACTOSE.		SACCHAROSE.		DULCIT.		INULIN.		GLUCOSE.		MANNIT.		SALICIN.		MALTOSE.		CASE FROM WHICH ISOLATED.
Days.	3	14	3	14	3	14	3	14	3	14	3	14	3	14	3	14	
XLII. 12.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	Diarrhoea.
XLII. 11.	–	A.	–	A.	–	–	–	A.	–	A.	–	–	–	–	–	A.	Diarrhoea.
XXXV. 14.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	Breast-fed Normal.
XXXV. 17.	–	A.	–	–	–	–	–	A.	–	A.	–	–	–	A.	–	–	Breast-fed Normal.
XXIX. 11.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	Diarrhoea.
XXXI. 8.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	A.	Bottle-fed Normal.

It will be seen from the above table that of the two organisms whose fermenting powers are slower and less universal than the others, one was isolated from a case of diarrhoea, the other from a normal breast-fed infant.

PATHOGENICITY.

A guineapig inoculated intraperitoneally with 4 cc. of a 4 days old glucose broth culture of XXXV. 17, (breast-fed) showed no ill efects.

Six mice fed for a week on bread soaked in glucose broth culture of XXXI. 8, (from a normal bottle-fed) showed no effect.

Two mice fed for 5 days on bread soaked in glucose broth culture of XXIX. 11, (from diarrhoea case) showed no effect.

THE/

THE LACTOSE-FERMENTING GROUP OF GRAM-NEGATIVE BACILLI.

A vast amount of work has been done on the intestinal organisms of this group, but unfortunately almost all the earlier work is now merely of historical interest.

Many early workers considered that intestinal B.Coli were the cause of diarrhoea, but there is no evidence of the true nature of the organisms with which they were working. Neither the Dysentery Bacilli nor Morgan's No I Bacillus had been discovered at this time, and it is possible that many so called B.Coli were organisms of this group.

Of late years there has been a tendency to regard intestinal B.Coli as being as a rule harmless and possibly even necessary, but others (20) have held that intestinal B.Coli may at times attain an exalted virulence and produce pathological results within the intestine.

McConkey's work (22 and 23) in the differentiation of intestinal B.Coli by means of the sugar and/

and other tests has shown that there are very numerous varieties of this group. Other workers, notably Twort, (21) have denied the value of McConkey's tests in the differentiation of organisms of this group on the ground that the reactions were not sufficiently stable.

McConkey himself regards the reactions as being sufficiently stable, even when the organisms are grown under very unfavourable conditions, and he is supported in his views by Savage (24) and Horrocks among others.

McConkey divided the lactose fermenting bacilli into 4 main groups according to their action on saccharose and dulcit, and further subdivided these groups by testing their motility, action on gelatin, production of indol, behaviour to Vosges and Proskauer's reaction, and action on adonit, inulin, and inosit.

By means of these tests 109 possible variations are separated. Actually McConkey only met with 36 varieties.

Out of 178 lactose fermenters isolated by McConkey (23) from human faeces, 42 were an organism called/

by McConkey No.71, 37 were the B.Coli Cummunis (No.34) 33 were B.Vesiculosus (No.5), while B.Neapolitanus (No.72), B.Schafferi (No.35), No.1, B.Grünthal (No.8) and B.Lactis Aerogenes (No.103) occurred with fair frequency. The other 28 varieties occurred with rarity.

In a previous work McConkey (22) examined the faeces of animals and human beings, and out of 480 coli-like organisms only 4 were the Bacillus Lactis Aerogenes.

He was unable to find any notable differences between the B.Coli of normal cases and those from 6 cases of diarrhoea.

In the present work I have endeavoured to ascertain:

I. Whether by the use of McConkey's tests one can differentiate between the Coliform organisms isolated from breast fed cases and those isolated from bottle fed and other cases.

II. Whether there is any difference between the Coli-like organisms isolated from normal cases and those isolated from cases of diarrhoea.

III./

III. Whether the reactions are stable as judged by a repetition or the tests after some months' cultivation on artificial media.

IV. Whether there is any difference in virulence to animals of the organisms isolated from diarrhoea cases and those from normal.

I also wished to ascertain if the agglutination tests of these organisms would confirm the differentiation into species by McConkey's series of reaction.

For this purpose four rabbits have been immunised for over a month by doses with three strains of coli-like organisms. Their sera, unfortunately have not yet acquired a sufficiently high agglutinating power for the purpose of differentiation between different strains, and the publication of the results must therefore be postponed.

The results of this research I have put in the form of tables.

All the organisms before being put through the series were plated at least once on McConkey Bile Salt Neutral Red Lactose Agar, most being plated twice. For inoculation of the various test media Agar Slope cultures not exceeding 3 days in age were used.

T A B L E   A.

REACTIONS OF LACTOSE-FERMENTING-BACILLI ISOLATED FROM NORMAL BREADT-FED INFANTS.

Number of Organism.	Motility. 4-6 hrs.	Indol 7 days.	Gelatin. 2 months	Lactose 7 days	Saccharose.	Dulcit.	Adonit.	Inulin.	Inosit.	Vosges & Proskauer. 7 dys.	Litmus Milk. 15 dys.	Name	No. of McConkey's Classification.
XXXV.2	−	+	−	AG	−	−	−	−	−	−	AC	B.Vesiculosus.	5.
XXXV.3	+	+	−	AG	AG	AG	−	−	−	−	AC	−	71.
XXXVII 14 A	+	+	−	AG	−	AG	−	−	−	−	AC	B.Coli Communis	34.
LI.1.	+	+	−	AG	−	−	−	−	−	−	AC	B.Grünthal	4.
LI.2.	+	+	−	AG	−	−	−	−	−	−	AC	B.Grünthal	4.
LI.3.	+	+	−	AG	−	−	−	−	−	−	AC	B.Grünthal	4.
LIV.1.	−	−	−	AG	AG	−	AG	−	AG	+	AC	B.Lactis Aerogenes	103.
LIV. 2 A.	−	−	−	AG	AG	−	AG	−	AG	+	AC	B.Lactis Aerogenes	103.
LIV. 2 B.	−	−	−	AG	AG	AG	AG	−	AG	+	AC	B.−	67.
LV.1.	−	+	−	AG	AG	−	−	−	−	−	AC	B.Coscoroba	107.
LV.2.	−	+	−	AG	AG	−	−	−	−	−	AC	B.Coscoroba	107.
LVI.1.	+	+	−	AG	−	AG	−	−	−	−	AC	B.Coli Communis	34.
LVI.2.	+	+	−	AG	−	AG	−	−	−		AC	B.Coli Com.	34.

The cases from which the organisms in the above table were isolated, were all healthy breast-fed children, their age varying from 3 to 10 days.

It will be seen that the Bacillus Lactis Aerogenes and a very closely allied form (No.67) were isolated from one case. Judging from the McConkey plates in this case a very large proportion of the lactose-fermenting bacilli were Bacillus Lactis Aerogenes or closely allied species. This tendency for one type of lactose-fermenting bacillus to dominate all other types of lactose-fermenting bacilli has been noticed in several cases. It will be noticed for example that in specimen LI. the three organisms selected at random proved to be identical, and similarly with specimens LV. and LVI. The deductions one draws from this will depend upon one's view of the stability of the reactions of the coli group on McConkey's test; thus one may deduce that the intestinal tract may have been originally infected by this type of bacillus, or on the other hand one may deduce that the particular conditions met with in thia intestine have moulded coli organisms to this particular type.

It will be noticed that 7 different varieties of colon bacilli have been isolated in these 6 breast cases. 3 of them were tested as to pathogenicity to guineapigs and all produced a fatal result.

T A B L E B.

REACTIONS OF LACTOSE-FERMENTING-BACILLI ISOLATED FROM NORMAL INFANTS ON BREAST AND BOTTLE.

Number of Organism.	Motility.	Indol.	Gelatin.	Lactose.	Saccharose.	Dulcit.	Adonit.	Inulin.	Inosit.	Vosges and Proskauer.	Litmus milk.	Name.	No. in McConkeys Classification.
XXXVI. 1.	–	+	–	A.G.	–	–	A.G.	–	–	–	A.C.	B.Acidi Lactici (Hüppe)	2
XXXVI. 2.	–	+	–	A.G.	–	–	A.G.	–	–	–	A.C.	B.Acidi Lactici	2
XXXVI. 3.	–	+	–	A.G.	–	–	A.G.	–	–	–	A.C.	B.Acidi Lactici	2
XXXVI. 9.	–	+	–	A.G.	–	–	A.G.	–	–	–	A.C.	B.Acidi Lactici	2.
XXXVIII. 3.	–	+	–	A.G.	A.G.	A.G.	–	–	–	–	A.C.	B.Neapolitanus	72
XXXVIII. 4.	–	+	–	A.G.	A.G.	A.G.	–	–	–	–	A.C.	B.Neapolitanus	72
XXXVIII. 5.	–	+	–	A.G.	A.G.	A.G.	–	–	–	–	A.C.	B.Neapolitanus	72
XXXVIII. 6.	–	+	–	A.G.	A.G.	A.G.	–	–	–	–	A.C.	B.Neapolitanus	72
LII. 1.	–	+	–	A.G.	–	A.G.	–	–	–	–	A.C.	B.Schafferi	35
LII. 2.	+	+	–	A.G.	A.G.	A.G.	–	–	–	–	A.C.	–	71

In the above table the reactions of 10 bacilli isolated from the faeces of 3 children on breast and bottle combined, are given.

It will be seen that the point noticed in breast-fed cases, that frequently all the colon bacilli isolated from one specimen are identical in their reactions, is again to be seen in this table.

Thus in Specimen XXXVI. the first 4 colon bacilli selected at random were found to be the Bacillus Acidi Lactici of Huppe, while from Specimen XXXVIII. the 4 colon bacilli were the Bacillus Neapolitanus.

The Bacillus Acidi Lactici was pathogenic to a guineapig, as was also the unnamed organism from Specimen LII. (No. 71 of McConkey).

A rabbit has been immunised with the Bacillus Acidi Lactici, 2 doses being given at interval of 10 days, both intraperitoneally. The first dose was 3 cc. of a 24 hours'broth culture, the second 4 cc. of a 24 hours'broth culture, both killed by heating at 56°c. for 1 hour in a water bath. Unfortunately the rabbit showed signs of illness a week after the second dose and had to be bled. The serum only agglutinates its own bacillus up to a titer of 1 in 20, so no fuller experiments are being made with it.

TABLE C.

REACTIONS OF LACTOSE-FERMENTING-BACILLI ISOLATED FROM "NORMAL" INFANTS ON BOTTLE ONLY.

Number of Organism.	Motility.	Indol	Gelatine.	Lactose	Saccharose.	Dulcit.	Adonit.	Inulin.	Inosit.	Vosges and Proskauer.	Litmus Milk.	Name.	No. in McConkey's Classification.
XI.1.	–	+	–	A.G.	A.G.	–	–	–	–	–	A.C.	B.Coscoroba	107.
XI.2.	+	+	–	A.G.	A.G.	–	–	–	–	–	A.C.	–	106.
XI.4.	+	+	–	A.G.	A.G.	A.G.	–	–	–	–	A.C.	–	71.
XI.5.	+	+	–	A.G.	A.G.	A.G.	–	–	–	–	A.C.	–	71.
XI.6.	+?	–	–	A.G.	A.G.	–	A.G.	–	A.G.	+	A.C.	B.Lactis aerogenes	103.
XVI.2.	–	+	–	A.G.	–	A.G.	–	–	–	–	A.C.	B.Schafferi	35.
XXXI.2.	–	+	–	A.G.	A.G.	A.G.	–	–	–	–	A.C.	B. Neapolitanus	72.
XL.1.	+	+	–	A.G.	–	A.G.	–	–	–	–	A.C.	B.Coli Communis	34.
XL.2.	–	+	–	A.G.	–	A.G.	–	–	–	–	A.C.	B.Schafferi	35.

TABLE C. (Cont.)

Number of Organism.	Motility.	Indol	Gelatin.	Lactose.	Saccharose.	Dulcit.	Adonit.	Inulin.	Inosit.	Vosges and Proskauer.	Litmus milk.	Name.	No. in McConkey's Classification.
XL.3.	+	+	–	A.G.	–	–	–	–	–	–	A.C.	B.Grünthal	4.
XL.4.	+	+	–	A.G.	–	–	–	–	–	–	A.C.	B.Grünthal.	4.
XLVII. 2.	–	+	–	A.G.	A.G.	A.G.	–	–	–	–	A.C.	B.Neapolitanus.	72.
XLVII. 3.	–	+	–	A.G.	A.G.	A.G.	–	–	–	–	A.C.	B.Neapolitanus.	72.
XLVII. 4.	–	+	–	A.G.	A.G.	A.G.	–	–	–	–	A.C.	B.Neapolitanus.	72.
XLVIII. 3.	+	+	–	A.G.	–	A.G.	–	–	–	–	A.C.	B.Coli Communis.	34.
XLVIII. 4.	+	+	–	A.G.	–	A.G.	–	–	–	–	A.C.	B.Coli Comunis.	34.
L.3.	–	+	–	A.G.	–	A.G.	–	–	–	–	A.C.	B.Schafferi.	35.
L.4.	–	+	–	A.G.	A.G.	A.G.	–	–	–	–	A.C.	B.Neapolitanus.	72.

Table C. shows the reactions of bacilli isolated from bottle-fed cases only. Specimen XI. was from a case which had had the breast once or twice, but artificial feeding had been so predominant that the Specimen is included in this list.

It will be seen that the varieties met with are not great, some types occurring with frequency.

In 18 organisms B. Neapolitanus has been met with 5 times, and other names are repeated 2 and 3 times.

Organism XI. 6, which is the Bacillus Lactis aerogenes was noted as being " very slightly or not at all motile" at the first examination; at the second, some months later, no motility was observed.

TABLE D./

T A B L E D.

REACTIONS OF LACTOSE-FERMENTING-BACILLI ISOLATED FROM "NORMAL" YOUNG CHILDREN ON A MIXED DIET.

Number of Organism.	Motility.	Indol.	Gelatin.	Lactose.	Saccharose.	Dulcit.	Adonit.	Inulin.	Inosit.	Vosges and Proskauer.	Litmus milk.	Name.	No.in McConkey's Classification.
XIV.1.	+	+	–	A.G.	–	–	–	–	–	–	A.C.	B.Grün-thal	4.
XIV.2.	+	+	–	A.G.	–	–	–	–	–	–	A.C.	B.Grün-thal	4.
XIV.3.	+	+	–	A.G.	–	–	–	–	–	–	A.C.	B.Grün-thal	4.
XXX.8.	+	+	–	A.G.	A.G.	A.G.	–	–	–	–	A.C.	–	71.
XXXII.2.	+	–	–	A.G.	–	–	–	–	–	–	A.C.	–	7.
XXXII.3.	+	–	–	A.G.	–	–	–	–	–	–	A.C.	–	7.
XXXIV.7.	+	+	–	A.G.	–	A.G.	–	–	–	–	A.C.	B.Coli Communis	34.
XXXIV.8.	+	+	–	A.G.	–	A.G.	–	–	–	–	A.C.	B.Coli Communis	34.

See MS.

T A B L E E.

REACTIONS OF LACTOSE-FERMENTING-BACILLI ISOLATED FROM DIARRHOEA CASES.

Number of Organism	Motility.	Indol	Gelatin.	Lactose	Saccharose.	Dulcit.	Adonit.	Inulin.	Inosit.	Vosges & Proskauer.	Litmus Milk.	Name	No. in McConkey's Classification.
X.3.	+	+	+	AG	AG	AG	AG	–	AG	+	AC	–	–
X.4.	+	+	+	AG	–	–	–	–	–	+	AC	–	–
X.10.	+	+	+	AG	–	–	–	–	–	+	AC	–	–
X.17.	+	+	–	AG	AG	AG	AG	–	–	–	AC	–	–
X.18.	+	+	–	AG	AG	–	AG	–	–	–	AC	–	100.
X.19.	+	+	–	AG	AG	AG	AG	–	–	–	AC	–	–
X.20.	+	+	–	AG	AG	–	–	–	–	–	AC	–	106.
XVII.1.	+	+	–	AG	–	–	AG	–	–	–	AC	–	1.
XVII.2.	+	+	–	AG	–	–	AG	–	–	–	AC	–	1.
XVII.3.	–	–	–	AG	AG	AG	AG	–	AG	+	AC	–	67.
XVIII.1.	–	+	–	AG	AG	AG	–	–	–	–	AC	B.Neapolitanus.	72.
XVIII.2.	–	+	–	AG	AG	AG	–	–	–	–	AC	B.Neapolitanus.	72.
XIX.1.	+	+	–	AG	–	AG	AG	–	–	–	AC	–	33.

TABLE E. (Cont.)

Number of Organism	Motility.	Indol	Gelatin.	Lactose	Saccharose.	Dulcit.	Adonit.	Inulin.	Inosit.	Vosges & Proskauer.	Litmus Milk.	Name	No. in McConkey's Classification.
XIX.2.	+	+	–	AG	–	–	AG	–	–	–	AC	–	1.
XX.1	+	+	–	AG	–	AG	–	–	–	–	AC	B.coli Communis.	34.
XX.2	+	+	–	AG	–	–	–	–	–	–	AC	B.Grünthal.	4.
XXI.12	–	+	–	AG	–	AG	–	–	–	–	AC	B.Schafferi	35.
XXIV.1	+	+	–	AG	–	AG	–	–	–	–	AC	B.coli Communis	34.
XXIV.2	+	+	–	AG	–	–	–	–	–	–	AC	B.Grünthal.	4.
XXIX.1	+	+	–	AG	AG	AG	–	–	–	–	AC	–	71.
XXIX.2	+	+	–	AG	AG	AG	–	–	–	–	AC	–	71.
XXXIII. 1 A.	–	+	–	AG	–	AG	–	–	–	–	AC	B.Schafferi	35.
XXXIII, 1 B.	–	+	–	AG	–	AG	–	–	–	–	AC	B.Schafferi	35.
XXXIII.2	+	+	–	AG	–	AG	–	–	–	–	AC	B.Coli Communis	34.
XLI.1	+	+	–	AG	AG	–	–	–	–	–	AC	–	106.

T A B L E E. (Cont.)

Number of Organism	Moti-lity.	Indol	Gela-tin.	Lac-tose	Sacc-har-ose.	Dul-cit.	Adon-it.	Inul-in.	Inos-it.	Vosges & Pros-kauer.	Litmus Milk.	Name	No. in McConkey's Classifi-cation.
XLI.2.	+	+	–	AG	–	–	–	–	–	–	AC	B.Grün-thal.	4.
XLII.1.	+	+	–	AG	–	–	–	–	–	–	AC	B.Grün-thal.	4.
XLII.2.	+	+	–	AG	–	–	–	–	–	–	AC	B.Grün-thal	4.
XLIII.1.	–	+	–	AG	–	–	–	–	–	–	AC	B.Schaf-feri.	35.
XLIII.2.	–	+	–	AG	–	AG	–	–	–	–	AC	B.Schaf-feri.	35.
XLIV.6.	+	+	–	AG	–	AG	–	–	–	–	AC	B.Coli Communis	34.
XLV.3.	+	+	–	AG	AG	–	–	–	–	–	AC	–	106.
XLV.4.	+	+	–	AG	AG	–	–	–	–	–	AC	–	106.
XLVI.3.	–	+	–	AG	–	AG	–	–	–	–	AC	B.Schaf-feri.	35.
XLVI.4.	+	–	–	AG	AG	–	–	–	–	–	AC	–	–

The above 35 organisms were isolated from cases of diarrhoea.

On analysing the list, the following organisms were found to have been obtained both from normal and diarrhoea cases - (the numbers are those of McConkey's list).

Nos. 106, 67, 72, 34, 4, 71, 35.

In the diarrhoea cases 4 unnamed varieties have been met with which were not met with in normal cases, and which are not in McConkey's list. Three of these were obtained from one specimen X. 3, X.4, and X.17, while one was obtained from case XLVI. (XLVI.4). The following organisms isolated from cases of diarrhoea are in McConkey's list, but were not found in the normal cases -

Nos. 100. 1.33.

No.100 was got from case X.

As the last three organisms were present in cases examined by McConkey , we are left with 4 organisms from diarrhoea cases which so far as I know have never been met with normal cases, three of these being obtained from one case.

These 3 I came to the conclusion had a connection with the diarrhoea (see specimen X.) as on/

on examination of the motion during the height of the diarrhoea only gram-negative bacilli and some streptococci were present in the films, while on further study only gelatin-liquefying bacilli with varying reactions on sugars were isolated; on a second examination after recovery from the diarrhoea the gram-negative forms had decreased markedly in the films and no liquefying organisms were isolated. These I believe belonged rather to the Proteus than to the Coli group.

The fourth abnormal organism was obtained from a case of diarrhoea in which dysentery bacilli were present in large numbers and there was no reason to suppose that the lactose-fermenter had any causal connection with the diarrhoea.

Therefore in one case only of diarrhoea was there any reason to suspect that abnormal lactose-fermenting bacilli might be the cause of the diarrhoea.

The view has been held in some quarters that strains of colon bacilli normally present might in some cases become endowed with special virulence, and cause diarrhoea. This point is gone into in the following table. Fairly common strains have been selected.

PATHOGENICITY OF COLON BACILLI FROM DIFFERENT CASES.

The following table shows the effects of inoculation of guineapigs with strains of lactose-fermenters from normal and diarrhoea cases.

TABLE F.

Name of Organism	Strain	Case	Dose	Mode of Inoculation.	Result.
B.Lactis Aerogenes	LIV.1.	Normal Breast-fed.	2cc. of 24 hours broth	Intraperitoneal.Inoculation.	Death.
No.71 McConkey.	LII.2.	" with bottle	"	"	Death.
B.Coscoroba."	LV.2.	Breast-fed.	"	"	Death.
B.Grunthal.	LI.1.	"	"	"	Death.
B.Coli Communis	LVI.1.	"	"	"	No result
B.Acidi Lactici	XXXVI.1	Normal Breast & bottle	"	"	Death.
B.Grünthal.	XLII.1.	Diarrhoea.	"	"	No result
No.I.McConkey.	XIX.2.	Diarrhoea.	"	"	No result
B.Coli Communis	XX.1.	"	"	"	No result
B.Schafferi.	XXI.12	"	"	"	No result

Out of 6 organisms isolated from normal cases, 4 being breast-fed and two breast with bottle, 1 only was non-pathogenic while 5 produced a fatal result.

Out of 4 organisms isolated from diarrhoea cases, none produced a fatal result.

It will be noticed that both strains of B.Coli communis were non-pathogenic, while of the two strains of B.Grünthal tested the one isolated from the normal breast-fed case produced a fatal result while the one isolated from the case of acute diarrhoea did not.

There is therefore no evidence of any of these colon bacilli having been endowed with special virulence.

RELATIVE FREQUENCY OF DIFFERENT TYPES.

Some types of bacilli have occurred with much greater frequency than others. I have, however, not put this in the form of a table as it is evident that the results might be very misleading. Thus, in some cases, if one studied20 organisms they might all prove to be of the same type in which case a table including these 20 organisms would/

would markedly exaggerate the relative frequency of this type. I shall content myself by saying that the varieties found most frequently by McConkey in the faeces of man and animals have also been the varieties found most frequently by myself under normal and abnormal conditions in the faeces of young children.

These organisms are B.Coli Communis, B.Neapolitanus, B.McConkey No.71, B.Grünthal, B.Schafferi, B.Vesiculosus and B.Lactis Aerogenes.

THE STABILITY OF THE SUGAR REACTIONS AND OTHER TESTS OF McCONKEY.

Doubt has been cast by many, notably Twort, on the right to group varieties of colon bacilli into species on the strengh of their behaviour towards McConkey's tests. They base their objection on the opinion that these reactions are only temporary acquirements, and that therefore one is not justified in making a classification upon them.

In the following series of experiments I have put organisms through the tests twice, at an interval/

interval of several months.

All were kept on either ordinary agar slopes or in ordinary broth, being kept for the most part at ordinary room temperature. Before being put through the tests a second time, all were replated on McConkey's medium once more, and young agar slope cultures used for inoculating the test media.

32 strains have been tested twice, and the results are shown in the following table.

(I also hoped by means of agglutination tests to be able to confirm or disprove the identity of types grouped by McConkey's tests. For this, 3 other rabbits in addition to the one mentioned above have been immunised highly agglutinating, and the experiments have been postponed.)

TABLE/

TABLE G.

	Motility.	Indol.	Gelatin.	Lactose.	Saccharose	Dulcit.	Adonit.	Inulin.	Inosit.	Vosges and Proskauer.	Litmus milk.	
XX.1.	+	+	-	A.G.	-	A.G.	-	-	-	-	A.C.	
6 mths. after	+	+	-	A.G.	-	A.G.	-	-	-	-	A.C.	No change.
XX.2.	+	+	-	A.G.	-	-	-	-	-	-	A.C.	
6 mths. after		+	-	A.G.	-	-	-	-	-	-	A.C.	No change.
XIX.1.		+	-	A.G.	-	A.G.	A.G.	-	-	-	A.C.	
6 mths. after	+	+	-	A.G.	-	-	A.G.	-	-	-	A.C.	No change.
XIX.2.	+	+	-	A.G.	-	-	A.G	-	-	-	A.C.	
6 mths. after		+	-	A.	-	-	A.	-	-	-	A.	Change in gas production.
XXI.12.	-		-	A.G.	-	A.G.	-	-	-	-	A.C.	
6 mths. after	-	+	-	A.G.	-	A.G.	-	-	-	-	A.C	No change.
XXII.1.	-	+	-	A.	-	A.	-	-	-	-	A.C.	
6 mths. after	-	+	-	A.	-	A.	-	-	-	-	A.C	No change.
XXXVIII.3		+	-	A.G.	A.G.	A.G	-	-	-	-	A.C.	
4 mths after	-	+	-	A.G	A.G.	A.G.	-	-	-	-	A.C.	No change.
XXXVIII.4		+	-	A.G	A.G.	A.G.	-	-	-	-	A.C.	
4 mths. after	-	+	-	A.G.	A.G.	A.G.	-	-	-	-	A.C.	No change.
XXXVIII.5		+	-	A.G.	A.G.	A.G.	-	-	-	-	A.C.	
4 mths. after	-	+	-	A.G.	A.G	A.G.	-	-	-	-	A.C.	No change.
XXXVIII.6		+	-	A.G.	A.G.	A.G.	-	-	-	-	A.C.	
4 mths. after	-	+	-	A.G.	A.G.	A.G.	-	-	-	-	A.C.	No change.
XL.1.		+	-	A.G.	-	A.G.	-	-	-	-	A.C.	
3 mths. after	+	+	-	A.G.	-	A.G.	-	-	-	-	A.C.	No change.
XL.2.	-	+	-	A.G.	-	A.G.	-	-	-		A.C.	
3 mths. after	-	+	-	A.G.	-	A.G.	-	-	-	-	A.C.	No change.
XL.3.		+	-	A.C.	-	-	-	-	-		A.C.	
3 mths. after.	+	+	-	A.G.	-	-	-	-	-	-	A.C.	No change.

T A B L E G. (Cont.)

	Motility.	Indol.	Gelatin.	Lactose.	Saccharose.	Dulcit.	Adonit.	Inulin.	Inosit.	Vosges and Proskauer.	Litmus milk.	
XL.4.		+	−	AG	−	−	−	−	−	−	AC	
3 mths. later.	+	+	−	AG	−	−	−	−	−	−	AC	No change
XL.1.	−	+	−	AG	AG	−	−	−	−	−	AC	
6 mths. later.	−	+	−	AG	AG	−	−	−	−	−	AC	No change
XI.4.	+	+	−	AG	AG	AG	−	−	−	−	AC	
6 mths later.	+	+	−	AG	AG	AG	−	−	−	−	AC	No change
XI.5.	+	+	−	AG	AG	AG	−	−	−	−	AC	
6 mths. later.	+	+	−	AG	−	AG	−	−	−	−	AC	One change
XLII.1.	+	+	−	AG	−	−	−	−	−	−	AC	
3 mths. later.	−	+	−	AG	−	−	−	−	−	−	AC	One change
XLII.2.	+	+	−	AG	−	−	−	−	−	−	AC	
3 mths. later.	+	+	∓	AG	AG	−	−	−	−	−	AC	One change
XLIII.1	−	+	−	AG	−	AG	−	−	−	−	AC	
3 mths. later.	−	+	−	AG	−	AG	−	−	−	−	AC	No change
XLIII.2.	−	+	−	AG	−	AG	−	−	−	−	AC	
3 mths. later.	−	+	−	AG	−	AG	−	−	−	−	AC	No change
XLIV.6.	+	+	−	AG	−	AG	−	−	−	−	AC	
3 mths. later.	+	+	−	AG	AG	AG	−	−	−	−	AC	One change
XXXVI.1.	−	+	−	AG	−	−	AG	−	−	−	AC	
4 mths. later.	−	+	−	AG	−	−	AG	−	−	−	AC	No change
XXXVI.9.	−	+	−	AG	−	−	AG	−	−	−	AC	
4 mths. later.	−	+	−	AG	−	−	AG	−	−	−	AC	No change

T A B L E G. (Cont.)

	Motility.	Indol.	Gelatin	Lactose.	Saccharose.	Dulcit.	Adonit.	Inulin.	Inosit.	Vosges and Proskauer.	Litmus milk.	
XIV.3.	+	+	–	AG	–	–	–	–	–	–	AC	
6 mths. later.	+	+	–	AG	–	–	–	–	–	–	AC	No change
XVIII.1.		+	–	AG	AG	AG	–	–	–	–	AC	
6 mths. later.	–	+	–	AG	AG	AG	–	–	–	–	AC	No change
XXX.8.	+	+	–	AG	AG	AG	–	–	–	–	AC	
4 mths. later.	–?	+	–	A	A	–	–	–	–	–	AC	3 changes
XXXI.2.	–	+	–	AG	AG	AG	–	–	–	–	AC	
4 mths. later.	–	+	–	AG	AG	AG	–	–	–	–	AC	No change
XXXIII.2	+	+	–	AG	–	AG	–	–	–	–	AC	
4 mths. later.	–	+	+	AG	–	AG	–	–	–	–	AC	One change
XXXIV.7.	+	+	–	AG	–	AG	–	–	–	–	AC	
4 mths. later.	+	+	–	AG	AG	AG	–	–	–	–	AC	One change
XXXIV.8.	+	+	–	AG	–	AG	–	–	–	–	AC	
4 mths. later.	+	+	–	AG	–	AG	–	–	–	–	AC	No change
XLI.1.		+	–	AG	AG	–	–	–	–	–	AC	
3 mths. later.	–	+	–	AG	AG	–	–	–	–	–	AC	No change

Out of 32 organisms 8 have shown changes. One showed a loss of the power of gas-production, one showed a change in dulcit-fermentation, 3 in saccharose, 2 in motility, while the last showed 3 different changes. 24 organisms showed no change at all.

S U M M A R Y.

I think that from the above and the preceding tables we may conclude:-

(1) That the sugars themselves are sufficiently stable: thus we may deduce from the fact that so often organisms isolated from the same case give identical reactions.

(2) That prolonged cultivation on the same artificial media has no tendency to mould colon bacilla to one particular type.

(3) That in the majority of cases the reactions of the colon group towards McConkey's tests are stable.

(4) That there is no evident difference between the varieties of the colon group present in/

in the intestines of breast-fed children and those present in artificially fed.

(5) That in no case of diarrhoea save one, was there evidence of the presence of unusual forms of colon bacilli which might be the cause of the diarrhoea.

(6) That in this one case the organism was rather of the Proteus than of the Colon group.

(7) That there is no evidence of an increased virulence of colon bacilli in cases of diarrhoea.

THE/

THE NON-LACTOSE-FERMENTING GROUP OF GRAM-NEGATIVE BACILLI.

The discovery of the dysentery bacilli of Shiga in Japan and by Flexner in Manilla first directed attention to this group of organisms in cases of infantile diarrhoea.

In summer of 1902 and 1903, a large number of workers in America, chiefly under the direction of Flexner at the Rockefeller Institute, examined the stools of a very large number of cases of epidemic infantile diarrhoea. These studies were conducted with the sole object of determining the presence or absence of the dysentery bacillus, and no attention was paid to other organisms, even to other organisms of the non-lactose-fermenting group. These workers were able to isolate dysentery bacilli, chiefly of the Flexner-Harris type, in 63% of the cases examined.

In attaching value to this result one must bear in mind that these workers had the one object in view of discovering the dysentery bacillus, and that cases are put down as giving a positive result when scores of plates had been made before even one dysentery bacillus was discovered.

As/

As previously mentioned, Duval and Schorer, following the same techinque were able to discover true dysentery bacilli in 2 perfectly healthy child-dren. Nevertheless the great prevalence of dysentery bacilli in the stools of children suffering from epidemic diarrhoea was proved.

Between 1905 and 1908 Morgan and his assistants made extensive studies of the stools of children suffering from epidemic summer diarrhoea, his attention being directed solely to the non-lactose-fermenting group.

As a result he found that Morgan's No.I. Bacillus was as prevalent in diarrhoea cases in England as the Dysentery Bacillus is in America. His results are briefly, that about 63% of children suffering from epidemic summer diarrhoea, contained Morgan's No.I. in the stools, and that the sera of many of these patients agglutinated the bacillus. He was able to give diarrhoea to monkeys by feeding them with strains of Morgan's No.I. Bacillus.

Morgan found, however, that there were many other varieties of non-lactose-fermenters which were present in fair frequency in these diarrhoea cases, which were very uncommon in normal. These organisms/

organisms were, roughly speaking, of three types, those resembling Morgan's No.I. Bacillus, those resembling dysentery bacilli, and those resembling the Gaertner group, while there were other quite atypical varieties.

Morgan was able to obtain Morgan's No.I. Bacillus in the stools of 5% of normal children during winter, and of 12% of healthy children during the epidemic season.

He was quite unsuccessful in discovering his bacillus in milk, and indeed was unable to get it to grow in unsterilised milk. He was, however, able to isolate it from flies.

Lewis in Birmingham made an extensive study of this group of organisms in the summers of 1910 and 1911. In the cold summer of 1910, in which the outbreak of summer diarrhoea was slight, he found non-lactose-fermenting bacilli in 77% of cases of summer diarrhoea: while in normal cases they were present in only 14%. Nearly half of those from the diarrhoea cases were Morgan's No.I. Bacillus while several showed bacilli with the cultural characteristics of Flexner's dysentery bacillus/

bacillus, and there were also various numerous varieties.

In 1911, in which there was a serious outbreak of summer diarrhoea, he found non-lactose-fermenting bacilli in the stools of 95% of children suffering from epidemic diarrhoea, and of 38% of children not suffering from diarrhoea. The enormous majority of the non-lactose-fermenting bacilli obtained from both normal and diarrhoea cases were Morgan's No.I. Bacillus, or closely allied forms.

He found that Morgan's No.I. Bacillus was considerably more pathogenic to mice by feeding than were other members of the non-lactose-fermenting group; but the strains of Morgan's No.I.Bacillus isolated from normal cases were fully as virulent as those isolated from diarrhoea cases.

He was able to isolate Morgan's No.I. Bacillus from a few samples of milk, but other non-lactose-fermenters were equally numerous. He was also able to isolate it from the intestines of normal mice.

By agglutination tests he found that all strains of Morgan's No.I. Bacillus were not identical

(I/

(I may say that I had noticed this independently before Lewis' work was published).

Bacilli resembling the Dysentery bacilli of Flexner-Harris type, he found with rarity in 1911, but bacilli resembling Shiga except in being motile were common.

In 1909 and 1910, Williams Murray and Rundle found in a few cases of epidemic summer diarrhoea an organism having the cultural characteristics of the Paratyphoid-Gaertner group but proved by agglutination reactions and absorption tests to be distinct from other members of this group. They were unable to find this bacillus, which they called Bacillus "F", in the stools of normal children.

In the cases of diarrhoea and ileo-colitis studied by me, none of them being cases of epidemic summer diarrhoea, the same group of organisms has been found, in some in very large numbers.

Morgan's No.I. Bacillus has been found in 2 absolutely healthy intestines, and in 1 of them was proved by agglutination tests to be identical with a strain isolated from a case of acute diarrhoea. It has been isolated from six specimens from 5 cases of diarrhoea.

Dysentery/

Dysentery bacilli have not been isolated from any normal cases. Bacilli, proved by agglutination tests to be of this group, have been found in 2 severe cases of ileo-colitis both of which died, and from a case which at previous examinations had shown Morgan's No.I. Bacillus in its stools.

Another bacillus (XX.3.) resembling Shiga but motile was found in large numbers in a case of acute diarrhoea: this organism was found by both Morgan and Lewis in several cases of summer diarrhoea.

An organism XIX.3. and XXXIV.3. was found in both a diarrhoea case and a normal case. Similar organisms were found by Morgan and Lewis in normal cases and in cases of diarrhoea.

Several strains resembling culturally the Paratyphoid-Gaertner group, but showed by agglutination tests to be distinct, have been isolated from normal cases.

A similar strain has been isolated by Lewis from a normal case and from a case of diarrhoea.

A strain (XXXIII.4.) which resembled the Paratyphoid/

Paratyphoid-Gaertner group but did not form gas was isolated from a case of acute diarrhoea. It was proved by agglutination reactions to be distinct from this group. This organism was not isolated by Morgan or Lewis.

The reactions of these isolated bacilli are given in tables in Part I. of the thesis under the discussion of diarrhoea.

PATHOGENICITY.

In studying the pathogenicity of different organisms at first the subcutaneous inoculated of agar slope cultures was adopted.

3 strains of Morgan's No.I. Bacillus from diarrhoea cases, 2 from normal cases, and 2 other non-lactose-fermenters from a case of diarrhoea (XIX. 3 and 4) were inoculated in this way, the whole of a 24 hours' culture being used, without producing more than superficial ulceration.

3 mice were fed on strains of Morgan's bacillus from diarrhoea cases, with no effect.

A mouse fed for 5 days on organism XXXIII.4 died.

The results of larger doses of non-lactose-fermenters inoculated intra-peritoneally to guinea-pigs/

guinea-pigs is given in the following table.

Guinea-pigs were used in all cases.

PATHOGENICITY OF NON-LACTOSE-FERMENTING BACILLI.

Number of Organism.	Name if any.	Case.	Method of Inocula-tion.	Dose of 24 hrs. broth.	Result.
XLV.1.	Dysen-tery B.	Diarr-hoea.	Intraper-itoneal.	2 cc.	Death.
XLVI.1.	"	"	"	"	"
XLIV.1.	"	"	"	"	"
XLII.5.	Morgan's No.I.	"	"	"	Death.
XLIII.4.	"	"	"	"	"
XXXIX.1.	"	"	"	"	"
XXIV.5.	"	"	"	"	Non-fatal.
XXI.5.	"	"	"	"	"
XLI.11.	–	"	"	"	"
XLVIII.1.	–	Normal	"	"	Death.
XLIX.1.	–	"	"	"	"
L.1.	Morgan's No.I.	"	"	"	Non-fatal.
XV.4.	"	"	"	"	"
XXXI.1.	–	"	"	"	"
XXXII.1.	–	"	"	"	"

It will be seen from the table that if we except the dysentery bacilli, not much information is to be derived from the relative pathogenicity to guinea-pigs of organisms from normal and diarrhoea cases.

AGGLUTINATION EXPERIMENTS WITH MORGAN'S NO.I. BACILLUS.

A rabbit was immunised with repeated doses of Morgan's No.I. Bacillus from a case of acute diarrhoea(XLII.6.) This organism was emulsified in saline, killed in a water bath at 56°c. for 1 hour and inoculated intra-peritoneally. 6 doses were given at intervals of 10 days, increasing from $\frac{1}{4}$ of an agar slope culture to two large platefuls.

At the end of two months vaccination, its serum was found to agglutinate its own bacillus at a dilution of 1 in 5000.

The following table shows the effect of this serum on Morgan's No.I. Bacilli from other cases. ++ stands for complete agglutination at the end of 2 hours' incubation, the macroscopic method being used.

SERUM XLIII.6.

	Case.	$\frac{1}{20}$ Dilution.	$\frac{1}{100}$	$\frac{1}{1000}$	$\frac{1}{5000}$	Control in Saline.
XLIII.6.	Diarrhoea.	++	++	++	++	0
XLIII.4.	Diarrhoea.	++	++	++	++	0
XLII.5.	Diarrhoea.	0	0	0	0	0
XXIV.5.	Diarrhoea.	++	++	++	++	0
XXXIX.1.	Diarrhoea.	0	0	0	0	0
XXI.5.	Diarrhoea.	0	0	0	0	0
XV.4.	Normal.	++	++	++	++	0

All these organisms had all the cultural characteristics of Morgan's No.I. Bacillus.

It is therefore evident that Morgan's No.I. Bacillus contains more than one variety of organism

The serum was tested also against organisms XXXIX.2. and XXXII.1. neither of which are Morgan's No.I. Bacillus, with negative results.

The following table shows the reaction of 4 organisms to sera of the paratyphoid-Gaertner group. Three of these organisms, XXXI.1, XLVIII.1, and XLIX.1, were isolated from normal cases and had all the cultural characteristics of Paratyphoid-Gaertner group. The 4 organisms isolated from a case/

case of acute diarrhoea, differed in not producing gas. (XXXIII.4.)

These sera had titers ranging from 1 in 4000 to 1 in 10,000.

	Serum Para B.			Serum Para A.	Serum Gaertner.
	$\frac{1}{100}$	$\frac{1}{1000}$	$\frac{1}{2000}$	$\frac{1}{100}$	$\frac{1}{200}$
XXXIII.4.	0			0	0
XXXI.1.		0		0	0
XLVIII.1.		0		0	0
XLIX.1.		0		0	0

These tests therefore give completely negative results. Aertrych serum has not yet been obtained, but they will be tested against it shortly.

The three organisms having the characteristics of the dysentery group, isolated from 3 cases of severe diarrhoea, were tested against the serum of Dysentery Bacillus Type "Y" of Hiss and Russell. The titer of this serum is 1-5000.

	1-20.	1-2000.	1-5000.	Control in Saline.
XLIV.1.	++	0	0	0
XLV.1.	++	0	0	0
XLVI.1.	++	0	0	0

It is evident they are not Type "Y" of Hiss and Russell as they do not agglutinate at 1 in 2000 (½ titer), but they agglutinate at 1 in 20 therefore I think that one is justified in calling them members of the Dysentery group. They will be tested shortly against serum of the Flexner-Harris Dysentery Bacillus.

EXPERIMENTS/

EXPERIMENTS have been carried out upon the growth of Morgan's No.I Bacillus along with other intestinal organisms and these are given separately.

RESULTS.

Non-lactose-fermenting bacilli have been obtained from 6 out of 21 normal specimens: two of these were Morgan's No.I Bacillus.

Non-lactose-fermenting bacilli have been obtained from 11 out of 14 diarrhoea specimens: 5 of these were Morgan's No.I Bacilli, 2 were Dysentery Bacilli. The others were unnamed varieties.

A third dysentery bacillus was obtained from a convalescent case of diarrhoea from which Morgan's No.I Bacillus had been obtained on two previous examinations.

3 Non-lactose-fermenting bacilli from normal cases and 1 from a diarrhoea case resembled closely the Paratyphoid-Gaertner group, but agglutination tests proved them to have no connection with B. Paratyphosus B., B.Paratyphosus A., or B.Gaertner.

No very marked difference in virulence was noticed/

noticed between organisms isolated from normal cases, and those from cases of diarrhoea.

Morgan's No.I Bacillus was shown by agglutination experiments to contain more than one variety.

The actions on members of the group on sugars was shown to be stable by repetition at the end of a few months.

THE COCCAL FORMS.

The presence of gram positive cocci in the intestinal tract is a fact which has been known for a very long time.

The first description of the cultural characteristics of the common intestinal diplococcus was given by Thiercelin, in 1899. He described this as an organism capable of becoming pathogenic, and he believed it was identical with an organism isolated by him from a case of cerebro spinal meningitis. He described his enterococcus as being highly pathogenic to mice, slightly to rabbits, and not at all to guinea-pigs.

He believed that this organism was capable of producing appendicitis, and claimed to have isolated it from the pus of an appendicular abscess.

Much work has been done on the coccal forms since this time. Unfortunately the subject is at present in a state of great confusion, owing to some authors calling one organism by several names while a still larger number call several organisms by one.

The enterococcus has been accused of producing a wide variety of conditions, in which either this/

this organism or a similar organism has been found, such as intestinal catarrh, infectious jaundice, chronic broncho pneumonia, dysentery, meningitis, post-typhoid suppuration, myelitis, otitis, conjunctivitis, vaginitis, methritis, etc. It has also been believed to be identical with the diplococcus rheumaticus, and it has been thought that possibly chorea was due to infection from the intestinal tract by this organism.

In cases of acute diarrhoea in infants, diplococci and strepto-cocci have been frequently seen in large numbers in the stools, and various authors, chiefly Borker and Escherich, have considered strepto-coccal infections to be the primary cause of the diarrhoea. Lately there has been more tendency to regard strepto-cocci as secondary invaders in cases of diarrhoea.

One fact only is certainly known: that gram positive diplocci of somewhat varied morphology form a constant part of the intestinal flora of young children.

Whether the enterococcus of Thiercelin is identical with the strepto-coccus enteritidis of Hirsch-Libmann/

Hirsch-Libmann, and whether both or either are the same as the strepto-coccus lacticus, and what the relation of these is to the strepto-coccus faecalis, and whether the strepto-cocci seen in cases of diarrhoea are foreign varieties, is at present quite unsettled.

Gram positive cocci which turn out to be staphylococcus albus are always present in the stools of nurslings and young children. It is probable that these are mainly contaminations from the skin.

In the present research I have been able to study three different types of cocci. The first type is a small gram positive diplococcus which is the enterococcus of Thiercelin. This type has been isolated from every case examined except one or two of the cases of very acute diarrhoea.

The second type is a considerably larger coccus; also gram positive. This has been met with in the stools of all the older children and also in smaller numbers in the stools of young bottle fed children. It has never been seen in the stools of children on the breast only.

This organism I have called in the descriptions/

descriptions "The Oval Gram Positive Coccus."

Another form which I think is the same organism resembles this last organism but occurs in pairs.

The third type has been met with in cases of acute diarrhoea associated with members of the non-lactose fermenting group. Sometimes they have occurred in diplos but frequently also in chains. On isolation of these only rather coarse diplococci have been obtained even in fluid media, and I have come to the conclusion that the occurrence of these cocci in chains in the faeces of diarrhoea cases is not evidence of their being a new form, but is simply the common phenomenon of diplococci growing in chains in a fluid medium.

These types of organisms have the following growth characteristics.

DIPLOCOCCI OF THE ENTEROCOCCUS TYPE.

The organisms which come under this heading show in the films of faeces a varied morphology. They/

They occur mainly as diplococci resembling somewhat pneumo-coccus but as a rule a little larger and plumper. They never occur in chains in the faeces but in the fluid media they sometimes do so, therefore the chains of cocci seen in the loose stools of diarrhoea cases may quite well belong to this group.

On solid media they almost always occur in pairs, sometimes with a few clusters, and occasionally a few short chains may be seen. The individual elements vary considerably in size and shape. Usually lanceolate, they are sometimes quite round, and may become reniform with adjacent flattened surfaces. Frequently the elements of each pair form an acute angle.

In fluid media occasionally fairly long chains may be seen. As a rule, however, even in fluid media they occur as diplococci.

They are always gram-positive except in old culture when they may become decolourised.

In almost all the cases an organism has been isolated from the faeces after pasteurisation at 80°C.

$80^{o}C$. for 10 minutes, which I see no reason to suppose is a different organism. This organism when pure is killed by pasteurisation. This has been noticed in the case of several other organisms - that before isolation from the faeces it is able to resist a much greater degree of heat than it can after isolation

Is a facultative anaerobe.

IN ORDINARY BROTH.

A not very strong growth was usually seen in 24 hours, consisting of slight cloudiness and a rather feeble deposit.

IN GLUCOSE BROTH.

In 24 hours marked cloudiness with a heavy deposit.

ON AGAR SLOPE.

A growth of minute spherical smooth edged dew-drop-like colonies of the usual strepto-coccus type.

ON BLOOD SERUM SLOPE.

Ditto.

ON BLOOD AGAR SLOPE.

Ditto.

IN/

IN PEPTONE WATER.

No growth.

IN GLUCOSE AGAR STAB.

A good growth always took place all down the stab.

IN GELATIN STAB. (room temp.)

A delicate growth all down the stab with no liquefaction.

IN LITMUS MILK.

Some strains produced acid and clot, others acid only.

5 strains were tested in different sugars in peptone water: no two strains gave identical reactions though all seemed identical in other ways and were from normal cases. The attempt to differentiate these organisms by means of sugar tests was abandoned.

Two mice fed for 5 days on bread soaked in broth cultures of enterococci showed no effect. One guineapig inoculated subcutaneously with 2 cc of the whey of a culture in milk showed a superficial ulcer with complete recovery.

The second type of coccus, namely the larger oval/

In/

oval gram positive coccobacillus isolated from bottle fed and older cases, showed in cultures identical growths with those obtained from the enterococcus: its pathogenicity was not tested. It always tended to grow in clusters,or isolated.

The other large gram coccus was like the last, but frequently grew in pairs; it may have been the same organism.

The streptococci in the diarrhoea cases when isolated always showed one or other of the above forms, and did not show any greater tendency, as a rule, to grow in chains.

CONCLUSIONS

Coccal forms have formed part of the flora of all cases studied.

In the breast fed cases these have all been gram positive diplococci of the enterococcus type.

In the bottle fed and older cases the coccal forms have shown a much more varied morphology and a rather large oval gram positive coccobacillus has been frequently met with.

In/

In the diarrhoea cases streptococci in chains have been seen in the films.

I am of opinion that these are not new varieties, but are intestinal diplococci whose morphology has been altered by the abnormal condition of the intestinal contents in diarrhoea.

SPORE BEARING BACILLI.

The Bacillus enteritidis sporogenes was first isolated from the stools of infants suffering from diarrhoea by Klein, and he was of opinion that an epidemic studied by him was due to this organism.

Tissier has described the Bacillus enteritidis sporogenes or Bacillus Perfringeus, as being met with in stools of older children on a mixed diet, but considered that there was a form of subacute enteritis in children, which is due to this organism.

He cited as proof, the following case - A healthy mother with a healthy infant A., suckled another child B. suffering from diarrhoea and vomiting with this organism in the stools. Infant A. contracted diarrhoea and vomiting and showed this organism in its stools also.

Apart from these two workers, I have not come across any other literature in which this organism has been cited as a cause of infantile diarrhoea.

In the present study, spore bearing organisms were only seen in a very small number of cases, and/

and were not isolated from any case. In the diarrhoea cases, they were never present, except in extremely small numbers in one case.

EXPERIMENT/

EXPERIMENT A.

GROWTH of B. COLI in LACTOSE PEPTONE WATER.

A fermentation tube containing about 5 cc. of lactose peptone water was inoculated with one loopful of an agar slope culture of an intestinal bacillus coli from a normal infant. The strain selected was XII.2, which is No.71 (McConkey), one of the most common of the intestinal coli.

From this, McConkey Plates were spread at intervals of 24 hours at first, one loopful of the shaken-up lactose peptone water being always used. Later, plates were made at less frequent intervals. The number of the colonies on the McConkey Plate, after 2 days' incubation, was taken as a rough indication of the number of living b.coli present in the lactose peptone water fermentation tube at the time of spreading the plate.

IN 1 DAY -

acid and gas were produced. The McConkey plate spread, showed after 2 days' incubation, a very thick growth of lactose fermenting colonies.

IN 2 DAYS -

The/

THE acidity was more pronounced and the amount of gas was greater, The plate showed a rich growth of lactose fermenting colonies.

In 3 DAYS. The tube showed the same appearance as yesterday. The plate showed a rich growth of lactose fermenting colonies.

IN 4 DAYS. The tube and plate were the same as yesterday's.

IN 5 DAYS. The gas in the tube seemed to be rather smaller in amount than yesterday. The plate showed a slightly thinner growth of lactose fermenting colonies.

IN 7 DAYS. The plate showed an appearance similar to that at the end of 5 days.

IN 9 DAYS. No marked change, perhaps slightly thinner.

IN 11 DAYS. The plate showed a thin growth of lactose fermenting colonies, all now well isolated.

IN 14 DAYS. The plate showed a thin growth of lactose fermenting colonies, considerably thinner, than at the end of the 11th day.

IN 18 DAYS. 90 Lactose-fermenting-colonies were present.

CONCLUSION.

Intestinal/

Intestinal B. coli in pure culture in lactose peptone water are capable of living for eighteen days at 37^{0} c. though much reduced in numbers.

The appearance of individual colonies on McConkey's medium is unchanged during the fortnight's growth.

EXPERIMENT B.

GROWTH of B. COLI in GLUCOSE PEPTONE WATER.

This experiment was carried out in exactly the same manner, the same organisms being used. The only difference was that the tube contained glucose instead of lactose.

IN 1 DAY. Tube showed acid and gas. The plate showed a rich growth of lactose fermenting colonies.

IN 2 DAYS. The tube showed an increase of acid and gas. The plate showed a rich growth of lactose fermenting colonies.

IN 3 DAYS. The tube showed an unchanged appearance. The/

The plate again showed a rich growth.

IN 4 DAYS. No further change in the tube or on the fresh plates.

IN 5 DAYS. The tube showed a slightly diminished amount of gas. The plate showed a slightly thinner growth, the colonies near the edge being well isolated.

IN 7 DAYS. The same appearance as at the end of 5 days.

IN 9 days. The plate showed only three lactose fermenting colonies in all.

IN 11 DAYS. The plate showed no growth.

CONCLUSIONS.

In glucose peptone water this intestinal B. coli produced an amount of acid sufficient to kill itself within 11 days.

The appearance of individual colonies on McConkey's lactose agar is unchanged up to the end.

EXPERIMENT C.

GROWTH/

GROWTH of MORGAN'S NO. 1 BACILLUS in LACTOSE PEPTONE WATER.

This experiment was carried out in an exactly similar manner.

IN 1 DAY. Tube showed no change. Plate showed thick growth of non-lactose-fermenting colonies.

IN 2 DAYS. As in 1 day.

IN 3 DAYS. As in 1 day.

IN 4 DAYS. As in 1 day.

IN 5 DAYS. As in 1 day.

IN 7 DAYS. As in 1 day.

IN 9 DAYS. As in 1 day.

IN 11 DAYS. Tube showed no change. Plate showed a very slightly thinner growth. The colonies showed some tendency to send out streamers.

IN 14 DAYS. Still a good growth of non-lactose-fermenting colonies, some at edge throwing out streamers.

IN 18 DAYS. Still a good growth of non-lactose-fermenting colonies.

CONCLUSION./

CONCLUSION.

The number of Morgan's No. 1 Bacilli when in pure culture in lactose peptone water is nearly as great at the end of a fortnight's incubation as at the end of one day.

There is no tendency to acquire lactose-fermenting powers during this growth.

EXPERIMENT D.

GROWTH of MORGAN'S NO. 1 BACILLUS in GLUCOSE PEPTONE WATER.

IN 1 DAY. Tube showed slight acid reaction and a very small amount of gas. The fermentation is considerably less than in the other tubes, except of course Tube C. The plate showed a rich growth of non-lactose-fermenting colonies.

IN 2 DAYS. Tube showed a stronger acid reaction and a greater amount of gas. The plate again showed a thick growth of non-lactose-fermenting colonies./

colonies.

IN 3 DAYS. The tube as before. The plate again showed a thick growth.

IN 4 DAYS. As in 3 days.

IN 5 DAYS. Plate shows a slightly less thick growth.

IN 7 DAYS. As in 5 days.

IN 9 DAYS. Plate showed a thinner growth.

IN 14 DAYS. Plate showed a very thin growth, 44 colonies in all being present, all non-lactose-fermenting.

IN 18 DAYS. No growth took place.

CONCLUSION.

Morgan's No. 1 Bacillus, in pure culture in glucose peptone water, is still alive, though numerically much diminished, after 14 days incubation, but is dead within 18 days.

No change takes place in the action on lactose during this period.

EXPERIMENT E./

EXPERIMENT E.

GROWTH of MORGAN'S NO. 1 and B. COLI together in LACTOSE PEPTONE WATER.

This experiment was done in a similar manner, the lactose peptone water being inoculated with one loopful of Morgan's No. 1 Bacillus (XLIII. 4.) and one loopful of B. Coli (LII. 2.)

IN 1 DAY. Tube showed acid and gas. The plate showed thick growth of lactose fermenting colonies, and non-lactose-fermenters. It was not possible to estimate the relative numbers.

IN 2 DAYS. The fermentation was more marked. The plate showed rich mixed growth.

IN 3 DAYS. Thick growth of lactose-fermenters with a not very large number of non-lactose-fermenters.

IN 4 DAYS. Same appearance.

IN 5 DAYS. Same appearance.

IN 7 DAYS. Same Appearance.

IN 9 DAYS. Slightly thinner growth of lactose-fermenting/

fermenting colonies; a very few non-lactose-fermenters.

IN 11 DAYS. Same appearance..

IN 14 DAYS. A much thinner growth of lactose-fermenters. Non-lactose-fermenters extremely scanty.

IN 18 DAYS. Only 74 colonies altogether. 72 of these are lactose-fermenters, 2 are non-lactose-fermenters.

CONCLUSION.

In a mixed growth of Morgan's No. 1 with B. Coli in lactose peptone water, B. Coli lives as long as it does when in this medium in pure culture.

Morgan's No. 1 Bacillus, on the other hand, when grown in mixed culture in lactose peptone water is greatly diminished at the end of 18 days as compared with pured cultures in the same medium.

EXPERIMENT F./

EXPERIMENT F.

GROWTH of MORGAN'S NO. 1 and B. COLI together in GLUCOSE PEPTONE WATER.

Same conditions as in Experiment E.

IN 1 DAY. The tube showed acid and gas. The plate showed a thick growth of lactose-fermenting and non-lactose-fermenting colonies.

IN 2 DAYS. The acid and gas were greater in amount. The plate as before.

IN 3 DAYS. Thick growth of lactose-fermenting colonies with a smaller number of non-lactose-fermenters.

IN 4 DAYS. No change.

IN 5 DAYS. Slightly thinner growth consisting mainly of lactose-fermenting colonies.

IN 7 DAYS. No change.

IN 9 DAYS. Plate showed 30 lactose-fermenting colonies with 2 non-lactose-fermenting.

IN 11 DAYS. Plate showed 1 lactose-fermenting colony. No non-lactose-fermenters were present.

IN 14 DAYS. The plate remained sterile.

CONCLUSION./

CONCLUSION.

In a mixed growth of Morgan's No. 1, and B. Coli in glucose peptone water, the B. Coli out-number and out-live the Morgan's No. 1 Bacilli.

EXPERIMENT G.

GROWTH of BACILLUS ACIDOPHILUS, MORGAN'S NO. 1 BACILLUS, and B. COLI in LACTOSE PEPTONE WATER.

This experiment was carried out in a similar manner. The B. Acidophilus used was XXXI. 8. Two loopfuls of a glucose broth culture of XXXI. 8, and one loopful each of agar slope culture of XLIII. 4, and LII. 2, were used. In this and the following experiments a glucose agar plate was spread at the same time as the McConkey Bile Salt Plate.

IN 1 DAY. The tube showed acid and gas. The McConkey Plate showed a thick growth of lactose-fermenting and non-lactose-fermenting colonies mixed./

mixed.

The glucose agar plate showed only large colonies of coli or Morgan's No. 1 type.

IN 2 DAYS. The McConkey plate showed as before a rich growth of Coli and Morgan's Bacillus mixed.

The glucose agar plate showed only large colonies.

IN 3 DAYS. A similar appearance.

IN 4 DAYS. The McConkey plate showed a slightly thinner growth, lactose-fermenting colonies being to non-lactose-fermenting in about the ratio of 30 to 1.

The glucose agar plate showed only large colonies.

IN 5 DAYS. No change in McConkey Plate.

The glucose agar plate showed a few minute colonies of the acidophilus type, in addition to the large colonies.

IN 7 DAYs. The McConkey plate showed a thin growth of lactose-fermenting-colonies with a few non-lactose-fermenters.

The glucose agar plate showed large colonies/

colonies of coli and Morgan's No. 1 type along with minute colonies of the acidophilus type.

IN 9 DAYS. The McConkey plate showed a thin growth of lactose-fermenting colonies with non-lactose-fermenting, the latter being in the proportion of about 1 in 20.

The glucose agar plate showed a good growth of minute acidophilus colonies, along with a few larger colonies.

IN 11 DAYS. The McConkey Plate remained sterile.

The glucose agar plate showed a pure growth of B. Acidophilus.

CONCLUSION.

The effect of the addition of B. Acidophilus to a mixed culture of Morgan's No. I and B. Coli in lactose peptone water is to shorten the lives of these organisms by more than a week, the B. Acidophilus remaining alive in pure culture at the end of of 11 days.

EXPERIMENT H./

EXPERIMENT H

GROWTH OF B.ACIDOPHILUS, MORGAN'S NO. 1 BACILLUS AND B.COLI TOGETHER IN GLUCOSE PEPTONE WATER.

Carried out in the same manner as Experiment G.

IN 1 DAY. The tube showed acid and gas. The McConkey plate showed a thick mixed growth of lactose-fermenting and non-lactose-fermenting colonies.

The glucose agar plate showed large colonies only.

IN 2 DAYS. No change in plates. The tube showed strong acid reaction and a larger amount of gas.

IN 3 DAYS. The McConkey plate showed a rich mixed growth of lactose-fermenting and non-lactose-fermenting colonies.

The glucose agar plate showed large colonies only.

IN 4 DAYS. The McConkey plate showed a slightly thinner growth. Non-lactose-fermenters were to /

to lactose- fermenters in about the ratio of 1 to 30.

The glucose agar plate showed a pure growth of large colonies.

IN 5 DAYS. The McConkey plate showed a growth consisting practically of lactose-fermenting colonies.

The glucose agar plate showed mainly large colonies with a very few minute colonies of the acidophilus type.

IN 7 DAYS. The McConkey plate showed only 12 lactose-fermenting colonies in all. No non-lactose-fermenters.

The glucose agar plate showed a thick growth of minute colonies of the acidophilus type, along with 11 large colonies.

IN 9 DAYS. The McConkey plate remained sterile.

The glucose agar plate showed a pure growth of minute colonies of B. Acidophilus.

CONCLUSION.

The/

The addition of B. Acidophilus to a mixed growth of Morgan's No. 1 and B. Coli in glucose peptone water shortens the lives of these organisms by at least 3 days.

The B. Acidophilus remains alive in pure culture at the end of 9 days growth.

EXPERIMENT K.

THE GROWTH OF B.ACIDOPHILUS, MORGAN'S NO.1 BACILLUS, AND B.COLI IN LACTOSE BROTH.

This experiment was identical with experiment G. except that in this case broth was used instead of peptone water.

IN 1 DAY. Tube showed acid and gas. The McConkey plate showed a rich mixed growth of lactose-fermenters and non-lactose-fermenters.

The glucose agar plate showed large colonies.

IN 2 DAYS. The McConkey plate showed a rich growth. Non/

Non-lactose-fermenters were to lactose-fermenters in about the ratio of 1 to 10.

The glucose agar plate showed large colonies only.

IN 3 DAYS. The McConkey plate showed a fairly thick growth of lactose-fermenters. Practically no non-lactose-fermenters were present.

The glucose agar plate as before.

IN 4 DAYS. The McConkey plate was unchanged.

The glucose agar plate showed a few minute colonies of B. Acidophilus as well as numerous large.

IN 5 DAYS. The McConkey plate showed only one colony, this being a lactose-fermenter.

The glucose agar plate showed a pure growth of acidophilus.

CONCLUSION.

In a mixed growth of B. Acidophilus, Morgan's No. 1 Bacillus, and B. Coli, in lactose broth, the acidity produced kills the first two named organisms within 6 days, the B. Acidophilus remaining alive in pure culture.

EXPERIMENT L.

THE GROWTH OF B.ACIDOPHILUS, B.MORGAN'S NO. 1, AND B.COLI TOGETHER IN GLUCOSE BROTH.

IN 1 DAY. The tube showed acid and gas The McConkey plate showed a rich growth of lactose-fermenters and non-lactose-fermenters

The glucose agar plate showed large colonies only.

IN 2 DAYS. The plate remained unchanged.

IN 3 DAYS. The McConkey plate showed a rather thinner growth, the non-lactose-fermenters being to lactose-fermenters in about the proportion of 1 to 30.

The glucose agar plate showed large colonies only.

IN 4 DAYS. The McConkey plate showed a thin growth consisting almost entirely of lactose-fermenters.

The glucose agar plate showed a good growth of large colonies, with a very few minute colonies.

IN/

IN 5 DAYS. The McConkey plate remained sterile.

The glucose agar plate showed a growth of B.Acidophilus in pure culture.

CONCLUSION.

In a mixed growth of B. Acidophilus, Morgan's No. 1 Bacillus, and B. Coli in glucose broth the acidity produced kills the two first named organisms within 5 days, the B. Acidophilus remaining alive in pure culture.

EXPERIMENT M.

SPECIMEN XXXV. emulsified in ordinary broth was shown by rich plating on McConkey's Medium to contain lactose-fermenting colonies only.

The emulsion was incubated for 3 weeks at 37 c and when replated at the end of this time numerous non-lactose-fermenting colonies grew on the plates.

One of these on study was found to consist of B. Faecalis Alcaligenes.

EXPERIMENT N.

Ordinary Broth emulsion of SPECIMEN XXXI.

This on plating showed numerous lactose-fermenting colonies, with one non-lactose-fermentér. After three weeks incubation the reaction was slightly alkaline, and plating showed numerous non-lactose-fermenting colonies. These on study were found to consist of organisms resembling culturally the Dysentery Bacillus Type "Y"., but agglutination tests proved it to be not a member of the dysentery group.

EXPERIMENT O/

EXPERIMENT O.

Ordinary Broth Emulsion of SPECIMEN XXIX.

This on plating showed numerous lactose-fermenting colonies, with no non-lactose fermenters.

After 3 weeks incubation the emulsion was slightly alkaline. On replating, it showed a pure culture of non-lactose- fermenting colonies, which on examination proved to be Morgan's No 1 Bacillus.

EXPERIMENT P.

Ordinary Broth Emulsion of SPECIMEN LV.

This on plating showed pure culture of lactose-fermenting colonies.

After 3 weeks incubation the emulsion was replated, when it was found to contain large numbers of non-lactose-fermenting organisms. These on study proved to have the reactions of B. Faecalis Alcaligenes.

ATTEMPT/

ATTEMPT AT STRENGTHENING THE INHIBITING FLORA OF MICE.

The following experiment was carried out in an attempt to transform the intestinal flora of mice and to strengthen the inhibiting power of the flora on foreign pathogenic organisms.

For the transforming agent Bacillus acidophilus (XXXI.8) was made use of.

As an infecting agent I made use of an organism which I had isolated from a mouse epidemic. This organism, which I recovered from the heart blood of nearly all the mice that died during this epidemic is, I believe, Bacillus Aertryck. It has the cultural characters of the Paratyphoid-Gaertner group and is agglutinated by Gaertner in low dilution, but I have not yet tested it against Aertryck serum. I selected it as the infecting agent, as I knew it was pathogenic to mice and was capable of producing diarrhoea.

For the experiment 10 mice were used.

5 of these were fed during 10 days on 6 doses of B. Acidophilus: bread soaked in glucose broth cultures of the organism being used. The other 5 were given ordinary food.

All/

All the mice were then fed on bread soaked in broth cultures of the infecting agent. After the first dose one of the normal mice died. A second dose of the infectious agent was given next day, and one of the normal mice and one of the acidophilus mice died. A third dose of the infecting agent was given 2 days later and the following day the three normal mice and 2 of the acidophilus mice died. The next day the remaining acidophilus mice were found dead.

Thus the experiment completely failed in its object.

This experiment seems scarcely worth repeating on such small animals as mice. It would, however, be interesting to conduct such experiments with monkeys with whom one could examine the intestinal contents from time to time.

BIBLIOGRAPHY/

B I B L I O G R A P H Y.

(1). TISSIER. "La reaction chromophile d'Escherich et le Bacterium Coli".

Comp.rend.Soc.de biol. 1899 LI. 943

(2). MORO. "Ueber die nach gram färbbaren Bacillus des Säuglingsstuhles".

Wiener Klinische Wochenschrift. LII. No.5, 1900.

(3). TISSIER. "Recherches sur la Flore intestinale normale et pathologique du Nourrisson."

Thèse de Paris (Carre et Naud) 1900.

(4). RODELLA. "Ueber die sogenannten säureliebenden Bacillen im Säuglingsstuhle".

Centralblatt.f.Bakt.Vol.XXIX.S.717. 1901.

(5). CAHN. "Ueber die nach gram färbbaren Bacillen des Säuglingsstuhles".

Centralblatt.f.Bakt. Vol.XXX. S.721. 1901.

(6). MERESHKOWSKY. "Zur Frage über die Rolle des Mikro-organismen in Darmkanal".

Centralblatt f.Bakt.I.Abt.Origin. Band XXXIX. Heft.4, 1905.

(7). " Band XXXIX. Heft 5, 1905.

(8). " Band XXXIX. Heft 6, 1905.

(9). " Band XL. Heft 1. 1905-1906.

(10). TISSIER. "Répartition des microbes dans l'intestin du Nourrisson."

Annales de l'Institut Pasteur. 1905 XIX. 109.

(11). TISSIER/

(11). TISSIER. "Traitement des infections intestinales par la méthode de transformation de la flore bactérienne de l'intestin".

Compt.rend. de la Soc.de Biol. T.LX. p.359. 1906.

(12). COBENDY. "Essais d'acclimatation microbienne persistante dans la cavité intestinale Délimitation du siège probable de cette acclimatation".

Compt.rend. de la Soc.de Biol. T.LX. p.364. 1906.

(13). " "Aperçus sur la morphologie de la flore intestinale de l'homme. Nombre respectif des anaerobies et des facultatifs dans les selles".

Compt.rend. de la Soc.de Biol.T.LX. p.415. 1906

(14). " "Description d'un ferment lactique puissant capable de s'acclimater dans l'intestin de l'homme".

Comp.rend. de la Soc. de Biol. T.LX. p.558, 1906.

(15). " "De la désinfection intestinale obtenue sans régime spécial par l'acclimatation d'un ferment lactique dans le gros intestin".

Compt. rend. de la Soc. de Biol. T.LX. p.602, 1906.

(16). " "Essai de traitement de l'intérite muco-membraneuse aigué, par l'acclimatation d'un ferment lactique dans le gros intestin".

Compt.rend. de la Soc. de Biol. T.LX. p.872, 1906.

(17). JACOBSON. "Contribution à l'etude de la flore normale des selles du nourrisson".

Annales de l'Institut Pasteur. 1908. XXI. 303.

(18).

(18).NOGUCHI. "Pleomorphism and Pleobiosis of Bacillus Bifidus communis".

Journal of Experimental Medicine. No.2. March 14th, 1910.

(19). DISTASO. "Sur les microbes acido-tolerants de la flore intestinale".

Centralblatt f.Bakt.Abt.1 Orig.Vol. LIX. p.48, 1911.

(20).HERTER. "Bacterial Infections of the Digestive Tract". The Macmillan Company, New York, 1907.

(21).TWORT. "The fermentation of Glucosides by bacteria of the typhoid-coli group and the acquisition of new fermenting powers by Bacillus dysenteriae and other organisms".

Proc. of the Roy.Soc. B.Vol.LXXIX. p.329.

(22).McCONKEY. "Lactose fermenting bacteria in faeces".

Journal of Hygiene Vol.V. p.333. 1905.

(23). " "Further observations on the Differentiation of Lactose-fermenting Bacilli with special reference to those of intestinal origin.

Journal of Hygiene Vol.IX. No.1 April 1909.

(24).SAVAGE. "Bacteriological Examination of Tidal Mud as an Index of Pollution of the River".

Journal of Hygiene. Vol.V. p.149.

(25).THIERCELIN. "Sur un diplocoque saprophyte de l'intestin susceptible de devenir pathogène".

Comptes rend.de la Soc.de Biol. LI. p.269. 1899.

(26)./

(26).HOUSTON. "Fourth Report of the Royal Commission on Sewage Disposal." Vol.III. P.105 1904.

(27). " "Fifth Research Report to the Metropolitan Water Board". June 1910.

(28).SCHMITZ. "Ueber Enterokokken".

Centralbl.f. Bakt. Nov.9th, 1912.

(29).GORDON. "The Differentiation of Streptococci".

Journal of Path. and Bact. Jan.1911.

(30).BEATTIE & YATES. "Sugar Tests and Pathogenicity in the Differentiation of Streptococci".

Journal of Path. & Bact. Oct.1911.

(31).KLEIN.* "Report of Medical Officer to Local Government Board. XXV. 171; XXVII.210.

(32).TISSIER. "Etude d'une varieté d'infection intestinale chez le nourrisson". Annales de l'institut Pasteur.XIX.1905.

(33).BIENSTOCK. "Bacillus Putrificus".

Annales de l'institut Pasteur. XX.1906.

(34).SHIGA.* "Centralblatt f.Bakteriol. und.Parasitenk. XXIII. 599.

(35).DUVAL & BASSETT. "The Etiology of Summer Diarrhoea in Infants". Studies from the Rockefeller Institute. Vol.ii. 1904.

(36).MASON KNOX. "Clinical Summary of Cases of Summer Diarrhoea studied at the Thomas Wilson Sanatorium, Baltimore, during summer of 1902".

Ibid.

(37).FLEXNER. "Investigation during summer 1903."

Ibid.

(38)./

(38).DUVAL & SCHORER. "Report"

Ibid.

(39).WOLLSTEIN & DEWEY. Ibid.

(40).GAY & STANTON. Ibid.

(41).CORDER. Ibid.

(42).WAITE. Ibid..

(43).KENDALL. Ibid.

(44).PAUL LEWIS. Ibid.

(45).BASSET. Ibid.

(46).PARK & HOLT. "Report upon the results with different kinds of pure and impure milk in infant feeding in tenement houses and institutions in New York City: a chemical and bacteriological study".

Reprint from "Archiv.of Pediat. Dec. 1903.

(47.)MORGAN. "Bacteriology of Summer Diarrhoea".

Brit.Med.Journ. 1906.i.p.1911.

(48).MORGAN & LEDINGHAM. "The Bacteriology of Summer Diarrhoea". Proc.Roy.Soc.of Med. ii.2. Epidem.Section (1909).

(49).WILLIAMS, MURRAY & RUNDLE. "Bacillus F.: an organism obtained in a case of epidemic diarrhoea".

The Lancet. Jan.31st, 1909, p.301.

(50).WILLIAMS, MURRAY & RUNDLE. "Further Researches into the Bacteriology of Epidemic Summer Diarrhoea".

Lancet, 1910. ii. p.730.

(51)./

(51).LEWIS. "Report on the results of an examination of the faeces of children with special reference to the occurrence of bacilli which neither liquefy gelatin nor ferment lactose".

Extract from Ann.Rep.of Med.Off.of Loc.Gov.Board for 1910-1911.

(52).LEWIS. "Report on the Prevalence and Characters of Non-lactose Fermenting and Non-liquefying aerobic Bacilli in the Faeces of Young Children under normal conditions and in Epidemic Diarrhoea during 1911".

Report of the Medical Officer 1911-12.

(53).BAINBRIDGE. "On the Paratyphoid and 'Food-poisoning' Bacilli, and on the nature and efficiency of certain rat viruses".

Journal of Pathology. Vol.XIII.1909.

(54).ECKLES & SHAW. "The Influence of the Stage of Lactation on the composition and properties of milk".

U.S.Depart. of Agricult.Bureau of Animal Industry. Bulletin 155.

(55).WOLLMANN. "Sur l'élevage des mouches steriles"

Annales de l'institut Pasteur. 1911.

(56).VEEDER, KILDUFFE & DENNY. "Studies in the Bacteriology of the Acute Intestinal Diseases of Infancy".

American Journ. of Dis.of Children. Vol.IV. No.3. 1912.

(57).ABT. "History of the Classification of gastro-intestinal Diseases in ancient and Modern Times".

American Journ. of Dis. of Chil.Vol. IV. No.3.1912.

(58)./

(58).CLEMESTRA. "Criticism of C.J.Houston's report on water analysis"

Journ.of Hygiene. Vol.12, No.4, 1912.

(59).METCHNIKOFF. "Etudes sur la flore intestinale".

Les Annales. Vol.XXII. 1908.

(60).METCHNIKOFF. "Etudes sur la flore intestinal (2ième mémoire).

Les Annales de l'institut Pasteur.19D

---

* The two marked with an asterisk have not been personally consulted.

PART III.

THE LABORATORY INVESTIGATION.

The first few months were spent in testing and devising methods of isolating intestinal organisms in order to have a satisfactory routine examination which might be applied to each case. This preliminary experimentation was the most laborious and difficult part of the work.

The chief difficulty was to formulate a scheme which would include the separation and study of the more important organisms, but which would yet be sufficiently brief to be applicable by a single worker.

The following scheme was finally adopted and applied to each case.

THE COLLECTION OF MATERIAL.

A specimen of the motion varying in age from a few minutes to a few hours was preserved for me on the child's napkin. The colour and consistence of the motion, the presence of blood or mucus, and any peculiarity of odour, were noted, along with other/

other details concerning the child. These details varied slightly with different cases, but always included the age, history, and method of feeding, along with details of the illness in pathological cases.

A small portion of the specimen was then collected in a sterile test-tube by means of an ordinary throat swab, and at once taken to the laboratory.

The part of the specimen selected was as representative of the whole as possible; two or three small portions from different parts of the motion being as a rule taken.

The specimen thus obtained was naturally contaminated to a certain extent by organisms from the skin of the child and from the air.

TECHNIQUE OF BACTERIOLOGICAL EXAMINATION.

A. PRELIMINARY STEPS.

Small representative portions of the specimen were rubbed up in distilled water on two or three slides, and these films were stained by Gram's method. Particular note was made of the relative number/

number in these films of: -

1. gram-negative bacilli and cocco-bacilli,
2. gram-positive bacilli,
3. coccal forms, and
4. spore-bearers.

A portion of the specimen was rubbed up in ordinary broth so as to form a slightly opaque emulsion, a second portion being similiarly treated in broth containing two per cent of Glucose. These two broth emulsions were then used for the inoculation of the other media.

McConkey's Bile Salt Lactose Neutral Red Agar was then poured into three Petri Capsules of the ordinary laboratory size (4" by $\frac{7}{8}$") and allowed to harden with the lids half off. The inhibiting action of this medium prevents the contamination by organisms from the air. The medium is sufficiently hard within half an hour for surface inoculation. This was done with a platinum loop from the ordinary broth emulsion, the drop or drops thus transferred being spread in all directions over the surfact of the agar by means of a thin glass rod bent at an angle and sterilised by flaming.

A/

A different amount of emulsion was used for each plate, the aim being to obtain a plate in which individual colonies were sufficiently widely separated to have their characteristics fully developed, but which contained a sufficient number of colonies to afford a moderately accurate computation of the relative number of different varieties.. In plates which were too thickly spread, it was found that numerous colonies failed to ferment lactose,which, transferred to a fluid medium contain lactose, fermented the sugar rapidly; this failure to ferment lactose on the solid medium was presumably due to exhaustion of the medium.

The plate was then inverted to prevent condensation water dropping on the surface of the medium, and incubated at 37 c.

The broth emulsion of faeceswas usually incubated at the same time to provide the material for another series of McConkey Plates in case the first series proved unsatisfactory. Some of these broth emulsions served a further purpose as will be described later.

These preliminary preparations for the separation of organisms of the coli-dysentery group being completed, the next steps were concerned with the/

the separation of anaerobic organisms, spore-bearers, coccal forms and organisms of the acid-tolerant group: for this the glucose broth emulsion of faeces was used.

Four tubes of medium were used which for clearness I shall call tubes A, B, C, and D. Tube A contained broth with 2% of glucose added, B contained also glucose broth with the addition of ·4% of acetic acid, C contained glucose broth, while D contained sterilised cow's milk.

Tubes A and B were inoculated from the glucose broth emulsion. The glucose broth emulsion was then pasteurised at 80°c. for 10 to 15 minutes in a water bath, and from the now pasteurised emulsion tubes C and D were liberally inoculated.

The four tubes A, B, C, D, were placed in a Buchner's tube containing 3 or 4 grammes of dry pyrogallol. A five per cent solution of sodium hydrate was then poured into the Buchner's tube through a funnel the tube plugged firmly with a rubber cork and hermetically sealed with plasticin .

By this means potassium pyrogallate is produced within the Buchner's tube which absorbs the oxgyen present: the glucose in the medium further/

further assists in this direction and a condition of anaerobiosis is produced.

B. FURTHER EXAMINATION of the LACTOSE FERMENTING GROUPS.

After two days incubation the McConkey Plates were examined, the appearance of the colonies noted, and the relative numbers of lactose-fermenting and non-lactose-fermenting colonies estimated. This, it was found, was most readily done by dividing the inverted plate into a series of squares by means of a glass pencil, numbering the squares and noting the relative number of colonies in each.

It was only after a considerable amount of experience had been gained with this medium that it was possible to do this accurately. Frequently it was found necessary to spread fresh plates from the original broth emulsion; and if the plates had been at all too thickly spread it was found useful to leave them in the incubator for four days, at the end of which time colonies were sometimes found to be lactose fermenters which at the end of two days incubation, had not shown themselves to be so.

Several colonies were selected and trans- ferred/

transferred to agar slopes, being rubbed up in the condensation water and all over the surface of the agar. Four or five of these colonies were lactose-fermenters, care being taken to select well isolated and pure-looking colonies. Non-lactose-fermenting colonies, if present, were also transferred to agar slopes, usually up to the number of five or six, if so many could be obtained.

In the later cases each of these presumably pure cultures was plated once more on McCorkey Bile Salt Lactose Agar. This replating served a double purpose; it ensured the purity of the culture, and it allowed one to observe the behaviour of the organism on the medium. I may mention here that several strains of the coli group have a consistently characteristic appearance on this medium.

A fresh isolated colony being transferred from each of these plates to an agar slope, these pure cultures were put through a series of tests. The tests for the lactose fermenter were those proposed by McCorkey and consisted of an examination of the sugar-fermenting and gas-producing power of the organism, along with its action on litmus milk, motility, power of producing indol, of liquefying gelatin, and of producing Vosges and Proskauer's reaction. The sugars/

sugars used, as suggested by McConkey, were lactose, saccharose, dulcit adonit, inulin and inosit, these being present in a strength of per cent in litmus peptone water.

The agar culture from which this series of tests was inoculated was always from 24-48 hours old. The fermentation tubes were examined at the end of the third or fourth and of the seventh day after incubation and the presence of acid or gas within this period was noted. For the indol reaction a tube of plain peptone water was inoculated, and this was tested for the presence of indol at the end of seven days' incubation by means of Ehrlich's test, paradimethyamidobenzaldeheyde and potassium persulphate being used.

For the Vosges and Proskauer reaction a tube of glucose peptone was inoculated, and to this at the end of four days' incubation a few drops of a fifty per cent solution of caustic potash were added and the tube allowed to remain at room temperature for 24 hours. The production of a pinkish colour was regarded as a positive reaction.

The gelatin tubes were inoculated by deep stab and allowed to grow at room temperature, for a period/

period of three months. Some have been kept for nine months and a few are only two months old at the present date.

Motility was tested for at the end of 4-6 hours incubation in a fluid medium; as a rule the opportunity was taken of staining the organism by Gram's method at the same time.

The litmus milk culture was examined for acid and clot at the end of 7 and 14 days incubation.

In addition to these tests of McConkey I have attempted to further identify different strains of lactose-fermenters by means of their agglutinating reactions. The sera used were obtained by vaccinating rabbits with strains from four cases.

The pathogenicity of various strains was also investigated.

C. FURTHER EXAMINATION OF THE NON-LACTOSE-FERMENTING GROUP.

For the identification of non-lactose-fermenting organisms the procedure advocated by Lewis was adopted. This procedure included the use of lactose, glucose, mannit, dulcit, saccharose, and/

and salicin in peptone water, as tests of the fermentative power of the organism along with its mobility, action on litmus milk, and ability to produce indol and to liquefy gelatin.

Agglutinating tests were used for members of this group also; some of the sera used having high agglutinative power over known organisms, others being prepared from rabbits by the use of vaccines from organisms isolated by myself.

The pathogenicity to animals of various members of this group also, was tested.

D. FURTHER EXAMINATION OF THE ACID-TOLERANT AND OTHER GROUPS.

The tubes A,B,C, and D, which had been inoculated as described above from the glucose broth emulsion were removed from the incubator after 4-6 days growth under anaerobic conditions: the amount of growth in each was noted, and a film made from each and stained by Gram's method.

From each a glucose agar plate was spread and incubated anaerobically; from these after 4-6 days growth individual colonies were picked off and transferred to deep glucose agar by stab. After a/

a few days growth their appearance was noted and a gram stained film made. A sub-culture in glucose broth was made, and this used as the source of inoculation of the various test media including the carbohydrate. Various feeding experiments were made on mice with organisms of the acid-tolerant group; and guinea-pigs were inoculated intraperitoneally with strains.

For the growth of organisms on plates under anaerobic conditions, various methods were tried. At first a Bulloch's Bell Jar holding approximately eight plates was used; the hydrogen was obtained by means of a hydrogen cylinder, partial exhaustion produced by a Geryk pump and caustic soda solution allowed to flow into the jar, where it mixed with a small heap of dry pyropellol previously placed there. An atmosphere completely devoid of oxygen is thus produced, but there is this disadvantage which is, I think, a very great one, that where several plates have to be made, those first spread are exposed to the air for a considerable time before all the operations are completed.

The method eventually adopted, which was found to be satisfactory was that invented by Lentz. A/

A ring of a composite material impregnated with 1 gramme of pyropellic acid is placed on a glass plate; a 1% solution of caustic soda is poured into the centre of this ring, the inoculated half of a Petri capsule inverted over the whole and luted down to the plated with plasticine. Surface growths of strict anaerobes can be obtained by this method.

THE WORKING VALUE OF THE SCHEME OF EXAMINATION.

No aerobic plates save those made with McConkey's Bile Salt Medium were used. The chief reason for this was the possibility of contamination of the original material by aerobic organisms. It would be preferable if specimens could be obtained in some way that would preclude all possibility of external contamination; it is conceivable that some method might be devised which would be applicable to in-patients of a hospital; but I was unable to think of a method which would also be applicable to out-patients and to children in their own homes.

In practice it was found that this drawback mattered little. All true intestinal organisms must of necessity be facultative anaerobes, therefore all/

all strict aerobes were at once discarded as contaminations, and, as the methods used for the isolation of groups by the elimination of other groups - I refer to the use of Bile Salt Media, of acid media and of pasteurisation - in themselves inhibited the growth of almost all contaminations, little extra labour was caused by the possibility of such contamination. The only group of intestinal organisms with the study of which skin and air contaminations might seriously interfere is the coccal group. For this and for another reason the cocci were the group to which least attention was paid in the present research. The other reason being the impossibility of differentiating between various strains of diplo- and strepto-cocci, failing the use of the sugar tests, on which so much doubt has been thrown lately on the grounds of the instability of the fermentative powers of these organisms.

The gram stained films from the specimen were found to be of very great value, the difference between some of the normal fields and those of cases of acute diarrhoea being very striking. Contamination, could of course, play practically no part in these fields, the exposure of the specimen being/

being as a rule so very brief.

The methods of isolating and identifying the lactose-fermenting and non-lactose-fermenting members of the coli-gaertner-dysentery group were found to be adequate though lengthy. The value of the sugar tests in the differentiation of these organisms is discussed elsewhere.

For the successful isolation of organisms of the acid-tolerant group, primary inoculation in a fluid medium before plating was found to be a necessity. This is due to the fact that if the emulsion of faeces is plated direct to anaerobic glucose agar plates, strong growths of organisms of the coli group occur, which completely override all other organisms. This was the stumbling block which for many years caused research into the intestinal flora to remain almost at a stand-still.

If acidified glucose agar plates are used for direct inoculation from the faeces the results are scarcely better, as in these cases as a rule no growth occurs, though sometimes colonies of yeasts may grow.

These difficulties can be over-come by the use of glucose broth and acidified glucose broth as primary/

primary culture media. At first in these tubes (the tubes A,B, of the scheme) strong growths of organisms of the coli group occur in normal cases; but after a few days the coli themselves as well as the acid-tolerant organisms produce large amounts of acid by fermenting the glucose, and a destruction of organisms which can only slightly resist acids takes place. The result after about a week is the dominance in these tubes of bacilli of the acid-tolerant group, which can then be successfully plated out on glucose agar.

The staining of the growth in tube A after 4-6 days anaerobic growth, I thought at first to be a valuable method of comparison between different cases as a result of numerous observations. This I no longer think to be the case. The growth in the glucose broth tube varies so much in its composition within short spaces of time owing to factors mentioned above, that estimations of the relative numbers of gram negative and gram positive bacilli and of coccal forms in these tubes are of little comparative value.

It is otherwise with the growth in tube B. In this only acid-tolerant organisms are able to/

to grow, and estimations of the amount of growth in the acid glucose brothwere found to corroborate the appearance of the gram stained films of faeces.

Tubes C,D, were not very satisfactory. Spore-bearing organisms were only seen in the original gram stained films of faeces in a very few cases, and the failure to isolate them may have been due to these organisms being dead, as such a very large percentage of organisms seen in faeces are. But in future work I intend to substitute a tube of glucose broth containing a portion of white of egg as used by Metchnikoff for tube C.

Another method of isolation which I have used in addition to the above scheme in some of the later cases,is the use of emulsions of faeces in broth for plating after a long period of growth; and a further addition which I shall in future add to the scheme,is the estimation of the reaction of the intestinal contents.

SCHEME/

SCHEME of EXAMINATION.

Gram-stained Films.	i. Gram-positive Bacilli of the Acid-tolerant group. ii. Gram-negative Bacilli & cocco-bacilli. iii. Coccal Forms iv. Spore-bearing Bacilli.
McConkey Plates	Lactose-fermenting Bacilli Non-Lactose-fermenting Bacilli
PRIMARY ANAEROBIC MEDIA.	TUBE A. = Glucose Broth TUBE B. = Glucose Broth +·4% acetic acid. TUBE C. = Glucose Broth pasteurised at 80°c. for 10 minutes. TUBE D. = Milk pasteurised at 80°c. for 10 minutes.
SECONDARY ANAEROBIC MEDIA.	PLATE A. = Glucose agar spread from TUBE A. PLATE B. = Glucose agar spread from TUBE B. PLATE C. = Glucose agar spread from TUBE C. PLATE D. = Glucose agar spread from TUBE D.

Lactose-fermenting Bacilli.

Non/

Non-lactose fermenting Bacilli.

Bacilli of the Acid-tolerant group.

Coccal Forms.

Spore-bearers.

General Results.

SPECIMENS I., - IX., XII., XXII and XXIII, XXVI., XXVII. and XXVIII.,

are not included in this thesis. It has been found necessary to retain the old numbers for the Specimens given, in order to avoid confusion, as all the organisms have, during the work been numbered according to their cases.

The earlier cases are not included, because they were not completely examined, as the full routine examination had not at that time been devised.

XXVI., XXVII., and XXVIII., were fully examined, but were older children, suffering from intestinal indigestion, and I reserve their publication until a larger series of cases has been examined.

SPECIMEN X.

A.M. aged 3 months.

Charteris Ward, Royal Hospital for Sick Children.

Examined June 29th, 1912.

STATE.

Suffering from acute diarrhoea.

METHOD OF FEEDING.

Has always been a bottle-fed shild. Is at present on albumen water.

HISTORY.

Diarrhoea started a few days ago and has been very acute, with vomiting. Blood in the motions for the last day or two.

MOTION.

Obtained June 29th, 1912. Is a stringy, brownish motion with blood in it.

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FILMS.

i. Gram-positive Bacilli: -

None are to be seen in films.

ii/

ii. Gram-negative Bacilli and Cocco-bacilli: -

Are presnnt in nearly pure culture. They are rather plump coliform bacilli.

iii. Coccal Forms: -

A few chains of rather coarse gram-positive cocci are the only other organisms seen besides the gram-negative bacilli.

iv. Spore-bearers.

Are absent.

MCCONKEY PLATES.

These showed a rather sticky peculiar type of growth with a tendency for the colonies to run together. All the plates showed the same appearance.

Many of the colonies were non-lactose-fermenting. Five of these picked off were found to be lactose-fermenting in the fluid medium.

PRIMARY ANAEROBIC MEDIA.

In this case no intermediary fluid media were used, the emulsions being plated direct.

A glucose agar anaerobic plate showed a thick growth of colonies of the "coli" type only.

A glucose agar anaerobic plate + ·1 per cent of acetic acid showed two colonies which contained/

contained yeasts.

A glucose anaerobic plate +·3 per cent of acetic acid remained sterile.

LACTOSE-FERMENTING-COLONIES.

Several strains were studied, and all proved to be liquefied of gelatin, though they did not all correspond in sugar reactions.

X. 3.

Replated on McConkey Plate, again gave the appearance of the original plate, fluid-looking colonies tending to run together, many apparently non-lactose-fermenting

Non-lactose-fermenting colonies were a second time picked off and these again in fluid medium fermented lactose.

These strains were motile gram-negative bacilli, which were indol positive and Vosges and Proskauer positive. They liquefied gelatin within 3 days at room temperature and produced acid and clot in litmus milk. They produced acid and gas in lactose saccharose, dulcit, adonit, and inosit, but no change in inulin.

This falls into group III. McConkey, but does not agree in reactions with any strain described by/

by him.

X.4. and X. 10.

which were also non-lactose-fermenting colonies on the primary McConkey Plate, also gave the peculiar growth on repurification, and fermented lactose in peptone water.

They were motile gram-negative rods, which were indol positive, Vosges and Proskauer positive, liquefied gelatin within three days, and produced acid and clot in litmus milk. They produce acid and gas in lactose, but no change in saccharose, dulcit, adonit, inulin and inosit peptone water.

These nearly corresponded to No. 3, group I McConkey from which they differed in not fermenting inulin.

It is possible that X. 3 also contained the same organisms along with another which would account for the difference of reaction. Though plated twice over the colonies had such a tendency to run together that it is quite possible X. 3 may have been mixed.

$\frac{1}{2}$ a 24 hours agar slope culture of X. 3 introduced subcutaneously to a guineapig produced a superficial ulcer with complete recovery.

NON/

NON-LACTOSE-FERMENTING-BACILLI.

No true non-lactose fermenters were isolated in this case.

BACILLI OF THE ACID-TOLERANT GROUP.

None were seen in the films.

COCCAL FORMS.

Streptococci were present in the films, but were not isolated for further study.

GENERAL RESULTS.

Bacilli of the acid-tolerant were diminished to vanishing point. Gram-negative bacilli were very numerous, those studied being gelatin-liquefying varieties of the lactose-fermenting group.

No true non-lactose-fermenting bacilli were isolated. Coccal forms were present in small numbers, as streptococci.

Sporebearers were absent.

Yeasts were isolated.

SPECIMEN XI.

M.D. aged 2 months.

Female.

Charteris Ward, Royal Hospital for Sick Children.

Examined July 13th 1912.

STATE.

Was born at the eighth month. Is small and rather puny, but has no diarrhoea.

METHOD OF FEEDING.

Has been mainly the bottle. Has had the breast sometimes. Cow's milk and water.

HISTORY.

Patient's mother has been ill, and patient was temporarily admitted to Charteris on July 8th, because there was no one at home to look after her.

AFTER HISTORY.

The child did badly while in the ward, did not take feeds of milk and water well, and lost weight. Was very difficult to keep warm. Had no diarrhoea till the last day or two when she/

she had slight terminal diarrhoea. Cause of death? Malnutrition? No P.M.

MOTION.

Obtained July 13th 1912. Well formed, grayish, rather putty-like stool. No abnormality of smell.

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FIELDS.

i. Gram positive bacilli: -

More than $\frac{19}{20}$ths of the organisms present fall into this group. They are slender, slightly irregular bacilli of the acid-tolerant type: some straight, others slightly curved. Many take up the gram stain irregularly, giving rise to punctate forms. Show a tendency to occur in clusters side by side. No bifid forms or long threads. No larger gram positive bacilli seen.

ii. Gram negative bacilli and cocco-bacilli: -

Are present in very small numbers, and consist of/

of coliform bacilli and cocco-bacilli.

iii. Coccal Forms.

Are also present in very small numbers. These are for the most part small diplo-cocci of the ordinary entero-coccus type.

IV. Spore-bearing Bacilli.

None present.

McCONKEY BILE SALT LACTOSE AGAR NEUTRAL RED PLATES.

These after two days incubation showed growths of lactose-fermenting colonies. One or two doubtfully lactose-fermenting colonies were found to ferment lactose rapidly in peptone water. Therefore no non-lactose-fermenting organisms grew on these plates.

PRIMARY ANAEROBIC MEDIA.

This case was more completely examined than most of the others, but I give the main facts as briefly as possible.

Tube A showed after 5 days incubation a growth/

growth consisting mainly of gram positive bacilli of the acid-tolerant type, along with some gram negative coliform bacilli.

Tubes B. In this case three acidified glucose broth tubes were used. And (i) contained glucose broth + ·4 per cent of acetic acid, a second (ii) contained glucose broth + ·6 per cent of acetic acid the third (iii) contained glucose broth + 1 per cent of acetic acid. These after three days incubation anaerobically showed considerable deposit at the foot, the fluid above remaining clear.

These gram stained showed gram positive bacilli of the type described in the original films. Tube C showed cloudiness and deposit after three days anaerobic growth. This was found to contain gram positive bacilli of the same type. No spore-bearers.

Four plates were also spread in this case direct from the faeces, and incubated anaerobically. These consisted of one plate containing glucose agar, and three containing glucose agar + ·4%, ·6% and 1% of acetic acid respectively. A mixture of gram negative and gram positive bacilli grew on the/

the glucose agar plate, the acidified plates remaining sterile. Sub-cultures were made from the glucose agar plate 13, 14, 15, 16, 17, in glucose agar stabs.

PRIMARY AEROBIC MEDIA.

An ordinary agar plate was also spread in this case. Five colonies XI, 8, 9, 10, 11, and 12, were picked off and examined.

These organisms (8-12) were identified as B.coli, enterococci, and staphylococcus albus.

SECONDARY ANAEROBIC MEDIA.

Plate A. (glucose agar) showed growth of minute colonies of acid-tolerant type, along with some larger colonies of coli type.

Plates B. (glucose agar + (i) ·4% (ii) ·6% and (iii) 1% of acetic acid) showed pure growths minute colonies.

Plate C. (glucose agar) showed growth of minute colonies.

LACTOSE-FERMENTING BACILLI.

Five lactose-fermenting colonies, labelled XI, 1, 2, 4, 5, and 6 were preserved from the McConkey/

McConkey Plates for further examination.

These in common were gram negative bacilli and coccobacilli: all produced acid and clot in litmus milk, none liquefied gelatin. All fermented lactose and saccharose with the production of acid and gas. No change was produced in adonit, inulin or inosit by any of them. All produced acid and gas in glucose and mannit.

XI. 1. was non-motile and produced indol.

XI. 2. was motile and produced indol.

XI. 4. was motile and produced indol.

XI. 5. was motile and produced indol.

XI. 6. was very slightly motile and did not produce indol.

XI. 4 and 5 produced acid and gas in dulcit, the other three did not.

<u>NON-LACTOSE-FERMENTING BACILLI.</u>

None were isolated.

<u>BACILLI OF THE ACID-TOLERANT GROUP.</u>

9 of these were selected for further study. XI. 18, 19, and 20 were selected from Plate A, 21 and 22 from Plate C, 23 and 24 from Plate/

Plate B i, 25 from Plate B ii, 26 from Plate B. iii.

These were all found to be bacilli of the acid-tolerant group, and facultative aerobes. On anaerobic glucose agar grew as minute round delicate colonies. In glucose agar stabs grew all down the stab, with no gas production, appearing in from 24 to 48 hours. Appeared on aerobic agar slopes as extremely minute scarcely visible colonies.

In glucose broth showed slight cloudiness with a whitish deposit.

On blood serum under anaerobic condition a scarcely visible growth took place.

Were all able to resist an addition of from ·4 per cent to 1 per cent of acetic acid to glucose broth and to glucose agar.

In their method of growth these strains were indentical. In their morphology however, they differed.

For the examination of their morphology 5 day old deep glucose agar stabs were taken and Gram's stain used.

XI. 18. Field shows a considerable amount of polymorphism. Bacilli are small, short, and slender/

26. These are shorter, thicker bacilli, gram positive.

It will be seen that the pleomorphism of these bacilli renders their morphology useless as a means of differentiating between different strains.

COCCAL FORMS.

Those isolated were the ordinary entero-coccus, and a white staphylococcus, the latter probably a contamination.

SPORE-BEARERS.

None isolated.

GENERAL RESULTS.

Members of the acid-tolerant group dominated all others in the original fields. The lactose-fermenters isolated were saccharose-fermenting strains of B.coli. No non-lactose-fermenters were isolated.

The acid-tolerant organisms isolated were of the B. Acidophilus type. Coccal forms were inconspicuous and were of the ordinary entero-coccus type. Sporebearers were absent.

SPECIMEN XIII. (From same case as SPECIMEN X.)

A.M. aged $3\frac{1}{2}$ months.

Charteris Ward, Royal Hospital for Sick Children

Examined July 12th 1912.

STATE.

Recovering from attack of diarrhoea.

METHOD.OF FEEDING.

Is now getting buttermilk.

HISTORY.

Since last specimen was obtained a fortnight ago the patient has done well, and diarrhoea is completely cured. Is still much under weight.

MOTION.

Obtained July 12th 1912, is a greenish motion of semi-solid consistence. No blood or mucus.

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FILMS.

i. Gram-positive Bacilli:-

These/

These are now numerous and are straight or slightly curved moderately slender bacilli of acid tolerant type.

ii. Gram-negative Bacilli and Coccobacilli:-

Are present in fair numbers but are markedly diminished compared with Specimen X.

iii. Coccal Forms:-

Diplococci of the ordinary enterococcus type have reappeared in the films, and the chains of cocci have disappeared.

iv. Spore Bearers:-

Absent

McCONKEY BILE SALT LACTOSE AGAR NEUTRAL RED PLATES.

After 2 days incubation showed lactose fermenting colonies of the usual coli type. None of the spreading, fluid-looking colonies of the Plates from Specimen X. were seen.

No non-lactose-fermenters on the Plates.

PRIMARY ANAEROBIC MEDIA.

No fluid media were used.

One glucose agar plate was spread from the/

the faeces emulsion and incubated anaerobically.

From this were obtained gram-positive diplococci of the enterococcus type, gram-negative coliform bacilli, and some rather large gram-negative bacilli of rather peculiar type having an unstained portion at the end like a spore. (See XLI. 15.)

One glucose agar plate + ·4% acetic acid was also spread from the faeces emulsion and incubated anaerobically. From this, only yeasts were obtained.

LACTOSE FERMENTING BACILLI.

Four strains were studied.

These all had it in common that they were motile gram-negative bacilli, were indol positive, did not liquefy gelatin, produced acid and clot in litmus milk.

Two strains called X. 17 and 19 produced acid and gas in lactose saccharose dulcit and adonit, but no change in inulin and inosit.

X. 18 produced acid and gas in lactose saccharose and adonit, but no change in dulcit inulin and/

and inosit, while X. 20 produced acid and gas in lactose and saccharose, but no change in dulcit, adonit, inulin and inosit.

They therefore fell into groups iii. and iv. McConkey.

Several other isolated strains were tested in gelatin but none proved to be liquefiers.

NON-LACTOSE-FERMENTING BACILLI.

None were present on Plates.

The other organisms isolated in this case were the enterococcus, yeasts, and the gram-negative bacillus of type XLI. 15.

An adequate routine examination for the isolation of bacilli of the acid-tolerant group had not been at this time devised.

GENERAL RESULTS

Bacilli of the acid-tolerant type were numerous in the films, whereas in Specimen X. they had been absent.

Gram-negative bacilli were much less numerous/

numerous than in X. and the gelatin-liquefying type of lactose fermenter had disappeared.

Non-lactose-fermenters as before absent.

Streptococci had disappeared and the enterococcus re-appeared.

Sporebearers as before absent.

Yeasts as before present.

SPECIMEN XIV.

R.R. aged 3½ years.

Male.

Charteris Ward, Royal Hospital for Sick Children.

Examined August 10th 1912.

STATE.

Is normal gastro-intestinally. Is suffering from spastic paraplegia.

METHOD OF FEEDING.

Is having an approximately adult diet.

HISTORY.

Has never had any diarrhoea of any severity.

MOTION.

Brownish motion, fairly solid.

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FILMS.

i. Gram-positive bacilli:-

These are present in moderate numbers. Many are straight or slightly curved moderately slender bacilli of the acid-ophilus type, and there/

there are also some larger and plumper bacilli.

ii. Gram-negative bacilli and cocco-bacilli:-

Are present in large numbers and are mainly coliform in type.

iii. Coccal Forms:-

These are numerous and varied. Most are rather large oval isolated gram-positive cocci, but there are also fairly numerous diplococci of enterococcos type. No streptococci in chains.

iv. Spore-bearers.

No organisms bearing spores are present.

McCONKEY PLATES.

These, two days old, showed numerous lactose fermenting colonies of the ordinary type. No non-lactose-fermenters were present.

PRIMARY ANAEROBIC MEDIA.

Tube A. four days old showed cloudiness with deposit.

Tubes B and C. also showed cloudiness with deposit.

SECONDARY ANAEROBIC MEDIA.

Plate/

Plate A. four days old showed colonies of coli type and also some small and minute colonies. From this plate coli and gram-positive diplococci were obtained.

Plate B. showed minute colonies and from these bacilli of the acid-tolerant group were obtained.

Plate C. showed some small colonies, and from this plate a gram-positive diplococcus was obtained.

LACTOSE-FERMENTING BACILLI.

Three colonies were selected for study called XIV i, 2 and 3.

Those were motile gram-negative bacilli which produced indol, did not liquify gelatin, and gave a negative Vosges and Proskauer reaction. They produced acid and clot in litmus milk, and acid and gas in lactose peptone water, but no change in saccharose, dulcit, adonit, inulin, and inosit.

Were therefore B. Grunthal. (No.4 McConkey.)

NON-LACTOSE/

NON-LACTOSE FERMENTING BACILLI.

None were present on the plates.

BACILLI OF THE ACID-TOLERANT GROUP.

Two strains called XIV 7 and 9 were isolated from plate B.

XIV.7 was a very polymorph gram-positive bacillus. It occurred in threads, clubs spirilla, and commaforms; some of the forms had lost the power of taking up the stain.

XIV.9 was much more regular in shape, occurring as short, straight, or slightly curved, moderately slender bacilli. They both grew under aerobic conditions; were acid-tolerant, but differed in their colonies on glucose agar plates.

XIV.7 grew as minute smooth-edged colonies, while XIV.9 occurred as feathery colonies like tangled threads. XIV.7 showed no growth in gelatine stab, while XIV.9 showed a feeble growth of a very few feathery colonies.

COCCAL FORMS.

XIV.5 was a strain of gram-positive diplococci/

diplococci isolated from plate A.

MORPHOLOGY. (Agar slope 3 days.)

Small rather plump diplococci with rather pointed ends.

(Glucose broth 4 days.) Gram positive diplococci mainly in pairs, a few in short chains.

AGAR SLOPE AEROBIC.

Very small and minute round smooth-edged discrete colonies.

ORDINARY BROTH.

In 2 days slight turbidity.

GELATIN STAB.

In 3 days a thin growth all down stab, with some discrete minute colonies near the foot: no liquefaction.

BLOOD SERUM.

Growth of minute colonies.

POTATO.

No growth.

LITMUS MILK 3 DAYS.

Acid, no clot.

LACTOSE PEPTONE WATER 3 DAYS.

Acid no gas.

SACCHAROSE PEPTONE WATER 3 DAYS.

Acid/

Acid no gas.

SALICIN PEPTONE WATER 3 DAYS.

Acid, no gas.

RAFFINOSE PEPTONE WATER 3 DAYS.

No change.

INULIN PEPTONE WATER 3 DAYS.

No change.

SORBIT PEPTONE WATER 3 DAYS.

No change.

GLUCOSE PEPTONE WATER 3 DAYS.

Acid, no gas.

This was the ordinary enterococcus.

SPORE BEARERS.

None seen in films or isolated.

GENERAL/

GENERAL RESULTS.

Gram positive bacilli of the acid tolerant type were not numerous in the films, but were isolated from Plate B.

Gram negative bacilli were numerous. Those on the McConkey Plate were all lactose fermenters of the ordinary type.

Coccal forms were numerous and varied; the strain studied was the enterococcus

Spore-bearers were not seen in the films or isolated.

SPECIMEN XV.

M.H. aged 1 month.

Female.

Charteris Ward. Royal Hospital for Sick Children.

Examined August 8th, 1912.

Normal Healthy child.

METHOD of FEEDING.

Patient was on the breast during the first three weeks of life. During the fourth week she was on Neslés milk. During the 24 hours prior to the passage of the motion examined, she was on cow's milk and water.

HISTORY.

Patient was healthy in every way, except that the mother thought she strained when the bowels moved, and it was for this reason that she was taken off the breast and sent up to hospital. While in hospital patient was healthy, took the bottle well and had no diarrhoea.

MOTION.

Obtained August 8th 1912. Normal colour, consistence and odour.

THE/

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FIELDS.

i. Gram-positive Bacilli.

The great majority of the organisms in the film fall into this group. They are bacilli of the type of the acid-tolerant group, slender, some straight, others slightly curved; some swollen in middle, a good many as diplo-bacilli end to end. No punctate and no bifid forms seen.

ii. Gram negative Bacilli and Coccobacilli.

These are present in fair numbers though very greatly outnumbered by the gram positive forms. Are of coliform type.

iii. Coccal Forms.

Are scanty. All those present are small oval or lanceolate diplococci and cocci, of the ordinary enterococcus type.

iv. Spore-bearing Bacilli.

No spore bearing organisms in films.

McCONKEY BILE SALT LACTOSE AGAR NEUTRAL RED PLATES.

These after two days growth showed large numbers of non-lactose-fermenting colonies as well as the usual lactose fermenters, about 10% of total colonies/

colonies being non-lactose-fermenters.

PRIMARY ANAEROBIC MEDIA.

Tubes A and B. (glucose broth and acidified glucose broth) were unfortunately inoculated by mistake from the pasteurised emulsion, and as a result no observations could be made on these tubes.

Tube C. (glucose broth from pasteurised emulsion) showed, after a few days anaerobic incubation, cloudiness with deposit. This, gram stained, was found to consist of small gram positive diplococci resembling the enterococcus.

SECONDARY ANAEROBIC MEDIA.

The glucose agar plate C. from Tube C. showed after a few days a growth of small colonies resembling those of streptococci.

LACTOSE-FERMENTING BACILLI.

Two colonies labelled XV.5 and 6, were selected from the McConkey Plates for examination. These were found to be small gram negative bacilli: they did not liquefy gelatin, nor produce indol, nor did they give a positive Vosges and Proskauer reaction/

reaction: in broth they produced turbidity, on agar slope an uncharacteristic growth of the usual coli type: in deep glucose agar stab, they grew to the bottom of the stab, but did not produce gas. In lactose and saccharose peptone water they produced an acid reaction but no gas: in dulcit a doubtful acid reaction, no gas: in adonit, inulin and inosit no change.

As regards their identity, one can only say that these are atypical and non-gas-forming varieties of the lactose-fermenting group of organisms to which b.coli belong.

NON-LACTOSE-FERMENTING BACILLI.

Four non-lactose-fermenting colonies were selected for study from the lactose agar Plate. These labelled XV.1.2.3. & 4. were all found to be identical in their reactions, and may be grouped together. They were motile-gram-negative bacilli, indol positive Vosges and Proskauer negative: growth in broth and on agar slopes uncharacteristic: they produced acid and gas in glucose peptone water, but no change in lactose, mannit, dulcit, saccharose and salicin. Gelatin/

Gelatin was not liquefied, and a slightly alkaline reaction was produced in litmus milk.

These then had all the characters of Morgan's No.1 Bacillus. As a final test, a rabbit was immunized with increasing intraperitoneal doses of a Morgan's No.1 Bacillus isolated from a case of acute diarrhoea (XLIII.6). The serum from this rabbit was found to agglutinate organism XV.4, in as high a dilution (1-5000) as it agglutinated its own bacillus.

There was thus no question that this bacillus isolated from this infant who was, and remained, free from diarrhoea, was identical with a strain of Morgan's No.1 Bacillus present in large numbers in a case of acute diarrhoea.

(I may mention here that Case XLIII. was admitted to the ward about 4 months after Case XV. had left it.)

Organism XV. 4, inoculated intraperitoneally to a guineapig in the dose of $\frac{3}{4}$ of a 24 hours' agar culture, produced death within 48 hours. On putting this organism through Lewis' tests, after a period of 6 months on agar and in broth, its reactions were unchanged.

COCCAL/

COCCAL FORMS.

Two strains of diplococci isolated from Plate C. were studied XV.8 and 9.

These were small gram-positive cocci, occurring as diplococci on solid media, while in fluid media short chains of usually not more than 4 elements were seen. Some polymorphism was observed, the elements varying slightly in size and also in shape, but for the most part were oval diplococci with slightly pointed ends, resembling pneumococci in appearance. On agar slope they grew as small strepto-coccus-like colonies, visible in 24 hours as minute dew-drop-like points. Later they attained a small pinhead size, being still dew-drop-like, transparent and smooth-edged: on glucose agar plate a similar appearance was observed. In glucose broth a uniform turbidity was produced, acid and clot were produced in litmus milk in 4 days, an acid reaction being produced in lactose glucose and saccharose peptone water. Strain 9 was found to be still alive after 17 days growth in milk. These organisms it will be noted before isolation from the faeces resisted pasteurisation at 80°c for 10 minutes.

SPORE/

SPORE-BEARERS.

No spore-bearing organisms were isolated or seen in films.

GENERAL RESULTS.

In this case the acid-tolerant group, as shown by the original films, made up the greater part of the intestinal flora.

The gram-negative organisms in the original films made a small part of the flora only, as was to be expected in a child who, a month old, had been fed for three weeks of that time on the breast alone.

The point of interest was the presence of such large numbers of Morgan's No.1 Bacillus. These bacilli had multiplied at the expense, not of the acid-tolerant group, as was noticed in the diarrhoea cases, but of the coli group.

Of the two lactose-fermenters selected at random and studied, neither was a typical intestinal B.coli.

Notwithstanding this departure from the usual, the child had no diarrhoea.

SPECIMEN XVI.

E.T. aged 4 months.

Female.

Medical Out-patient Department. Sick Childrens Hospital.

STATE

Healthy child.

Method of Feeding.

Bottle, cow's milk and water.

THE BACTERIOLOGICAL EXAMINATION.

GRAM-STAINED FILMS.

I. Gram-positive Bacilli.

Formed the majority of the organisms present. These are mainly straight or slightly curved, moderately slender bacilli of the acidophilus type.

II. Gram-negative Bacilli and coccobacilli.

These are scanty and are coliform in type.

III. Coccal Forms.

Are also only present in moderate numbers/

numbers and are diplococci of the usual entero-coccus type.

IV. Spore-bearers

Are absent.

<u>McCONKEY PLATES.</u>

After 2 days incubation showed lactose fermenting colonies only.

<u>ORDINARY AGAR PLATES.</u>

This incubated under aerobic conditions showed three types of colonies. Large dense white colonies, colonies of coli type, and small dense colonies. From these respectively were obtained straphylococcus albus, coliform bacilli and yeasts.

<u>PRIMARY ANAEROBIC MEDIA.</u>

In this case the faecal emulsion was plated direct to the anaerobic plates without the intervention of fluid media.

On Plate A. grew colonies of medium size with also some minute colonies. From these colon bacilli and diplococci of the enterococcus type were obtained/

obtained.

On Plate B. rather small dense colonies grew, and from these yeasts were obtained.

On Plate C. a few small colonies appeared which were found to consist of rather coarse gram-positive diplococci.

LACTOSE-FERMENTING BACILLI.

One colony was selected for study from the McConkey Plates.

This was found to consist of motile gram-negative bacilli which produced indol, were negative to Vosges and Proskauer's test, did not liquefy gelatin, and produced acid and clot in litmus milk.

They produced acid and gas in lactose and dulcit, but no change in saccharose, adonit, inulin or inosit.

This organism was therefore Bacillus Coli communis. (No 34 McConkey.)

NON-LACTOSE-FERMENTING BACILLI.

None were present on the McConkey Plates.

BACILLI OF THE ACID-TOLERANT GROUP.

Though/

Though very numerous in the films none were isolated in this case owing to no primary fluid media being used.

COCCAL FORMS.

The diplococci isolated were the enterococcus of Thiercelin.

SPORE-BEARERS.

None were present in the films or isolated.

GENERAL RESULTS.

Gram-positive bacilli of the acid-tolerant group were predominant in the fields, but were not isolated owing to faulty technique.

Gram-negative bacilli were scanty.

The lactose fermenter studied was the Bacillus coli communis.

No non-lactose-fermenters were present on the McConkey plates.

Coccal forms were scanty and were of the enterococcus type.

Spore-bearers were absent.

SPECIMEN XVII.

S.T. aged 1 year 9 months.

Male.

Medical Out-Patient Department, Sick Children's Hospital.

Examined August 15th 1912.

STATE.

Suffering from diarrhoea and vomiting.

METHOD OF FEEDING.

"Anything that is going."

HISTORY.

Patient has had diarrhoea and some vomiting during the past week. Has been having 4 or 5 motions a day. Had a similar attack about 4 months ago. No blood in the motions.

Patient on examination looked healthy.

MOTION.

Brownish motion, solid.

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FILMS.

i. Gram-positive bacilli:-

Are/

Are numerous in the field and varied. Some are moderately slender bacilli of the acidophilus type, some are very small and slender, while there are also large and plump gram-positive bacilli. Numerous others are intermediate between these.

It is not easy to guess how many are of the acid-tolerant group, but they are probably numerous.

ii. Gram-negative bacilli and coccobacilli:-

Are present in fair numbers though not so numerous as the gram-positive.

iii. Coccal Forms:-

Are numerous and varied. Some are small cocci of the enterococcus type. Very many are fairly large oval cocci, either isolated or in pairs. Other varieties also present.

iv. Spore-bearers:-

No organisms seen in the film are bearing spores.

McCONKEY PLATES.

These/

These after 2 days growth showed lactose-fermenting colonies of the ordinary type and also large raised pink colonies.

No non-fermenting colonies present.

PRIMARY ANAEROBIC MEDIA.

Tube D. showed no clotting, and no organisms were found in the film.

A glucose agar plate anaerobic showed colonies of coli type along with very small and minute colonies.

A glucose agar plate + ·4% acetic showed a few colonies which contained yeasts.

. The full routine examination was not applied in this case.

LACTOSE-FERMENTING BACILLI.

These colonies were selected for study. XVII. 1 and 2, from the colonies of the ordinary type, XVII. 3, from the large pink variety.

XVII. 1 and 2, were found to be identical in their reactions. They were motile gram-negative bacilli which were indol positive, Vosges and Proskauer negative, did not liquefy gelatin and produce/

produce acid and clot in litmus milk. They produced acid and gas in lactose and adonit peptone water, but no change in saccharose, dulcit, inulin or inosit.

They are thus No. 1, McConkey, & differ only from Bacillus Acidi Lactici in being motile.

XVII. 3, was a large pink colony on McConkey Plate. On further replatings it always preserved an appearance unlike that of B. coli, showing on McConkey large fluid looking colonies some strongly lactose fermenting, others of a pale pink colour, with a tendency to run together.

They were non-motile gram-negative bacilli which were indol negative, Vosges and Proskauer positive, did not liquefy gelatin, and produced acid and clot with an unusual amount of whey in litmus milk. The growth on agar slope was thicker than the ordinary coli growth.

They produced acid and gas in lactose, saccharose, dulcit, inosit, glucose, mannit, salicin, sorbit, raffinose, galactose, maltose and dextrin; but no change in inulin peptone water.

It thus corresponds to No. 67, McConkey, differing/

differing only from the Bacillus Lactis Aerogenes in fermenting dulcit.

I have one strain of Bacillus Lactis Aerogenes, LIV. 2, which sometimes ferments dulcit, and at other times does not, therefore, I believe that XVII. 3, is the Bacillus Lactis Aerogenes.

NON-LACTOSE-FERMENTING BACILLI.

None were present on the Plates.

BACILLI OF THE ACID-TOLERANT GROUP.

None were isolated. Primary fluid media, which are necessary for the isolation of these organisms, were not used in this case.

COCCAL FORMS.

A strain of diplococci had the following characteristics:-

MORPHOLOGY. (Agar slope.)

Gram-positive oval or lanceolate cocci growing diplos for the most part; a few clusters. (Glucose broth.) Gram-positive oval diplococci some in short chains.

AGAR SLOPE.

Minute/

Minute discrete colonies of streptococcus type.

ORDINARY BROTH.

Cloudiness and deposit in 24 hours.

GLYCERINE AGAR SLOPE.

Minute discrete colonies.

GLUCOSE BROTH.

Cloudiness with deposit.

LITMUS MILK.

Acid and clot in 2 days.

LACTOSE PEPTONE WATER.

Acid but no gas in 2 days.

SACCHAROSE PEPTONE WATER.

Acid but no gas in 2 days.

GLUCOSE PEPTONE WATER.

Acid but no gas in 2 days.

POTATO.

No visible growth.

GENERAL RESULTS.

Gram-positive bacilli were dominant in the fields./

fields.

Gram-negative bacilli were fairly numerous.

The Lactose Fermenting Bacilli isolated were No. 1, McConkey, which is closely allied to, or identical with, B.Acidi Lactici; and No 67, McConkey which is closely allied to, or identical with, B. Lactis Aerogenes.

Non-Lactose-Fermenting Bacilli were not present on the plates.

Coccal Forms were numerous and varied; the strain studied was the enterococcus.

Spore-bearers were not isolated.

SPECIMEN XVIII.

T.H. aged 1 year 4 months.
Male.

Medical Out-Patient Department, Sick Children's Hospital.

Examined August 15th 1912.

STATE.

Suffering from diarrhoea.

HISTORY.

Patient was well up till a few days ago when he began to have diarrhoea; no vomiting. Has been having 5 or 6 motions a day.

METHOD OF FEEDING.

Was getting milk, porridge, puddings, etc.

MOTION.

Yellowish green; no blood or mucus.

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FILMS.

i. Gram-positive bacilli:-

Are scanty. None of the usual type of the acid-tolerant group are to be seen, those present/

present being rather large, straight, plump gram-positive rods.

ii. Gram-negative bacilli and coccobacilli:-

These are present in large numbers, dominating all others. They are of coliform type.

iii. Coccal Forms;-

Are fairly numerous. They are very variable in size, but are for the most part diplococci. A fair number of moderately large isolated oval cocci are also present.

No chains.

iv. Spore-bearers:-

Are absent.

McCONKEY PLATES.

These after 2 days incubation showed lactose fermenting colonies only. They were of the usual type.

PRIMARY ANAEROBIC MEDIA.

Tube C. showed no curdling. Gram stained contained no organisms.

SECONDARY/

SECONDARY ANAEROBIC MEDIA.

Plate A. 5 days old showed colonies of coli type, as well as small and minute colonies.

Plate B. remained sterile.

LACTOSE-FERMENTING BACILLI.

Two colonies selected for study called XVIII. 1 and 2, were found to be identical.

They were non-motile gram-negative bacilli which produced indol, were negative to Vosges and Proskauer's test, did not liquefy gelatin, and produced acid and clot in litmus milk.

They produced acid and gas in lactose, saccharose, and dulcit peptone water, but no change in adonit, inulin or inosit.

They were thus B.Neapolitanus (No. 72, McConkey).

NON-LACTOSE-FERMENTING BACILLI.

None were present on the McConkey Plates.

BACILLI OF THE ACID-TOLERANT GROUP.

None were isolated.

COCCAL FORMS.

Two/

Two strains isolated from Plate A. were diplococci of the enterococcus type.

SPORE-BEARERS.

None seen in films or isolated.

GENERAL RESULTS.

Gram-positive bacilli of the acid-tolerant type were scanty.

Gram-negative bacilli and coccobacilli were numerous.

The lactose-fermenting bacilli isolated were the Bacillus Neapolitanus.

No non-lactose-fermenting bacilli were present on the plates.

Coccal forms were numerous and varied, but no streptococci in long chains were present.

Spore-bearers were absent.

SPECIMEN XIX.

A.H. aged $4\frac{1}{2}$ months.

Male.

Charteris Ward, Royal Hospital for Sick Children.

Examined August 20th 1912.

STATE.

Suffering from diarrhoea.

METHOD OF FEEDING.

Bottle-fed since birth; cow's milk and water. At present albumen water and whiskey.

HISTORY.

Was operated on for hernia in the McKay Smith ward, and after dismissal had diarrhoea. Was admitted for this cause to Charteris on August 3rd and dismissed improved on August 13th. Diarrhoea again became bad, and Patient was readmitted to Charteris on August 19th. Is having 5 or 6 motions a day.

MOTION.

Obtained August 20th, is a pale green semi-solid motion.

THE/

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FILMS.

i. Gram-positive Bacilli: -

Are scanty, and are of two types. The slender, straight or slightly curved type of acidophilus, and the plumper straight forms of the same organism. Both are present in very small numbers.

ii. Gram-negative Bacilli and Coccobacilli: -

Are present in a considerable majority and are of coliform type.

iii. Coccal Forms: -

Are present in small numbers, and are moderate sized gram-positive cocci and diplococci mainly of the enterococcus type.

iv. Spore-bearers: -

Are absent.

McCONKEY BILE SALT LACTOSE NEUTRAL RED AGAR PLATES.

These after 2 days growth showed lactose-fermenting colonies of the ordinary type and a considerable number of non-lactose-fermenting colonies. The/

The non-lactose-fermenting colonies formed about 5-10 per cent of the total colonies.

PRIMARY ANAEROBIC MEDIA.

Tube A, 4 days old, showed cloudiness and deposit. This, gram-stained, showed gram-negative coliform bacilli along with a few gram-positive oval cocci occurring singly, in pairs, and in short chains.

Tube B. also showed cloudiness and deposit which consisted of somewhat irregular slender coccobacilli occurring in pairs and singly. No definitely bacillary forms.

SECONDARY ANAEROBIC MEDIA.

Plate A, 5 days old, showed colonies mainly of coli type with a few more minute.

Plate B. showed one minute colony.

From Plate A were obtained coli bacilli and bacilli of the acid-tolerant type. From Plate B was obtained the very irregular cocco-bacillary form of acidophilus.

LACTOSE-FERMENTING BACILLI.

Two colonies were selected for study XIX. 1 and 2. XIX. 1 and 2 were both motile gram-negative bacilli which were indol-positive, Vosges and/

and Proskauer negative, liquefied gelatin and produced acid and clot in litmus milk.

XIX. 1, produced acid and gas in lactose, dulcit, and adonit, but no change in saccharose, inulin and inosit.

6 months later it had lost the power of producing acid and gas in dulcit peptone water.

XIX. 2, produced acid and gas in lactose, and adonit, but no change in dulcit, saccharose, inulin or inosit.

6 months later it had lost the power of producing gas.

NON-LACTOSE-FERMENTING BACILLI.

Two colonies kept for study XIX. 3 and 4. These were identical in their reactions. They were non-motile gram-negative bacilli, which produced indol, did not liquefy gelatin, and produced no change in litmus milk. They produced acid and gas in glucose, mannit, dulcit and salicin, but no change in lactose and saccharose.

Both produced acid but no gas in lactose peptone water in a fortnight.

Half a 24 hours agar slope cultured introduced subcutaneously, produced a superficial ulcer with recovery.

BACILLI/

BACILLI OF THE ACID-TOLERANT GROUP.

The two strains isolated were the extremely irregular cocco-bacillary form of acidophilus.

COCCAL FORMS.

None studied.

SPORE-BEARERS.

None seen in films or isolated.

GENERAL RESULTS.

Gram-positive bacilli of the acid-tolerant type were scanty.

Gram-negative coliform bacilli were numerous. Of these, non-lactose-fermenters formed about 5-10 per cent on the McConkey Plates.

The non-lactose-fermenting were unnamed varieties, falling into Group I of Lewis' classification. Coccal forms were present in small numbers and were mainly moderately plump enterococci.

Spore-bearers were absent.

SPECIMEN XX.

G.C. aged 6 months.

Medical Out-Patient Department, Sick Children's Hospital.

Examined August 20th 1912.

STATE.

Suffering from diarrhoea.

METHOD OF FEEDING.

Has been on the bottle since birth.

HISTORY.

Patient has had diarrhoea with some vomiting for 3 or 4 days, several motions a day.

MOTION.

Greenish, loose, no blood or mucus.

THE BACTERIOLOGICAL EXAMINATION

GRAM STAINED FIELDS.

i. Gram-positive bacilli:-

Are extremely scanty. Those present are straight or slightly curved bacilli of acidophilus type.

ii./

ii. Gram-negative bacilli:-

Are the dominant organisms in the field. They are of rather plump coliform type.

iii. Coccal Forms:-

Are numerous and varied; some small of entrococcus type, many larger. Most are diplos, some isolated. No chains.

iv. Spore-bearers:-

Are absent.

McCONKEY PLATES.

Three days old, showed numerous lactose-fermenting colonies and a few non-lactose-fermenting colonies. The latter formed about 3 - 4 per cent of the total.

PRIMARY ANAEROBIC MEDIA.

Tube A. 4 days old showed cloudiness with deposit.

Gram stained showed coliform gram-negative bacilli, and a considerable number of gram-positive cocci in chains.

Tube B. showed cloudiness and deposit. Gram stained showed yeasts.

SECONDARY/

SECONDARY ANAEROBIC MEDIA.

Plate A. showed (5 days old) colonies of coli type and small and minute colonies. From these only B coli and diplococci were obtained.

Plate B. showed small and minute colonies. From these yeasts only were obtained.

LACTOSE-FERMENTING BACILLI.

Two colonies were kept for study, XX. 1 and 2. These were motile gram-negative bacilli, indol positive, Vosges and Proskauer negative, which did not liquefy gelatin, and produced acid and clot in litmus milk. They produced acid and gas in lactose but no change in saccharose, adonit, inulin, or inosit.

XX. 1, produced acid and gas in dulcit peptone water, whereas XX. 2, produced no change.

XX. 1, was thus B.coli Communis (No. 34 McConkey) while XX. 2, was B.Grünthal (No. 4 McConkey)

A guineapig inoculated intraperitoneally with 2 cc. of a 24 hours broth culture of XX. 1, showed no sign of illness.

NON-LACTOSE-FERMENTING BACILLI.

Two/

Two non-lactose-fermenting colonies were kept for study, and were found to be identical in their reactions. XX. 3 and 5, were motile gram-negative bacilli which were indol negative and Vosges and Proskauer negative. They did not liquefy gelatin and produced a slightly alkaline reaction in litmus milk.

In glucose and maltose they produced an acid reaction but no gas, whereas in lactose, saccharose, dulcit, mannit, salicin, adonit, inulin, inosit, sorbit, raffinose and dextrin they produced no change.

They might thus have been considered to be the Shiga-Kruse type of the Dysentery Bacillus had they not been motile.

BACILLI OF THE ACID-TOLERANT GROUP.

None were isolated in this case.

COCCAL FORMS.

Were present in Tube A. as chains of streptococci, but only diplococci of the enterococcus type were isolated.

SPORE-BEARERS./

SPORE-BEARERS.

Were absent.

GENERAL RESULTS.

Gram-positive bacilli of acid-tolerant type were very scanty in this case.

Gram-negative bacilli were numerous.

The Lactose fermenting bacilli isolated were B.coli communis and B. Grünthal.

Non-lactose-fermenting bacilli were present on the McConkey Plates, forming about 3 - 4 per cent of the total colonies. Those isolated had a resemblance to the Dysentery group in cultural characteristics.

Coccal forms were fairly numerous.

Spore-bearers were absent.

SPECIMEN XXI.

M.S. aged 11 months.

Male.

Dundas Ward. Sick Children's Hospital.

Examined August 20th 1912.

STATE.

Suffering from acute diarrhoea.

METHOD OF FEEDING.

Was bottle fed. Lately getting porridge, milk puddings etc. Is at present on albumen water.

HISTORY.

Patient started an attack of diarrhoea and vomiting 2 days ago, and has become very collapsed. Has been having 5 or 6 motions a day. Was admitted to Hospital yesterday.

AFTER HISTORY.

The patient recovered very quickly and was out of hospital in a few days.

MOTION.

Obtained August 20th. Greenish semi-solid Stool, no blood or mucus.

THE/

THE BACTERIOLOGICAL EXAMINATIONS

GRAM STAINED FILMS.

I. Gram-positive Bacilli;-

These are present in fair numbers.

Two types are present, the straight or slightly curved moderately slender type of acidophilus, and also the straight slightly plumper variety.

II. Gram-negative Bacilli and Coccobacilli:-

These are the dominant organisms in the film and are of coliform type.

III. Coccal Forms:-

Are scanty. Those present are diplococci of the enterococcus type as well as some larger cocci.

IV. Spore-bearers:-

Are absent.

McCONKEY PLATES.

2 days old showed lactose fermenting and non-lactose-fermenting colonies.

The non-lactose-fermenting colonies formed over 50 per cent of the total.

PRIMARY ANAEROBIC MEDIA.

Tube A/

Tube A. 6 days old showed cloudiness with deposit, and contained large numbers of gram-negative coliform bacilli along with small numbers of gram-positive bacilli of the acidophilus type.

Tube B. showed a sediment but the supernatant fluid remained clear. The sediment gram-stained contained gram-positive bacilli. Straight and fairly regular for the most part, but some irregular and approaching a coccal type.

A glucose broth tube + 1% acetic acid remained sterile.

Tube C. showed slight cloudiness with deposit, and gram-stained showed gram-positive diplococci.

Tube D. showed slight curdling. Gram-stained, it contained some small gram-positive cocci growing in diplos and short chains of 4.

SECONDARY ANAEROBIC MEDIA.

Plate A. 5 days showed some colonies of coli type and also some small and minute colonies.

Plate B. showed no growth.

Plate C. showed some very small, smooth edged colonies from which diplococci were obtained.

Plate/

Plate D. showed very small smooth edged colonies, and from this also gram-positive diplococci were obtained.

LACTOSE-FERMENTING BACILLI.

One colony selected for study from the McConkey Plate had the following reactions.

It was a non-motile gram-negative bacillus, which was indol positive, Vosges and Proskauer negative, did not liquefy gelatin and produced acid and clot in litmus milk. It produced acid and gas in lactose and dulcit peptone water, but no change in saccharose, adonit, inulin or inosit.

Was therefore B.Schafferi (No 35 McConkey)

A guineapig inoculated intraperitoneally with 2cc. of a 24 hours'broth culture of this organism (XXI.12) showed no sign of illness.

NON-LACTOSE-FERMENTING BACILLI.

Four colonies kept for study, XXI. 1, 2, 4 and 5.

These were identical in their reactions.

They were motile gram-negative rods which were indol positive, did not liquefy gelatin and produced/

produced a slightly alkaline reaction in litmus milk.

They produced acid and gas in peptone water but no change in lactose, mannit, dulcit, saccharose or salicin.

They were therefore Morgan's No 1 Bacillus.

On XXI.5 being tested as to its agglutinability by a serum obtained through another strain of Morgan's No 1 (XLIII. 6), it gave a completely negative result. It is, therefore, evident that the name "Morgan's No 1 Bacillus" covers more than one variety of organism.

A guineapig inoculated subcutaneously with a 24 hours' sloped agar culture of XXI. 1 in normal saline developed a superficial ulcer, with complete recovery.

An exactly similar experiment with XXI. 2 gave the same result.

A guinea-pig inoculated intraperitoneally with 2cc. of a 24 hours' broth culture of XXI. 5, showed no sign of illness.

A mouse fed for three successive days on bread soaked in broth cultures of XXI. 2 remained well/

well.

BACILLI OF THE ACID-TOLERANT GROUP.

Though these were present in fair numbers in the primary films, Plate B. remained sterile, therefore no members of this group were isolated.

COCCAL FORMS.

Two strains XXI. B. and C. were both isolated from Plate D.

They were oval gram-positive diplococci of the enterococcus type, but no particular study was made of them.

SPORE-BEARERS.

None were seen in the films or isolated.

GENERAL RESULTS.

Gram-positive bacilli of the acid-tolerant group were present in fair numbers in the original films.

Gram-negative bacilli were, however, considerably more numerous.

More than half the colonies on the

McConkey/

McConkey Plates were non-lactose-fermenting, and all of these which were studied were Morgan's No 1 Bacillus.

Coccal forms were scanty and were of the usual diplococcal type.

Spore-bearers were absent.

SPECIMEN XXIV.

A.F. aged 5 months.

Male.

Charteris Ward, Royal Hospital for Sick Children.

Examined November 3rd 1913.

STATE.

Severe recurrent diarrhoea.

METHOD OF FEEDING.

Has been on the bottle since birth; cow's milk and water. At present on albumen water.

HISTORY.

Patient has had frequent attacks of vomiting and diarrhoea during the last 2 months. The present one started a few days ago and is severe; had 6 motions and vomited 3 times yesterday. Blood has never been noticed in the motion.

MOTION.

A very loose greenish motion. No blood or mucus present.

THE BACTERIOLOGICAL EXAMINATION.

GRAM/

GRAM STAINED FILMS.

i. Gram-positive bacilli:-

Are extremely scanty. Those present are rather short squat bacilli of the occasionally met with, plump type of acidophilus.

ii. Gram-negative bacilli and coccobacilli:-

Are the dominant variety present. Vary somewhat in their morphology, but are coli-form.

iii. Coccal forms:-

Are fairly numerous. Most are gram-positive diplos of the enterococcus type, but there is a considerable amount of variation in size. Some isolated oval cocci.

iv. Spore-bearers:-

Are absent.

MCCONKEY PLATES.

These after 2 days incubation showed lactose-fermenting colonies. The latter formed about 5 or 6 per cent of the total colonies.

PRIMARY ANAEROBIC MEDIA.

Tube/

Tube A. showed cloudiness and deposit, and the film contained gram-negative coli-form bacilli, and diplococci of the enterococcus type.

Tube B. showed some deposit, and gram stained showed short, moderately plump gram-positive bacilli.

Tube C. showed slight cloudiness and deposit, and it showed in the film small gram-positive diplococci.

SECONDARY ANAEROBIC MEDIA.

Plate A. showed come colonies of coli type and a few small and minute colonies.

Plate B. showed a pure growth of minute smooth-edged colonies of the acidophilus type.

Plate C. showed some small colonies, and also some large white colonies, probably contaminations, from which staphylococcus albus was obtained.

LACTOSE-FERMENTING BACILLI.

Two colonies were selected for study from the McConkey plate called XXIV. 1 and 2.

They were motile gram-negative bacilli which were indol positive, Vosges and Proskauer negative,

negative, and did not liquefy gelatin. They produced acid and clot in litmus milk, acid and gas in lactose peptone water, but no change in saccharose, adonit, inulin, inosit. XXIV. 1, also produced acid and gas in dulcit peptone water while XXIV. 2, produced no change.

XXIV. 1, was thus B.coli communis (No. 34 McConkey), while XXIV. 2, was B. Grünthal (No. 4 McConkey).

NON-LACTOSE-FERMENTING BACILLI.

Two studied in this case XXIV. 5 and 6 were found to be identical in their reactions.

They were motile gram-negative bacilli, indol positive, Vosges and Proskauer negative, which produced a slightly alkaline reaction in litmus milk and did not liquefy gelatin. They produced acid and gas in glucose peptone water but no change in lactose, mannit, dulcit, saccharose and salicin.

They had thus all the reactions of Morgan's No. 1 Bacillus. XXIV. 6, was tested as to its agglutinability by a serum prepared by myself in a rabbit with a strain of Morgan's No. 1, isolated from/

from another case of diarrhoea, (XLIII. 6,) with positive results; complete agglutination being produced in a dilution of 1 - 5000, which was the full titer of the serum.

A guineapig was inoculated subcutaneously with a 48 hours agar slope culture in saline of XXIV. 6, with no apparent effect beyond slight superficial ulceration.

A guineapig was inoculated intraperitoneally with 2 cc. of a 24 hours broth culture of XXIV. 5, with no apparent effect.

BACILLI OF THE ACID-TOLERANT GROUP.

Those isolated from Plate B. were short bacilli of the acidophilus type, though rather plumper than usual. They resisted the addition of ·4 per cent of acetic acid to glucose broth and to glucose agar.

COCCAL FORMS.

Those isolated were diplococci of the enterococcus type and the staphylococcus albus.

SPORE-BEARERS.

None/

None seen in films or isolated.

GENERAL RESULTS.

In the films gram-negative bacilli were in a great majority over gram-positive bacilli of the acid-tolerant type.

The lactose-fermenting organisms isolated were varieties met with in normal cases.

Non-lactose-fermenting were present in large numbers, forming 5 - 6 per cent of the colonies on the McConkey Plates.

Those isolated were Morgan's No. 1 Bacillus.

Coccal Forms were fairly numerous in the films but streptococci in long chains were absent.

Spore-bearers were absent.

SPECIMEN XXV. (from same case as XIX.)

A.H. aged 5 months.

Male.

Charteris Ward, Royal Hospital for Sick Children.

Examined September 6th, 1912.

STATE.

Recovered from diarrhoea.

METHOD OF FEEDING.

Is now getting milk and water once more.

HISTORY.

Since date of obtaining the last specimen, patient has recovered from the attack of diarrhoea, but is still very thin. Only 1 or 2 motions in the 24 hours now.

MOTION.

is a yellowish-green semi-solid motion.

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FILMS.

i. Gram-positive Bacilli: -

are now much more numerous than in specimen XIX. They are of the moderately slender, straight/

straight or slightly curved acidophilus type.

ii. Gram-negative-bacilli and Cocco-bacilli: -

Are much less numerous than in XIX. Are of coliform type.

iii. Coccal Forms: -

Are moderately numerous. Are mainly diplococci of the entero-coccus type. Are on the average smaller organisms than the coccal forms seen in XIX.

iv. Sporebearers: -

As before, are absent.

MCCONKEY PLATES.

After 2 days growth showed numerous lactose-fermenting colonies with 2 non-lactose-fermenters.

One of these proved in fluid medium to be a lactose fermenter.

Proportion of non-lactose-fermenters to total colonies was under ·1 per cent.

NON-LACTOSE-FERMENTING BACILLI.

The non-lactose-fermenting bacillus selected for study was found to be a lactose-fermenter in fluid medium.

GENERAL/

GENERAL RESULTS.

In comparison with specimen XIX the differences are: -

i. large increase of bacilli of acid-tolerant group.

ii. a decrease in the films of gram-negative bacilli.

iii. a great decrease on McConkey Plates of non-lactose-fermenting colonies.

SPECIMEN XXIX.

M.M. aged 4 months.

Female.

Charteris Ward. Royal Hospital for Sick Children.

Examined October 15th 1912.

STATE.

Suffering from acute diarrhoea.

METHOD OF FEEDING.

Bottle-fed since birth. On albumen water at the date of examination.

HISTORY.

Was admitted to the ward on the 9th October. suffering from acute diarrhoea, which had started a few days previously. Has been having 5 or 6 motions in the 24 hours. Very collapsed.

MOTION.

A large slimy stool of olive-green colour. Smell only slight. Some mucus. No blood.

THE/

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FIELDS.

i. Gram positive bacilli:-

Are present in fair numbers but are considerably out-numbered by the gram negative. Those present are rather stout straight bacilli some of fair length; larger than the usual acidophilus type. None of the slender slightly curved variety are present. No headlet, no bifid forms, no threads.

ii. Gram negative bacilli and cocco-bacilli:-

These constitute the greater number of the organisms present. Vary considerably in size, some being small, others fairly large and plump.

iii. Coccal Forms;-

These are fairly numerous, being more numerous than the gram-positive bacilli, but less numerous than the gram-negative. Are almost all gram-positive.

iv /

iv. Sporebearers:-

None present in films.

McCONKEY BILE SALT LACTOSE AGAR NEUTRAL RED PLATES.

Showed at a rough estimate about 1,500 lactose fermenting colonies.

No non-lactose fermenters present.

PRIMARY ANAEROBIC MEDIA.

Tube A. 4 days old showed considerable cloudiness with white deposit. Gram-stained showed gram-negative coliform bacilli as the chief organism. Gram-positive diplococci was also fairly numerous, those being mainly rather plump and not lanceolate.

There were a very few stout gram-positive bacilli, isolated, or in pairs end to end, probably of the acidophil group, but these are relatively very scanty. A very few stout rather thick gram-positive bacilli were present.

Tube B. showed fairly marked cloudiness with deposit/

deposit. Gram stained showed short fairly slender gram-positive rods, straight for the most part, ends square or slightly rounded off, many occurring in diplos or short chains. There are also one or two larger plumper gram-negative bacilli which seem to be a different species. None of the gram-positive bacilli show bifurcation or branching.

Tube C. also showed cloudiness with deposit. Organisms are for the most part gram-positive cocci of different kinds, some isolated spherical cocci, and diplococci. One or two gram-positive bacilli.

Tube D. showed curdling; gram-stained the whey showed some large and long gram-positive bacilli, along with some small diplococci.

SECONDARY ANAEROBIC MEDIA.

All 5 days old.

Plate A. showed a growth of colonies the great majority of which were small and minute, up to a very small pin head size, a few larger of the coli type. Under the low power all the minute and very small colonies resembled each other in appearance being plain round colonies slightly ragged in the centre/

centre, differing only in that some are translucent while others are much darker and more opaque.

Plate B. showed a growth of small and minute colonies. The minute under the low power were of the translucent type described on Plate A., there being a very small number of the opaque variety present. The larger colonies are dark brown and opaque under the low power.

Plate C. showed a fair number of colonies mainly of the coli type with a small number of small and minute colonies.

Plate D. showed a small number of small colonies. These under low power were opaque spherical brownish colonies.

LACTOSE FERMENTING BACILLI.

The reactions of two of these XXIX.1 and 2 were as follows. They were identical in all tests. They were motile gram-negative bacilli, which did not liquefy gelatin in 5 months, produced indol, were negative to Vosges and Proskauer, produced acid and clot in litmus milk.

Growth on agar and in broth uncharacteristic.

Produced/

Produced acid and gas in lactose, saccharose and dulcit peptone water, the acid in the dulcit fermentation tube being present in the anaerobic part of the tube only, in both cases; no change in adonit inulin or inosit peptone water.

In addition they both produced acid and gas in glucose, mannit, subit, raffinose, gelectose, maltose, and dextrin, but no change in salicin.

These corresponded in reactions to Bacillus 71. group III of McConky's classification.

A mouse was fed for 5 days with bread soaked in broth cultures of XXIX.2. without fatal result.

Put through the tests 4 months later these organisms were found to be unchanged.

NON-LACTOSE-FERMENTING BACILLI.

None were present on the McConkey Plate.

BACILLI OF THE ACID-TOLERANT GROUP.

Six cultures of organisms of this group were stidoed, XXIX. 10, 11, 12, 13, 15, and 16.

These were of two types, XXIX. 10 and 15 belonging/

belonging to one type, while XXIX. 11, 12, 13 and 16 belonged to the other.

XXIX. 10. (Isolated from Plate B.)

MORPHOLOGY. (Glucose agar stab 4 days old)

Is a very slender gram-positive bacillus; a few rather thicker forms: one or two headlet bacilli. Have a tendency to occur in clusters side by side.

(Glucose broth anaerobic 4 days old) slender gram-positive bacilli; most are only of moderate length, but there are several long thread forms.

(Glucose broth anaerobic 1 month old) slender gram-positive bacilli, straight or slightly curved, moderate length, a great many partly or completely decolourised. No bifid forms.

GLUCOSE AGAR PLATE (ANAEROBIC) -

Minute and very small, smooth edged.

GLUCOSE AGAR STAB -

Showed in 2 days strong growth starting about ¾ inch from the surface.

GLUCOSE/

GLUCOSE BROTH ANAEROBIC -

Cloudiness with heavy whitish deposit in 3 days. (Non-motile bacilli.)

GELATIN STAB. (Room temp.)

In 2 days showed a delicate growth right up to surface.

GLUCOSE BROTH + ·4% ACETIC ACID ANAEROBIC -

A good growth occurred with cloudiness and deposit.

GLUCOSE AGAR STAB -

Repeated after 1 months artificial cultivation. A very feeble growth occurred in the upper part of the stab; a thick growth below.

XXIX. 15. (Isolated from Plate B.)

MORPHOLOGY. (Glucose agar stab 4 days old)

Gram-positive bacilli, most of them thin, of moderate length. Resembles XXIX. 10.

(Glucose broth anaerobic 4 days) long slender bacilli gram-positive, non-motile.

(Glucose broth anaerobic 1 month old) a very few slender gram-positive bacilli, but the great majority are decolourised degenerate or dead forms./

forms.

GLUCOSE AGAR PLATE ANAEROBIC -

Minute smooth edged colonies.

GLUCOSE AGAR STAB -

In 2 days faint growth appeared, in 4 days there was a strong growth starting $\frac{3}{4}$ of an inch down the stab, a very feeble growth near the surface.

GLUCOSE BROTH ANAEROBIC -

Showed after 4 days slight cloudiness with considerable deposit.

GLUCOSE BROTH + ·4% ACETIC ACID -

Slight cloudiness with deposit.

GELATIN STAB - (Room temp.)

Feeble growth extending up to the surface.

XXIX. 11.

MORPHOLOGY. (Glucose agar plate anaerobic 4 days old)

Short straight slender gram-positive bacilli: a few are slightly curved: a few are diplobacilli.

GLUCOSE/

GLUCOSE AGAR PLATE ANAEROBIC-

Small and minute colonies, white, moderately dense. Under low power pale delicately granular smooth edged colonies tending to remain discrete.

GLUCOSE AGAR STAB -

In 4 days thick growth extending right up to the surface.

POTATO ANAEROBIC -

Growth of small white colonies.

AGAR SLOPE ANAEROBIC -

Growth of minute smooth edged colonies visible to the naked-eye in 2 days.

GELATIN STAB -

In 2 days strong ribbon growth extending up to surface.

GLUCOSE BROTH + ·4% ACETIC ACID -

Cloudiness with deposit.

GLUCOSE BROTH -

Cloudiness with deposit. Produced strongly acid reaction in 3 days in lactose, saccharose dulcit, inulin, glucose, mannit, salicin and maltose broth.

A mouse fed for 5 successive days with bread soaked in glucose broth cultures of XXIX. 11 remained/

remained well.

XXIX. 12, 13 and 16 were also of the facultative anaerobic type.

COCCAL FORMS--

Two types of diplococci also were studied, XXIX. 6 and 18 belonging to the first, XXIX. 7 and 8 to the second. Both types were fairly numerous in the original gram-stained fields of faeces.

XXIX. 6. (Was isolated from Plate B.)

MORPHOLOGY. (Glucose agar stab 4 days old)

Is a plump gram-positive coccus occurring mainly in pairs. They are a little larger than the usual enterococcus, but it is quite possible they are varieties of this organism. Most are oval with rather pointed ends.

GLUCOSE AGAR STAB -

Fairly thick growth all down the stab.

GLUCOSE AGAR PLATE -

Small colonies of very small pin head size.

AGAR/

<u>AGAR SLOPE ANAEROBIC</u> -

Growth of very small colonies visible in 2 days.

<u>GELATIN STAB</u> -

This growth all down. No liquefaction. Before isolation it resisted Pasteurisation at 80° c. for 5 minutes.

<u>XXIX</u>. <u>18</u>. (Isolated from Tube C.)

<u>MORPHOLOGY</u>. (Glucose agar stab 4 days)

A gram-positive coccus of the enterococcus type occurring mainly in pairs but also in small clusters.

(Glucose broth 24 hours) gram-positive coccus in diplos and small clusters and also in short chains.

<u>GLUCOSE AGAR STAB</u> -

In 24 hours thick growth all down the stab.

<u>AGAR SLOPE ANAEROBIC</u> -

Spherical smooth edges isolated colonies of the usual strepto-coccus type.

ORDINARY/

<u>ORDINARY</u> <u>BROTH</u> -

Slight cloudiness with deposit in 24 hours.

<u>GLUCOSE</u> <u>BROTH</u> -

In 24 hours marked cloudiness with deposit.

<u>GELATIN</u> <u>STAB</u> -

Delicate growth all down. No liquefaction.

<u>BLOOD</u> <u>SERUM</u> -

Small round discrete colonies.

<u>LITMUS</u> <u>MILK</u> -

Acid and clot in 3 days.

<u>PEPTONE</u> <u>WATER</u> -

No growth.

In lactose, salicin, saccharose, raffinose, inulin and subit peptone water, no acid or gas was produced. It was doubtful whether any growth took place in these tubes although the inoculating culture was tested and found to be alive. No reduction of neutral red.

A mouse fed for 5 successive days on bread soaked in glucose broth cultures of this organism suffered no ill effect.

<u>XXIX</u>. <u>7</u> /

XXIX. 7. (Isolated from Tube D.)

MORPHOLOGY. (4 days old glucose agar stab)

Is a gram-positive coccus or cocco-bacillus, occurring in pairs, isolated and in clusters. Is a much more delicate organism than XXIX. 6 or 18.

GLUCOSE AGAR STAB -

Visible in 24 hours as a delicate growth all down the stab. This always remains a delicate growth.

GLUCOSE AGAR PLATE -

Small streptococcus-like colonies, regular border, small pin-head size.

AGAR SLOPE ANAEROBIC -

A faint filmy growth was visible in 24 hours, and this had the same appearance at the end of 24 hours. Colonies flat and filmy and showed a marked tendency to coalesce. In 3 days isolated colonies were considerably larger than the ordinary streptococcus type: still remained flat and filmy.

GELATINE STAB -

In 2 days a very delicate growth all down the stab was visible. No liquefaction of the gelatin/

gelatin took place.

SPORE BEARERS -

None were seen in films or were isolated in this case.

GENERAL RESULTS.

Gram-negative Bacilli of coliform type were the dominant organisms in the field. Those isolated and studied were found to be Lactose-Fermenting- Bacilli of the type No. 71 of McConkey's Classification. Non-Lactose-Fermenting Bacilli were not found on the Special Media used for their isolation.

Gram-positive Bacilli of the acid-tolerant type were present in fair numbers. Members of both the main varieties of this group were isolated, the anaerobic type or Bacillus Bifidus of Tissier, and the facultatively anaerobic type or B. acidophilus of Moro.

Coccal forms were numerous and 2 varieties were isolated, one being of the type of the enterococcus, the other being a not hitherto described variety./

variety.

Spore Bearers were absent.

ADDENDUM.

The ordinary broth emulsion of faeces of this case was left in the incubator for 3 weeks, when two McConkey Plates were spread.

These after 2 days incubation showed pure growth of Non-Lactose-Fermenting Colonies.

Two picked out, called XXIX. 22 and 23 were found to be identical in their reactions.

They were motile gram-negative bacilli which produced indol, were negative to Vosges and Proskauer test, and did not liquefy gelatin. Both produced an alkaline reaction in litmus milk in a week. Both produced acid and gas in glucose and galactose, no change in lactose, saccharose, dulcit, adonit, inulin, inosit, mannit, salicin, sorbit, raffinose, galactose, maltose and dextrin, peptone water.

Were both therefore Morgan's No. 1 Bacillus. That is to say that the faecal emulsion from which when fresh only lactose fermenting colonies were obtained,/

obtained, contained after 3 weeks incubation Morgan's No. 1 Bacillus in pure culture.

To the general results one must therefore add, that Morgan's No. 1 Bacillus was also present in the faeces in extremely small numbers.

SPECIMEN XXX.

A.B. aged 4 years.

Charteris Ward, Royal Hospital for Sick Children.

Examined October 12th, 1912.

STATE.

Acute Pyelitis. (coli.)

A special catheter specimen of urine and a specimen of faeces were obtained from this case with the object of comparing the coli of the faeces and those of the urine.

The McConkey plates from both urine and faeces showed only lactose-fermenting colonies.

One selected from the plate from urine called XXX, 2, was a non or very slightly motile gram-negative bacillus, which was indol-positive, Vosges and Proskauer negative, did not liquefy gelatin, and produced acid and clot in litmus milk. It produced acid and gas in lactose, dulcit, glucose, mannit, sorbit, gelactose, salicin, maltose and dexterin peptone water, but no change in saccharose, adonit, inulin, inosit, and raffinose.

XXX. 8, from the faeces differed in being actively/

actively motile and in producing acid and gas in saccharose and raffinose peptone water.

XXX. 2, was therefore B.coli communis though less motile than usual for this type, while XXX. 8, was No. 71. McConkey.

2 cc. of a 24 hours broth culture of XXX. 2, introduced intra-peritoneally to a guineapig produced no effect.

A mouse fed for 5 consecutive days with bread soaked in broth cultures of XXX. 2, showed no sign of illness.

SPECIMEN XXXI.

M.L. aged 7 months.

Female.

Charteris Ward, Royal Hospital for Sick Children.

Examined October 17th, 1912.

STATE.

Case of empyema. Is normal gastro-intestinally.

METHOD of FEEDING.

Has had the bottle since birth: cow's milk and water.

HISTORY.

Patient was admitted in September suffering from empyema, which has been drained. Bowels are regular and she has never suffered from diarrhoea.

MOTION.

Obtained October 17th, is fairly solid, yellowish, odour slight.

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FIELDS.

i. Gram-positive Bacilli.

Are present in considerable numbers. Are mainly/

mainly of the straight or slightly curved moderately slender acidophilus variety, others of the slender straight "B.Exilis" type.

ii. Gram-negative Bacilli and Coccobacilli.

These are numerous and varied, out-numbering the gram-positive. Are all of the coli-form type. Some very small, others of moderate size and thickness.

iii. Coccal Forms.

Are fairly numerous of varied morphology. Many are small lanceolate diplococci of enterococcus type. Some are rather large oval cocci, isolated for the most part. Some very slender diplococci. Most are gram-positive, but there are a few gram-negative.

iv. Spore Bearers

Are absent.

McCONKEY PLATES.

Showed after 2 days incubation a good growth of lactose-fermenting colonies of the ordinary type, and one non-lactose-fermenting.

This gave on counting, a percentage of ·1% of non-lactose fermenting colonies to the total.

PRIMARY/

PRIMARY ANAEROBIC MEDIA.

Tube A. 6 days old showed cloudiness and deposit.

Gram stained, a mixture of gram-positive bacilli of acidophilus type with gram-negative bacilli coliform, along with a few diplococci of enterococcus type.

Tube B. showed slight cloudiness with deposit. Gram-stained showed straight or slightly curved slender gram-positive bacilli of acidophilus type.

Tube C. showed slight cloudiness with deposit. Gram-stained showed some coliform gram-negative bacilli, and some gram-positive diplococci.

Tube D. showed slight clotting,gram-stained showed gram-positive diplococci.

SECONDARY ANAEROBIC MEDIA.

Plate A. 8 days old showed round whitish colonies of coli type, small pin-head size: also still smaller grayish colonies very thin: and numerous very small and minute colonies. These last constitute much the greatest number of the colonies.

Plate B. showed rather degenerate colonies of/

of pinhead size, in fair numbers and a large number of very small filmy colonies rather irregular in outline. From both B.acidophilus was obtained.

Plate C. showed some colonies of coli type and small colonies of streptococcus variety from which diplococci were obtained.

Plate D. showed mainly very small colonies which under the low power had the outline of a rosette.

LACTOSE-FERMENTING BACILLI.

Two lactose-fermenting colonies were selected from the McConkey Plates for further study, named XXXI., 2 & 3.

XXXI,2, was a non-motile gram-negative bacillus which produced indol, was negative to Vosges and Proskauer's test, did not liquefy gelatin, and produced acid and clot in litmus milk. It produced acid and gas in lactose, saccharose and dulcit peptone water, but no change in adonit, inulin or inosit.

Put through these tests again, after an interval of four months, its reactions were identical, except that it gave very slightly Vosges and Proskauer's reaction.

NON-LACTOSE-FERMENTING BACILLI.

The single non-lactose-fermenting colony on the McConkey Plates was kept for study, XXXI,1.

This/

This was a motile gram-negative bacillus which did not produce indol, nor Vosges and Proskauer's reaction, did not liquefy gelatin, and produced acid, but no clot in litmus milk in a fortnight. It produced acid and gas in glucose mannit and dulcit, but no change in lactose, saccharose, salicin, adonit, inulin or inosit. It had thus the cultural characteristics of B.Paratyphosus A. but when tested as to its agglutinability by Para A.serum of a Titer of 1-6000, it was completely negative, even in 1-100.

2 cc. of a 24 hours' broth culture of this organism introduced intra-peritoneally to a guinea-pig, produced no apparent effect.

<u>BACILLI of the ACID-TOLERANT GROUP</u>.

Three of these were selected for study XXXI. 7, 8 and 9. 7 was isolated from Plate A., 8 and 9 from Plate B.

These were morphologically very similar and though it was at first thought they were different, owing to difference in appearance of the original selected colonies on the glucose agar plates, and also some difference in glucose agar stab cultures, on further subculturing they appeared to be identical

XXXI. 8.

MORPHOLOGICALLY is a moderately slender gram-positive bacillus/

bacillus of the usual straight or slightly curved acidophilus type. This organism has been repeatedly examined during 5 months artificial cultivation, and has always preserved the same appearance when in young culture.

GLUCOSE AGAR PLATE ANAEROBIC.

Colonies varying in size from minute to a very small pinhead size. They are smooth-edged and rather more dense than the usual type.

GLUCOSE AGAR PLATE AEROBIC.

Similar appearance. Colonies rather more delicate.

AGAR SLOPE AEROBIC.

Minute delicate colonies, poor growths.

ORD BROTH AEROBIC.

Very feeble growth.

GLUCOSE BROTH AEROBIC.

Cloudiness and deposit in 2 days.

GLUCOSE BROTH +·4% of acetic acid.

Cloudiness and deposit.

GLUCOSE AGAR STAB.

Thick growth all down stab, growing up to the surface.

POTATO/

POTATO AEROBIC.

Growth of whitish colonies.

GELATIN STAB.

No growth.

ACID PRODUCTION.

In Lactose, saccharose, dulcit, inulin, glucose, mannit, salicin and maltose broth, it produced a very strong acid reaction in all in 3 days: litmus milk acid and clot.

6 Mice fed for 7 days on bread soaked in glucose broth cultures of this organism, remained well and lively.

XXXI., 9, showed a great tendency to grow in chains, as strepto-bacilli.

COCCAL FORMS.

3 Strains of cocci were studied XXXI.4, 5 and 11. XXXI 4 & 5 were isolated from Plate A., XXXI 11, from Plate D.

XXXI. 4 and 5 turned out to be identical in all respects and were of the ordinary enterococcus type.

MORPHOLOGY.

They occurred in glucose agar stabs as diplos/

diplos and short chains. In glucose broth some fairly long chains were seen in films.

GLUCOSE AGAR PLATE.

Small smooth-edged dew-drop-like colonies of small pinhead size.

GLUCOSE AGAR STAB.

Good growth all down stab.

BLOOD AGAR AEROBIC.

Growth minute colonies.

GLYCERINE AGAR AEROBIC.

In 2 days no visible growth.

ORDINARY AGAR STAB.

In two days no growth.

POTATO AEROBIC.

No growth.

LITMUS MILK.

Acid in 2 days.

ORD. BROTH.

No growth.

GELATIN STAB.

No growth.

GLUCOSE BROTH ANAEROBIC

Cloudiness/

Cloudiness with deposit in 2 days.

AGAR SLOPE AEROBIC.

Feeble filmy growth of colonies which show no tendency to remain discrete.

In peptone water XXXI., 5, produced acid but no gas in lactose and saccharose, no change in salicin, raffinose and sorbit.

The other type of coccus isolated was XXXI. 11. This morphologically differed from XXXI. 4 & 5, in being much coarser and more irregular in films made from the glucose agar stab culture, but in a film made, a glucose broth culture, it appeared identical with XXXI.4 & 5. It showed the same cultural characteristics, but its action on sugars was not tested. It also, did not grow in gelatin stab.

SPORE BEARERS.

None were seen in this case or isolated.

GENERAL RESULTS.

Acid-tolerant Bacilli were present in large numbers in this case, and were of acidophilus type. Gram-negative bacilli were present in still larger numbers./

numbers. Of these lactose-fermenting b.coli were in an enormous majority: non-lactose fermenting bacilli were present in very small numbers, there being about 1 to 1000 lactose fermenters. A bacillus indistinguishable culturally from B.Paratyphosus A. was isolated, but its agglutination reactions proved it to be quite distinct.

Coccal forms were fairly numerous. Varieties both of the regular enterococcus type and of the irregular heat resisting type were isolated.

SPORE BEARERS were absent.

SPECIMEN XXXII.

- H. aged 1 year 6 months.

Male.

Charteris Ward, Sick Children's Hospital.

Examined October 16th 1912.

STATE.

Patient is normal gastro-intestinally. Is in hospital for poliomyelitis.

HISTORY.

Has never suffered from diarrhoea to any extent.

METHOD OF FEEDING.

Has been getting a mixed diet lately.

MOTION.

Obtained October 16th 1912; is brownish in colour, of firm consistence.

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FILMS.

i. Gram-positive Bacilli: -

Are scanty. A small number of these are moderately slender bacilli of the acidophilus, and there are also some very small delicate bacilli/

bacilli.

ii. Gram-negative Bacilli and Coccobacilli: -

Coliform bacilli are present in considerable numbers, being much more numerous than the gram-positive bacilli of acid-tolerant type.

iii. Coccal Forms: -

Are numerous and varied. Many are diplococci of the enterococcus type; others are of the same shape but larger, while there are some very small diplococci with pointed ends. In addition to these gram-positive forms there are also gram-negative diplococci of varying size, some with very pointed ends. There are also oval isolated gram-positive cocci.

iv. Spore-bearers: -

Are present in fair numbers. They are large gram-negative bacilli bearing an oval spore at the end. Morphologically they resemble the Bacillus Putrificus of Bienstock.

McCONKEY PLATES.

Showed numerous lactose-fermenting colonies and also a very few white colonies.

The number of non-lactose-fermenting colonies/

colonies is about ·2 per cent of the total.

PRIMARY ANAEROBIC MEDIA.

Tube A. 4 days old showed cloudiness with deposit. Gram-stained, it showed gram-positive diplococci of enterococcus type, occurring in pairs, clusters and short chains; and gram-negative bacilli coliform. These 2 varieties are in about equal numbers. No gram-positive bacilli present.

Tube B. showed cloudiness and deposit, and contained rather delicate and polymorph gram-positive cocco-bacilli, many occurring as diplos.

Tube C. showed cloudiness and deposit, and contained gram-negative coliform bacilli, along with gram-positive diplococci.

Tube D. showed clotting with a large amount of whey. In films spread from the whey, only gram-positive diplococci were to be seen.

SECONDARY ANAEROBIC MEDIA.

Plate A. 4 days old showed small and minute colonies only, smoothedged and delicate. From this plate oval gram-positive diplococci were isolated.

Plate B. showed good growth of minute colonies with a few small white denser colonies.

From/

From these polymorph gram-positive cocci-bacilli were obtained.

Plate C. showed very small colonies, and from these gram-positive diplococci were obtained.

Plate D. showed growth of minute colonies with slightly larger colonies as well. From these large oval gram-positive diplococci were obtained.

LACTOSE-FERMENTING BACILLI.

2 colonies, XXXII. 2 and 3 were selected for study from the McConkey Plates. These were identical in their reactions.

They were motile gram-negative bacilli, which did not produce indol, were Vosges and Proskauer negative, did not liquefy gelatin and produced acid and clot in litmus milk. They produced acid and gas in lactose peptone water, no change in saccharose dulcit, adonit, inulin, or inosit.

They were thus No. 7 McConkey, an unnamed and rather rare variety.

NON-LACTOSE-FERMENTING BACILLI.

One colony called XXXII. 1 selected for study.

This was a motile gram-negative bacillus which/

which was indol negative, Vosges and Proskauer negative, did not liquefy gelatin, and produced acid but no clot in litmus milk in 3 weeks.

It produced acid and gas in glucose, mannit, sorbit, galactose, maltose and dextrin peptone water, but no change in lactose, dulcit, saccharose, salicin, adonit, inulin, inosit, or raffinose.

A guineapig inoculated intraperitoneally with 2cc. of a 24 hours' broth culture of XXXII. 1, showed no sign of illness.

XXXII. 1, was tested as to its agglutinability by a Morgan's No. 1 Bacillus serum with negative results.

Is therefore an unnamed variety belonging to group H. of Lewis's classification.

BACILLI OF THE ACID-TOLERANT GROUP.

3 strains XXXII. 4, 5, and 6 were isolated from Plate B. They were all of the irregular coccobacillary type of acidophilus, were acid-resistant, grew badly in ordinary broth, but well in glucose broth and on glucose agar, and their colonies on glucose agar plates were of the smooth edged variety.

COCCAL FORMS./

COCCAL FORMS.

One strain XXXII. 9, isolated from Plate D, was a rather coarse irregular gram-positive cocco-bacillus, but after artificial cultivation for a short time it changed to a regular oval coccus occurringin diplos clusters and short chains.

Its growth characteristics in glucose agar stab, on glucose agar plate, in glucose broth, in ordinary broth and on ordinary agar slope were not distinguishable from those of the enterococcus.

SPOREBEARERS.

Though present in the original films, no spore-bearers were isolated from plates C and D.

GENERAL RESULTS.

The flora was very varied in this case, and it was not easy to guess the relative numbers of different varieties.

Gram-positive bacilli of the acid-tolerant type were scanty in the films, but were isolated from Plate B.

Gram-negative bacilli were numerous.

The/

The lactose-fermenting bacilli isolated were No. 7 McConkey.

A very small number of non-lactose-fermenting bacilli were present on the Plates, and the one strain studied was a unnamed variety.

Coccal forms were numerous and varied.

Spore-bearers of the B.Putrificus type were seen in the films but were not isolated.

SPECIMEN XXXIII.

R.K. aged 4 years.

Charteris Ward Royal Hospital for Sick Children.

Examined October 18th, 1912.

STATE.

Acute Diarrhoea.

METHOD of FEEDING.

At the time the diarrhoea commenced she was on a milk diet.

HISTORY.

Came into the ward suffering from nephritis. While in the ward started an attack of acute diarrhoea with blood in the stools. (Recovered completely).

MOTION.

Loose slimy with red blood.

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FIELDS.

---

i. Gram positive bacilli are present in fair numbers. They are of very varied morphology. Some, not very numerous, are slender bacilli of the acid-tolerant type. Some are much larger plumper bacilli which seem to be of another variety.

ii. Gram negative Bacilli and Coccobacilli. Are numerous/

numerous and are also very varied. Some are coliform, others are almost coccal. There are also some very large gram-negative bacillary forms, some of these in chains.

iii. Coccal Forms.

Are fairly numerous. Many are oval gram-positive cocci or coccobacilli, mainly as diplos. Some resemble the enterococcus. Many are diplococci resembling the enterococcus in shape but much larger.

iv. Sporebearers.

None of the organisms in the film are carrying spores.

<u>McCONKEY PLATES.</u>

Showed lactose-fermenting colonies, and non-lactose fermenters, the latter forming about 7% of the total.

Some of the non-lactose-fermenters turned out in fluid medium to be slow lactose fermenters.

<u>PRIMARY ANAEROBIC MEDIA.</u>

Tube A. 7 days old showed cloudiness and deposit. The film contained mainly gram-negative coliform bacilli; a few long, slender gram-positive bacilli probably decolourised forms of the same; and a few oval gram-positive cocci, isolated and in diplos/.

diplos.

Tube B. showed cloudiness and deposit. Gram-stained, it showed long, slender gram-positive bacilli, and some decolourised forms of the same.

Tube C. showed cloudiness and deposit and the organisms in the film were small gram-positive diplococci, many of them in chains.

Tube D. showed curdling and the whey contained some oval gram-positive diplococci.

SECONDARY ANAEROBIC MEDIA.

Plate A. 6 days old showed dense colonies of coli type with also some very small and minute colonies. From the small and minute, bacilli of the acid-tolerant group were isolated.

Plate B. showed small and minute colonies only. From these, bacilli of the acid-tolerant group were obtained.

LACTOSE-FERMENTING BACILLI

Two of the non-lactose fermenting colonies selected for study were found to ferment lactose in fluid medium. When replated on McConkey's medium in the usual routine, it was found that one, XXXIII. 1, fermented lactose slowly and feebly even on the solid medium./

medium.

In their reactions these 2 were identical (XXXIII. 1 & 2.). They were motile gram-negative bacilli, which were indol-positive, Vosges & Proskauer negative, did not liquefy gelatin, and produced acid and clot in litmus milk. They produced acid and gas in lactose, dulcit, glucose, mannit, galactose, maltose, and dextrin peptose water, but no change in saccharose, adonit, inulin, inosit, salicin, sorbit, raffinose.

The amount of gas produced in the lactose peptone water tube of XXXIII.2, after a week's incubation was very small.

These organisms thus coincided with the B. coli.communis in their reactions. XXXIII.2, especially was a variety which was either feebly lactose fermenting, or was in process of losing the power of fermenting lactose.

NON-LACTOSE FERMENTING BACILLI.

One strain XXXIII.4, was a motile gram-negative bacillus, which was indol negative, Vosges & Proskauer negative, did not liquefy gelatin, and produced no change, or a very slightly alkaline reaction in lutmus milk. It produced acid but no gas in glucose mannit, sorbit, and galactose, no change in lactose/

lactose, saccharose, adonit, inulin, inosit, salicin, raffinose, and dextrin, and a doubtful acid reaction in dulcit and maltose.

When these reactions were repeated twice over at intervals of some months, it was found that it did not ferment dulcit.

Its agglutination reactions to Para B. and Gaertner serum gave negative results.

It corresponds more closely in reactions to the Dysentery group, from which it differed in being motile. Further agglutination tests are being carried on, and will be included in another part of the Thesis, if completed in time.

A mouse fed for 5 successive days on bread soaked in broth cultures of XXXIII.4 died 3 weeks later, having shown signs of illness after the first dose. The organism was not recovered from the heart blood.

A guineapig inoculated subcutaneously with an agar slope culture, 24 hours old, of the same organism, died 6 days later, having shown signs of illness within 24 hours. The organism was not recovered from the heart blood.

BACILLI of the ACID TOLERANT GROUP.

In this case strains corresponding morphologically to the three main sub-divisions of the acid/

acid-tolerant group were isolated.

XXXIII.12, isolated from Plate A. was the irregular, very polymorph type, which Tissier called the Bacillus Bifidus, and corresponded to this bacillus in its anaerobic tendencies.

MORPHOLOGY.

(Glucose agar stab 4 days old). Gram-positive bacilli in chains and long threads; very polymorph, but all are bacilli. Some fairly large and thick, some decolourised. A great many coils and spirilla.

(Glucose broth 13 days old). Very much the same appearance; still in coils, some long chains of streptobacilli. Still very irregular.

GLUCOSE AGAR PLATE ANAEROBIC. Small and minute colonies. Edge became wrinkled. Had not the 'ball of wool' appearance.

GLUCOSE BROTH ANAEROBIC. Cloudy with a tenacious sediment at the foot.

GLUCOSE AGAR STAB. In 3 days a rather delicate growth in the anaerobic part of the tube only. Never grew right up to the surface.

LITMUS/

LITMUS MILK ANAEROBIC. In 5 days acid and clot.

BLOOD SERUM ANAEROBIC. In 5 days, very poor growth.

POTATOE ANAEROBIC. No growth.

GLYCERIN AGAR ANAEROBIC. Poor growth of minute irregular colonies.

GELATIN STAB. No growth.

GLUCOSE BROTH +·4% of ACETIC ACID. Cloudiness and deposit.

XXXIII. 13.

Was of the more regular, straight or slightly curved facultative aerobic type called by Moro the Bacillus Acidophilus.

MORPHOLOGY. (4 days old glucose agar stab) Straight moderately slender gram-positive bacilli rounded ends regular in appearance.

(13 days old glucose broth anaerobic) straight moderately slender bacilli, lying side by side.

GLUCOSE/

<u>GLUCOSE AGAR PLATE ANAEROBIC.</u>

Minute colonies, edge slightly irregular.

<u>GLUCOSE BROTH ANAEROBIC.</u> Cloudy with whitish tenacious deposit.

<u>GLUCOSE AGAR STAB.</u>

In 2 days moderate growth all down. In 4 days heavy growth above, getting thinner below.

<u>ORDINARY BROTH AEROBIC.</u>

No growth.

<u>PEPTONE WATER AEROBIC.</u>

No growth.

<u>ORDINARY AGAR SLOPE AEROBIC.</u>

Good growth of minute colonies.

<u>GLYCERIN AGAR SLOPE AEROBIC.</u> "

<u>BLOOD AGAR SLOPE AEROBIC.</u> "

<u>ORDINARY AGAR STAB.</u>

Good growth all down.

<u>ACTION on SUGARS in PEPTONE WATER</u> - lactose, glucose saccharose, mannit, salicin, inulin, and dulcit. No change was produced in a week.

<u>ACTION on SUGARS in BROTH,</u> - lactose, glucose, maltose and salicin were fermented within 3 days with a strong production/

production of acid. Saccharose, mannit, dulcit, adonit, inulin and inosit were unchanged after a week's incubation.

POTATO AEROBIC.

Good growth of white raised colonies.

GELATIN STAB.

Delicate growth of minute colonies. No liquefaction.

GLUCOSE BROTH + ·4% of ACETIC ACID.

Cloudiness with deposit.

XXXIII. 14.

Resembled XXXIII. 13, in cultural characteristics, but differed morphologically in being the small very slender straight, delicate, gram-positive bacillus, called by Tissier the Bacillus Exilis.

COCCAL FORMS.

2 Strains isolated from a gelatin plate culture, (XXXIII. 9 & 10) were found to be diplococci of the enterococcus type.

SPORE BEARERS.

None isolated.

GENERAL RESULTS.

As this specimen was isolated from a case of 4 years old, the films showed a very varied flora. Acid-tolerant bacilli were isolated, types of the Bacillus Bifidus, the Bacillus Acidophilus, and the Bacillus Exilis being obtained.

Gram-negative bacilli were numerous in the films.

Lactose-fermenting-bacilli of the B.coli Communis type were isolated.

Non-lactose fermenting bacilli of group C. of Lewis' classification were isolated.

Enterococci were isolated.

Spore bearers were not seen or isolated.

SPECIMEN XXXIV.

E.B. aged 2 years 5 months.

Dundas Ward, Royal Hospital for Sick Children.

Female.

Examined October 18th 1912.

STATE.

Suffering from coli pyelitis.

HISTORY.

Bowels have always been regular.

METHOD OF FEEDING.

Mixed diet, at present on milk.

A catheter specimen of urine, and a specimen of faeces was obtained.

From the urine the following organism was obtained (XXXIV. 1,). Was an non-motile bacillus, gram negative, indol positive, Vosges and Proskauer negative, which did not liquefy gelatin and produced acid and clot in litmus milk. It produced acid and gas in lactose, dulcit, glucose, mannit, salicin, sorbit, galactose, maltose, and no change in saccharose, adonit, inulin, inosit, or raffinose.

It differed therefore, from B.coli Communis in/

in being non-motile, and was B. Schafferi(No. 35 McConkey).

In the McConkey Plates spread from the faeces, both lactose-fermenters and non-lactose-fermenters were present, the latter forming about 1 per cent of the total colonies.

Two lactose-fermenting colonies were studied, XXXIV. 7 and 8.

They were motile gram-negative bacilli which were indol positive, Vosges and Proskauer negative, did not liquefy gelatin and produced acid and clot in litmus milk.

They produced acid and gas in lactose and dulcit peptone water, but no change in saccharose, adonit, inulin, or inosit.

Were therefore B.Coli Communis. (No. 34 McConkey).

One non-lactose-fermenting colony studied, XXXIV. 3, was a non-motile gram-negative bacillus which was indol positibe, Vosges and Prokauer negative, did not liquefy gelatin and produced an alkaline reaction in litmus milk. It produced acid and gas in glucose, mannit, dulcit and salicin but no change in/

in lactose and saccharose.

Had thus a resemblance to the Paratypoid-gaertner group from which it differed in fermenting salicin and in being indol positive.

GENERAL RESULTS.

B. Schafferi was obtained from the urine.

B. Coli Communis was obtained from the faeces.

An unnamed variety of non-lactose-fermenting bacillus was also obtained from the faeces.

SPECIMEN XXXV

- aged 10 days.

Maternity Hospital.

Examined October 28th 1912.

STATE.

Healthy full time child.

METHOD OF FEEDING.

Breast only.

MOTION.

Fairly solid greenish-yellow motion.

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FIELDS.

i. Gram-positive Bacilli: -

The enormous majority of the organisms present are of the irregular and irregularly-staining bifidus type. No bifid forms seen but all have a degenerate appearance.

ii. Gram-negative Bacilli and Cocco-bacilli.

Are extremely scanty and are only to be found after search.

iii. Coccal Forms/

iii. Coccal Forms.

A few Gram-positive Coccus of the enterococcus type are present, and a very few clusters of minute gram-negative cocci. Some Sarcinae are also present.

iv. Spore bearers.

Absent.

McCONKEY PLATE.

After 2 days incubation showed a very few colonies of the Coli type. No non-lactose-fermenters.

PRIMARY ANAEROBIC MEDIA.

TUBE A. 5 days old showed cloudiness with deposit. Gram stained showed mainly gram-negative Coli-form bacilli; also present gram-positive diplococci of enterococcus type but most of those rather large and plump. Some almost bacillary.

TUBE B. showed cloudiness deposit, and the films contained gram-positive bacilli, of very irregular appearance, some curved, others straight, in chains, swollen forms. etc. No bifid forms and all took the stain well.

TUBE C. showed slight cloudiness with deposit. Gram stained showed gram-positive diplococci.

TUBE D./

TUBE D. showed no clot, and no organisms were found in the film.

SECONDARY ANAEROBIC MEDIA.

PLATE A. 5 days old showed a growth of minute colonies, under low power these were smooth-edged finely granular colonies. Subcultures from several colonies showed them to consist of bacilli of the acid-tolerant group, and one contained enterococci.

PLATE B. showed also a growth of minute colonies. Subcultures from these also contained bacilli of the acid-tolerant group.

PLATE C. Also showed small and minute colonies. Subcultures from these showed diplococci of enterococcus type.

PLATE D. showed no growth.

LACTOSE FERMENTING BACILLI.

2 strains were studied, XXXV. 2 and 3. Both were indol-positive. Vosges and Proskauer negative. Neither liquefied gelatin and both produced acid and clot in litmus milk. XXXV.2 was non-motile/

non-motile produced acid and gas in lactose peptone water, but no change in saccharose, dulcit, adonit, inulin, or inosit. XXXV. 3 was motile, and produced acid and gas in lactose and saccharose peptone water but no change in dulcit, adonit, inulin or inosit.

NON-LACTOSE-FERMENTING BACILLI.

None were present on the McConkey plates.

BACILLI OF THE ACID-TOLERANT GROUP.

One of the isolated strains was particularly studied in this case.

XXXV.17.

Isolated from Plate B.

MORPHOLOGY. (Glucose agar stab 8 days old) is a very delicate straight small gram-positive bacilli, many occurring in masses side by side.

(Glucose broth 4 days old) small slender straight or slightly curved gram-positive bacilli, many in clusters side by side.

(Glycerin agar slope aerobic 4 days old) small moderately slender straight or slightly curved bacilli and strepto-bacilli.

(Blood/

(blood agar anaerobic 4 days old) occur as cocco-bacilli in chains. Many would in ordinary conditions be classified as cocci.

(Agar slope aerobic 4 days old) Small short slender bacilli straight uniform in size, occurring in chains as strepto-bacilli.

Non-motile in 4 days old glucose broth.

GLUCOSE-AGAR-PLATE ANAEROBIC.

Small and minute, smooth edged colonies.

GLUCOSE-BROTH ANAEROBIC

Cloudiness and deposit in 2 days.

GLUCOSE BROTH ANAEROBIC +·4 PER CENT ACETIC ACID.

Cloudiness and deposit in 2 days.

ORDINARY BROTH.

No growth.

AGAR SLOPE AEROBIC.

Faint Film.

GLYCERIN AGAR SLOPE AEROBIC.

(In 4 days faint growth of minute irregular colonies)

BLOOD AGAR SLOPE AEROBIC.

In 4 days faint film.

BLOOD/

BLOOD SERUM SLOPE ANAEROBIC.

In 4 days no visible growth.

GELATIN STAB.

Growth of minute isolated colonies all down.

No liquefaction.

PEPTONE WATER ANAEROBIC.

No growth.

POTATO AEROBIC.

No visible growth.

GLUCOSE AGAR STAB.

In 4 days moderate growth all down, much stronger below.

On the following sugars in peptone water no change was produced after 4 days incubation under anaerobic conditions -- Lactose, saccharose, dulcit, adonit, inulin, inosit, glucose, mannit, salicin, sorbit, raffinose, galactose, maltose, dextrin.

IN LITMUS MILK.

Acid and clot was formed under anaerobic conditions.

On the following sugars in broth no change was produced after 4 days incubation under aerobic conditions, but a strongly acid reaction was produced within 8 days. -- lactose, glucose, salicin, inulin,

In/

In Maltose mannit, dulcit, adonit, inosit no change was observed at the end of 8 days.

2.cc. of a 4 days old glucose broth culture of XXXV. 17, introduced intraperitoneally to a guineapig produced no effect.

XXXV. 14.

Another acid-tolerant strain isolated, XXXV.14 produced a strong acid reaction in lactose, saccharose, dulcit, inulin, glucose mannit salicin and maltose within 3 days: the medium in this case was broth.

COCCAL FORMS.

3 strains were isolated, one particularly studied XXXV.11. from Plate C. proved to be the usual type of enterococcus.

SPORE BEARERS.

None seen or isolated.

GENERAL/

GENERAL RESULTS

Gram-positive Bacilli of the acid-tolerant type were in an enormous majority in the original films. Those isolated were acid-tolerant and acidogenetic bacilli which preferred anaerobic conditions. Gram-negative Bacilli were scanty and consisted of the ordinary intestinal B.coli.

No non-lactose-fermenters grew on the plates.

Coccal forms were scanty and those isolated were of the ordinary enterococcus type.

SPECIMEN XXXVI.

- aged 7 days.

Maternity Hospital.

Examined October 28th 1912.

STATE.

Healthy full-time child.

METHOD OF FEEDING.

Is on breast and bottle as mother has not enough milk.

MOTION.

Pale green semi-solid.

BACTERIOLOGICAL EXAMINATION.

i. Gram-positive Bacilli: -

These are present in very large numbers. They are moderately slender bacilli, some straight, some slightly curved. All take up the stain well, and there are no bifed forms.

ii. Gram-negative bacilli and cocco-bacilli.

These are present in fair numbers and are of the usual coliform type.

iii/

iii. Coccal Forms: -

Are present in small numbers and are mainly diplococci of the enterococcus type.

iv. Spore-bearers; -

Spore-bearers are present in fair numbers and are large racquet-shaped gram-negative bacilli, large but somewhat slender.

McCONKEY PLATES.

After two days' growth the plates show numerous lactose-fermenting colonies which were all of one particular type. They were colonies with a round dark red centre and a pale regular periphery.

PRIMARY ANAEROBIC MEDIA.

Tube A. showed after four days' growth cloudiness with deposit. Gram-stained, this showed mainly gram-positive diplococci along with a few gram-negative bacilli. No gram-positive bacilli present.

Tube B. showed some deposit but no cloudiness. Gram-stained no organisms were found in the films.

Tube C. showed slight cloudiness with deposit/

deposit. Gram-stained, this showed some gram-negative coliform bacilli.

No spore-bearers in the film.

Tube D. showed clotting of the milk; the whey gram-stained showed a few gram-negative bacilli.

SECONDARY ANAEROBIC MEDIA.

Plate A. five days' old showed a mixture of large, small and minute colonies. The minute are all smooth-edged.

Plate B. showed very small and minute colonies.

Plate C. also showed some small colonies.

Plate D. showed some colonies of pin-head size and some other very small colonies.

LACTOSE FERMENTING-BACILLI.

Three colonies were kept from the McConkey plates for further study called XXXVI. 1, 2, and 3. These were found to be identical in their reactions. They were non-motile, gram-negative bacilli which were indol-positive, Vosges and Proskauer negative, and did not liquefy gelatine. They produced acid and clot in litmus milk and acid and gas in lactose, adonit, glucose, mannit, salicin, sorbit, galactose, maltose/

maltose and dextrin peptone water, but no change in saccharose, dulcit, inulin and inosit. They were therefore B.Acidi lactici (No.2 McConkey). 2cc. of a twenty-four hours' broth culture of XXXVI. 1, introduced intra-peritoneally into a guineapig produced death within forty-eight hours.

NON-LACTOSE-FERMENTING BACILLI.

None were present on the plates.

BACILLI OF THE ACID-TOLERANT GROUP.

One strain was particularly studied XXXVI. 14, isolated from plate B. This in glucose agar stab showed a growth of minute colonies in the upper part of the stab, and a growth of much coarser colonies in the lower part. This appearance is often to be seen in pure cultures of the members of the acid-tolerant group of bacilli. In this case the glucose agar tube was purposely broken, and fresh sub-cultures made and re-plated from the growth in the upper part, and from the growth in the lower part of the stab. Isolated colonies were then selected from each of these plates and put into glucose agar stabs once more, when it was found that this peculiarity of appearance was repeated in each of these re-purified cultures. This peculiarity of growth/

growth is probably due to the favouring influence of an oxygen-less atmosphere on the colonies in the deep part of the stab. These cultures were found to be acid-tolerant and consisted of gram-positive bacilli. Some of the sub-cultures showed slender bacilli while in others the bacilli were considerably plumper. On glucose agar plates colonies of these sub-cultures all showed the same appearance resembling small balls of tangled thread.

In glucose agar stabs a fairly strong growth of minute colonies was visible in two days. This grew right up to the surface.

COCCAL FORMS.

One strain XXXVI. 11, isolated from plate A, was a diplococcus which also grew in short chains in fluid media. The elements in chains were round, while those in diplos were elongated and resembled the enterococcus.

ANOTHER TYPE.

A rather large gram-negative bacillus which seemed to be the same organism as will be described under the name XLI. 15, was also isolated from Plate D.

MORPHOLOGY./

MORPHOLOGY.

A rather large gram-negative bacillus, many end to end in pairs, some in rather long threads. The peculiarity is that portions at the end are unstained, giving the appearance of a spore.

RESISTANCE TO HEAT.

This organism resisted pasteurisation at 80^{o}c. before separation, but when pure was killed at this temperature.

The peculiar staining reaction was only present in glucose agar and glucose broth cultures. When sub-cultured on ordinary agar and in ordinary broth, only coliform gram-negative bacilli and cocco-bacilli, not particularly large or plump were to be seen.

On sub-culturing back into glucose agar and glucose broth, the appearance was not repeated, only coliform bacilli being present.

The organism was plated on McConkey's Plate on which it grew in lactose-fermenting colonies.

These sub-cultured once more again gave an ordinary coliform appearance.

Its action on McConkey's tests was identic-

al/

identical with the action of XXXVI. 1, 2, and 3. That is to say it was the Bacillus acidi Lactici.

The question therefore, is whether i. the original culture from Plate D. was mixed, or ii. the Bacillus Acidi Lactici is capable of taking the stain in this peculiar manner, and assuming this peculiar shape and size, under certain conditions.

SPORE-BEARERS.

None were isolated.

GENERAL RESULTS.

Gram-positive bacilli of acid-tolerant type were dominant in the fields, and were isolated.

Gram-negative bacilli were present in fair numbers. These on McConkey Plates proved to be all lactose-fermenters, and those isolated were the Bacillus Acidi Lactici of Hüppe (No.2 McConkey).

Coccal forms were present in small numbers and were enterococci.

Spore-bearers were seen in films but were not isolated.

SPECIMEN XXXVII.

aged 5 days.

Charteris Ward, Royal Hospital for Sick Children.

Examined October 28th, 1912.

STATE.

Healthy full time child.

METHOD OF FEEDING.

Breast only.

HISTORY.

No diarrhoea.

MOTION.

Rather dark-green in colour.

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FILMS.

i. Gram-positive Bacilli: -

The field seems to be composed of the irregular type of acid-tolerant bacillus only. Most take up the stain very irregularly and are very degenerate looking. No bifid forms seen.

ii./

ii. Gram-negative Bacilli: -

Are scanty and many of those present seem to be degenerate forms of the gram-positive which have lost the power of taking up the stain.

iii. Coccal Forms: -

None are to be seen in films.

iv. Spore-bearers: -

Absent.

McCONKEY PLATES.

These though very thickly spread, remained sterile.

PRIMARY ANAEROBIC MEDIA.

Tube A, 5 days old, showed cloudiness and deposit. The film gram-stained showed only gram-negative bacilli of coliform type.

Tube B. showed deposit but no cloudiness. Gram-stained showed some very irregular and decrepit looking gram-positive bacilli.

Tube C, showed slight cloudiness with deposit. Gram stained; this showed small gram-positive diplo- and strepto-cocci, along with some long, very slender gram-negative threads.

Tube D, showed slight curdling, but no organisms/

organisms were found in the film.

SECONDARY ANAEROBIC MEDIA.

Plate A showed one or two pin-head colonies, and numerous very small and minute colonies. From these enterococci grew in all the subcultures.

Plate B showed some dense rather large pin-head size colonies: From these in subculture only yeasts grew.

Plate C. showed colonies from pin-head size downward. In subculture gram-negative gas-forming bacilli grew in all.

Plate D showed some minute colonies, from which enterococci were obtained.

PRIMARY AEROBIC MEDIA.

Ordinary Agar Plates were made straight from the faeces. From these were isolated i. yeasts, ii. staphylococcus albus, iii. entercocci.

LACTOSE-FERMENTING-BACILLI.

None were isolated from the McConkey Plates, but four strains XXXVII. 11, 12, 13, and 14, were isolated from Plate C.

XXXVII. 14 further studied was found to be that form of gram-negative bacillus which takes the strain/

stain, under certain conditions, in a peculiar manner so as to leave portions at the end unstained. Though isolated from Plate C, it was killed by pasteurising at 80°c. for 10 minutes when in pure culture.

It was replated on McConkey's medium and was then found to be indol-positive, Vosges and Proskauer negative, produced acid and clot in litmus milk, and acid and gas in lactose. dulcit, glucose, mannit and salicin peptone water, but no change in saccharose, adonit, inulin or inosit.

Morphologically it resembled XXXVI. 9 and XLI. 15.

NON-LACTOSE-FERMENTING BACILLI.

None on plates.

BACILLI OF ACID-TOLERANT GROUP.

None grew on artificial media, though almost in pure culture in the films.

COCCAL FORMS.

The enterococcus was isolated in 6 different strains XXXVII. 5 from agar plate aerobic, XXXVII. 6, 8, 9, and 10 from Plates A. and D.

YEASTS.

Were/

Were obtained from the aerobic agar plate and from Plate B.

GENERAL RESULTS.

Gram-positive bacilli of punctate bifidus type formed the enormous majority of the organisms in the films. These were probably all strictly anaerobic and were probably all dead before the examination was commenced.

Lactose-fermenting bacilli were extremely scanty.

Non-lactose-fermenting bacilli were absent.

Coccal forms were scanty and were of enterococcus type.

Yeasts were isolated.

Spore-bearers absent.

SPECIMEN XXXVIII.

F.I. aged 9 weeks.

Male.

Charteris Ward Royal Hospital for Sick Children.

Examined October 29th 1912.

An ill-nourished child, but vigorous.

METHOD of FEEDING.

Child was on the breast till admission to hospital, 3 days prior to date of examination of the Specimen. During the last three days has had peptogenic milk.

HISTORY.

Child was brought up to Hospital because the mother thought he was not thriving as he ought. Since admission has done well, takes bottle well, no diarrhoea. Has never had diarrhoea, or any other gastro-intestinal disorder.

MOTION.

Rather greenish normal motion.

THE/

THE BACTERIOLOGICAL EXAMINATION.

GRAM-STAINED FIELDS.

i. Gram-positive Bacilli.

A very large majority of the organisms present are gram-positive bacilli of the usual type. All take the stain well. No bifid or punctate forms. Fairly uniform in size. No long threads.

ii. Gram-negative Bacilli and Coccobacilli.

Are scanty. Those present are coliform.

iii. Coccal Forms.

Are also scanty and consist of diplococci and some isolated cocci. The diplococci are for the most part rather plump, the isolated cocci are oval: all these are gram-positive. There are also a very few gram-negative diplococci, slightly larger than the gram-positive, with pointed ends.

iv. Spore bearers.

No spore-bearing organisms seen.

McCONKEY PLATES.

These after three days growth showed upward of 500 lactose-fermenting colonies, no non-lactose fermenters being present.

PRIMARY/

PRIMARY ANAEROBIC MEDIA.

Tube A. after 8 days growth showed marked cloudiness and deposit. Gram-stained consists chiefly of two organisms i. coliform coccobacilli, gram-negative, of moderate size: ii. very slender and long gram-positive bacilli. Some losing the power of taking up the stain, and some completely gram-negative but all evidently the same organism. These were evidently old forms of the acid-tolerant group. No headlet or bifid forms.

A very few gram-positive diplococci also present.

Tube B. 8 days old, also showed two varieties. The one was a slender gram positive bacillus, like those of Tube A., but if anything slightly plumper: the other a very small gram-negative bacillus, staining peculiarly, so as to leave a portion at one end usually unstained.

Tube C. 8 days old. Showed slender gram-positive bacilli, like those of Tube A. many decolourised, along with some small gram negative coccobacilli.

Tube D. 8 days old, showed clotting. Gram-stained showed a similar appearance to Tube C.

SECONDARY/

SECONDARY ANAEROBIC MEDIA.

Plate A. 10 days old, showed a growth of rather dense colonies varying in size from large pinhead to very minute colonies. Some of the large colonies were white, others gray: all were spherical and smooth-edged.

Plate B. 10 days old, shows a growth of very small and minute colonies of acid-tolerant type: edge comparatively smooth in all.

Plate C. No growth took place.

Plate D. 10 days old, showed colonies varying in size from a pinhead downwards, moderately spherical and moderately dense.

LACTOSE-FERMENTING BACILLI.

Four of these were selected for study from the Bile Salt Plates, named XXXVIII, 3, 4, 5, & 6. These were found to be identical in all reactions, and may therefore be given together.

They were non-motile gram-negative bacilli of the ordinary coliform type, produced indol, were negative to Vosges and Proskauer's test, did not liquefy gelatin, but produced acid and clot in litmus milk. They produced acid and gas in lactose saccharose and dulcit, but none in adonit, inulin or inosit. The gas produced by these bacilli was considerably greater/

greater in amount than that usually produced by intestinal b.coli.

These are No.71 Group III. McConkey.

NON-LACTOSE-FERMENTING BACILLI.

None were present on the McConkey Plates.

BACILLI of the ACID-TOLERANT GROUP.

Several strains were isolated, 5 being preserved for further examination, named XXXVIII. 7, 8, 9, 10, & 15. Of these 7, 8, and 9 were isolated from Plate A. 10 from Plate B., 15 from Plate D.

XXXVIII. 7, morphologically is a short, straight or slightly curved gram-positive bacillus, with square or rounded ends, occurring for the most part in clusters side by side, but also singly and end to end. This film was made from an eight days old glucose agar stab culture.

On a glucose agar plate anaerobic colonies varied in size up to a small pin-head and were rather dense and smooth-edged.

In glucose agar stab culture a strong dense growth occurred all down the stab.

This bacillus was able to resist the addition/

addition of a ·4% of acetic acid to glucose broth.

XXXVIII. 8, morphologically is a very irregular gram-positive bacillus. Some are long and curved: others short and straight; some thin, some thick, some common forms, spirillae, and others very irregular shapes. Many have lost the power of taking up the stain.

This film also was made from an 8 days old glucose agar stab culture.

On a glucose agar anaerobic plate, colonies were minute, smooth-edged and delicate, being much more translucent than those of XXXVIII. 7.

<u>IN DEEP GLUCOSE AGAR STAB</u> a delicate growth occurred all the way down after 2 days' incubation.

<u>IN GLUCOSE BROTH</u>

produces cloudiness and deposit, growing equally well under aerobic and anaerobic conditions.

<u>ON AGAR SLOPE AEROBIC</u> grows as very minute colonies scarcely visible naked eye; under the low power these are fairly translucent roundish colonies with a not very regular outline.

<u>ON POTATO AEROBIC</u>

produces a dry white growth, raised from the surface, visible in 2 days. It produces neither acid/

acid nor gas in 3 days from the following sugars tested in peptone water, - lactose, saccharose, dulcit, adonit, inulin, inosit, glucose, mannit, salicin sorbit, raffinose, galactose, maltose and dextrin. (This was before I discovered that those organisms grew with great difficulty, in peptone water).

IN GELATIN STAB.

At room temperature, no growth.

XXXVIII. 9, is morphologically like XXXVIII. 8. On glucose agar plate anaerobic, and in deep glucose agar stab, the growth resembled that of XXXVIII. 8.

XXXVIII. 10. is morphologically like XXXVIII. 7.

ON GLUCOSE AGAR ANAEROBIC PLATE

Smooth-edged colonies up to the size of a pinhead and fairly dense were seen.

IN DEEP GLUCOSE AGAR STAB.

A strong growth was visible, all down, in 2 days growing up to, but not on the surface.

IN GLUCOSE BROTH ANAEROBIC

Slight cloudiness with deposit.

ON ORDINARY AGAR SLOPE ANAEROBIC

No growth.

ON/

ON ORDINARY AGAR SLOPE AEROBIC.

No growth.

IN ORDINARY BROTH AEROBIC.

No growth.

ON BLOOD SERUM AEROBIC.

A doubtful growth took place.

IN ORDINARY AGAR STAB.

A very delicate growth of minute colonies took place after 2 days incubation.

IN GELATIN STAB.

(At room temperature) No growth.

IN GLUCOSE BROTH + ·4% acid.

Cloudiness and deposit.

IN LITMUS MILK.

Acid and clot in 5 days.

XXXVIII. 15. is morphologically like XXXVIII 8 & 9, being of the extremely irregular type.

ON GLUCOSE AGAR PLATE ANAEROBIC

Grew as minute colonies rather fine and translucent, smooth-edged, some attaining the size of a small pinhead.

IN DEEP GLUCOSE AGAR STAB.

A very delicate growth of feathery colonies was visible in 2 days.

IN/

IN GLUCOSE BROTH ANAEROBIC.

Slight cloudiness with considerable deposit.

ON ORDINARY AGAR SLOPE AEROBIC.

A growth of minute colonies was visible in 2 days.

POTATO ANAEROBIC.

No visible growth in 5 days.

ORDINARY AGAR STAB AEROBIC.

In 4 days a scarcely visible growth of minute colonies was visible.

LITMUS MILK ANAEROBIC.

In 5 days no change.

ORDINARY BROTH AEROBIC.

No growth.

IN GELATIN STAB.

At room temperature, no growth.

Two types of acid-tolerant organisms were then isolated which may possibly be distinct.

i. Comprising 7 & 10 is a morphologically regular form, producing fairly dense colonies on glucose agar, and a dense growth in glucose agar stab culture:

ii. Comprising 8, 9, & 15, morphologically extremely irregular, and producing thin translucent colonies/

colonies and a delicate growth in stab culture.

COCCAL FORMS.

Two cultures which turned out to be identical in all respects were isolated, XXXVIII, 11 from Plate A. XXXVIII. 17 from Plate D.

These morphologically were egg-shaped gram-positive cocci or coccobacilli, occurring mainly in clusters. No tendency to occur in diplos or chains. Some decolourised, some distinctly bacillary (a very few of XXXVIII. 17, were noticed to be in diplos.)

Their reactions and cultural characteristics were found to be identical and are therefore, described together.

ON GLUCOSE AGAR PLATE ANAEROBIC

Occurred as smooth-edged colonies varying in size from minute up to small pin-head. Not distinguishable from acidophilus on the one hand, or entero-coccus on the other.

IN GLUCOSE AGAR STAB.

Fairly strong growth all down, visible in two days.

IN GLUCOSE BROTH ANAEROBIC.

Cloudiness and deposit.

ORDINARY AGAR SLOPE ANAEROBIC.

Minute, round, regular, translucent, rais-

ed/

raised, dew-drop-like colonies.

GLUCOSE AGAR SLOPE ANAEROBIC.

Similar appearance.

BLOOD AGAR SLOPE ANAEROBIC.

Similar appearance.

BLOOD SERUM SLOPE ANAEROBIC.

Similar appearance.

POTATO ANAEROBIC.

No visible growth.

LACTOSE PEPTONE WATER ANAEROBIC

No acid or gas in 5 days.

GLUCOSE PEPTONE WATER ANAEROBIC.

No acid or gas in 5 days.

LITMUS MILK ANAEROBIC.

Acid and clot in 5 days.

ORDINARY AGAR SLOPE AEROBIC.

Minute, round, regular, translucent, raised, dew-drop-like colonies.

ORDINARY AGAR STAB.

Vague growth all down stab. No isolated colonies seen.

GELATIN STAB.

At room temperature.

SPORE BEARERS.

None were isolated.

GENERAL/

GENERAL RESULTS.

Gram-positive Bacilli of the acid-tolerant type were the dominant organisms of the intestinal flora.

Gram-negative bacilli were scanty and those isolated were all non-motile members of Group III. McConkey.

No non-lactose-fermenters were present on the plate.

Coccal Forma were scanty and were of the usual enterococcus type.

Spore-bearers were absent.

SPECIMEN XXXIX. (from same case as XXIX. See also XLV.)

M.M. aged $4\frac{1}{2}$ months.

Female.

Charteris Ward, Royal Hospital for Sick Children.

Examined October 30th, 1912.

STATE.

Convalescent from acute diarrhoea.

METHOD OF FEEDING.

Is now getting Nâher Maltose.

HISTORY.

Since last specimen was obtained, patient has not recovered completely from diarrhoea, but is much improved.

MOTION.

Obtained October 30th, 1912. Is a rather large, pale, fatty stool of fairly solid consistence.

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FIELDS.

i. Gram-positive Bacilli: -

Are/

Are present in considerable numbers. Compared with specimen XXIX. they are distinctly more numerous than the gram-negative. Are mainly also of a more slender variety than those seen in XXIX, being of the straight or slightly curved acidophilus type.

ii. Gram-negative Bacilli and Cocco-bacilli: -

Are relatively less numerous than in specimen XXIX, but are still present in considerable numbers. Are mainly less plump and altogether smaller than those of specimen XXIX.

iii. Coccal Forms: -

These are fairly numerous, many being of the rather large oval type, some isolated, some in pairs.

iv. Sporebearers: -

None present in Films.

McCONKEY PLATES.

Showed numerous lactose-fermenting colonies along with a fair percentage of non-lactose fermenters.

On counting it was found that non-lactose fermenters formed about 5% of the total colonies.

PRIMARY ANAEROBIC MEDIA.

Tube/

Tube A, 4 days old, showed cloudiness and deposit. Gram-stained showed gram positive bacilli of the acetogenes type, along with gram-negative coliform bacilli in about equal numbers. A few gram-positive diplococci also present.

TUBE B. showed slight cloudiness with deposit. Gram-stained showed gram positive diplobacilli and short bacilli.

Tube C showed cloudiness and deposit. Gram-stained showed mainly gram negaitve bacilli coliform.

Tube D showed clotting. Gram-stained showed some gram positive diplococci.

SECONDARY ANAEROBIC MEDIA.

Plate A, 4 days old, showed growth of small and minute colonies mainly of the enterococcus and acid-tolerant type.

Plate B, 4 days old, showed very small and minute colonies of the acid-tolerant.

Plate C showed a few pin-head white colonies with numerous minute colonies. The latter turned out to consist of coccal forms.

Plate D showed some large fairly dense pin-head/

pinhead colonies, and some minute. These also were found to be coccal forms.

NON-LACTOSE-FERMENTING BACILLI.

3 colonies were kept for study, called XXXIX 1, 2, 3. Two, XXXIX 1 and 3 were found to be identical in their reactions while XXXIX 2 differed slightly. XXXIX 1 and 3 was motile gram-negative bacilli, which did not liquefy gelatin, which produced indol and were negative to Vosges and Proskauer test, and which produced an alkaline reaction in litmus milk in 7 days. They produced acid and gas in glucose peptone water, but no change in lactose, mannit, dulcit, saccharose and salicin.

XXXIX 2 differed only in producing acid and gas in mannit peptone water.

After an interval of 5 months XXXIX 1 and 2 were again put through the tests. XXXIX 1 was found to be quite unchanged, but XXXIX 2 now fermented dulcit with acid and gas production, otherwise remaining as before.

Thus Morgan's No.1 Bacillus was got from this specimen in the Primary McConkey Plates, whereas/

whereas from specimen XXIX. it was absent from these plates and was only got by the use of the old broth emulsion.

XXXIX. 2, on its second testing seemed to be more akin to the Paratyphoid-gaertner group, but differed from these in being indol-positive. 2 cc. of a 24 hours broth culture inoculated intra-peritoneally to a guineapig produced death within 24 hours.

BACILLI OF THE ACID-TOLERANT GROUP.

Several varieties were isolated from Plates A and B. To one type belonged XXXIX. 9, 12 and 13 from Plate B.

XXXIX. 9, 12, and 13.

MORPHOLOGICALLY. A small extremely irregular gram-positive coccus and cocco-bacillios.

GLUCOSE AGAR PLATE.

Very small spherical, smooth-edged colonies.

GLUCOSE AGAR STAB.

All these showed an indentical growth. Consisted of a smooth dark line all down the stab, with on one side of it only, an out-growth all down of minute colonies, grows right up to surface.

GLUCOSE/

<u>GLUCOSE BROTH.</u>

Cloudiness, with deposit.

<u>GLUCOSE BROTH + ·4 OF ACETIC ACID.</u>

Cloudiness with deposit.

To the second type belonged XXXIX. II.

<u>XXXIX. II.</u>

Was isolated from Plate A.

<u>MORPHOLOGICALLY.</u>

Consisted of delicate gram-positive bacilli some of fair length.

<u>GLUCOSE AGAR PLATE.</u>

Minute colonies of the usual type.

<u>GLUCOSE AGAR STAB.</u>

a very delicate growth all down the stab took place.

<u>GLUCOSE BROTH + ·4% OF ACID.</u>

<u>COCCAL FORMS.</u>

Two types of this also were isolated. The first type, isolated from the Pasteurised emulsions; Tubes A and B, was a large rather plump gram-positive diplo-coccus, some growing in short chains. Three organisms of this type were studied, XXXIX. 15, 17, 18. These were ascertained to be of the same/

same type as XXIX. 6 and 18.

The second type was also a gram-positive egg-shaped coccus; a good many decolorised. It occurred singly and in clusters. It was discovered to be identical as far as cultural characteristics and morphology go, with a variety of coccus or cocco-bacillus which is described more fully under XXXVIII. 15.

NOTE.

It is probable that these two types were identical as later one strain was isolated which at times gave the morphology of the one variety, and at other times of the other, on the same media.

SPORE-BEARERS.

None were seen in the films or isolated.

GENERAL RESULTS.

Morgan's No. 1 Bacillus was isolated from the McConkey Plates with ease in specimen XXXIX, whereas it was not present on the Plates of specimen XXIX. and was only isolated from it by means of other special methods.

Yet the inhibiting gram-positive flora was considerably/

considerably greater relatively to the potentially pathogenic gram-negative flora in specimen XXXIX, than in specimen XXIX, and coccal forms showed practically no difference. That is to say, in specimen XXXIX. the non-lactose-fermenting group had increased at the expense of the lactose-fermenting group, and not of the acid-tolerant group. Therefore during the height of the diarrhoea, lactose-fermenting-bacilli of the type of No. 71 of McConkey's classification were present in abnormally large numbers, and decrease in their numbers synchronized with recovery from diarrhoea.

ADDENDUM.

It may be noted here that diarrhoea again became very severe a few weeks later.

SPECIMEN XL.

M.B. aged 6 weeks.

Charteris Ward, Royal Hospital for Sick Children.

Examined November 1st 1912.

STATE.

Somewhat puny child, normal gastro-intestinally

METHOD OF FEEDING.

Bottle fed since birth, cow's milk and water.

HISTORY.

Has never suffered from diarrhoea. Was brought up to Hospital because she did not seem to be thriving well.

MOTION.

Obtained November 1st 1912, is pale green and of firm consistence.

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FILMS.

i. Gram-positive bacilli:-

Form an enormous majority of the organisms present. They are short bacilli of the/

the acidophilus type, straight or slightly curved. A little plumper than usual.

ii. Gram-negative bacilli and cocco bacilli.

These are present in relatively very small numbers. They are plump and rather larger than the usual coliform type. Many of them give the impression of being decolourised forms of the gram-positive.

iii. Coccal forms.

Some of the gram-positive and also of the gram-negative forms are so short that they could be classed as cocci, but diplococci of the enterococcus type are extremely scanty.

iv. Spore-bearers.

Are absent.

McCONKEY'S PLATES.

These after two days incubation showed numerous lactose fermenting colonies of the ordinary type. Non-lactose fermenters were absent.

PRIMARY ANAEROBIC MEDIA.

Tube A. 5 days old showed cloudiness with deposit. Gram-stained it showed large numbers of rather/

rather plump coliform gram-negative bacilli, a few rather coarse gram-positive diplococci, and numerous gram-positive bacilli of two kinds, one type consisting of long slender regular bacilli, the other of shorter, thicker bacilli.

Tube B. showed cloudiness with deposit. The film showed slender gram-negative bacilli and stouter gram-positive bacilli. The gram-negative were possibly degenerate forms of the gram-positive.

Tube C. showed slight cloudiness with deposit. The film showed gram-negative bacilli of coliform type, along with a few short moderately slender gram-positive bacilli, some as diplos.

Tube D. showed some clotting. The whey gram-stained showed some coliform gram-negative bacilli and one or two gram-positive cocci.

SECONDARY ANAEROBIC MEDIA.

Plate A. 5 days old showed some colonies of the coliform type, but much more numerous, small and minute colonies.

Plate B. showed very small and minute colonies. One type being very dense, the other being/

being very delicate, filmy. Plump acid-tolerant bacilli were obtained from the dense type, slender from the filmy.

Plate C. showed some pin-head colonies with numerous very small. Diplococci and plump acid-tolerant bacilli were obtained by subculture.

Plate D. showed rather dense white small pin-head colonies. The oval gram-positive type of cocco-bacillus was got by subculture.

LACTOSE FERMENTING BACILLI.

Four colonies were selected from the McConkey Plates for further study, XL. 1, 2, 3, and 4.

These were all motile gram-negative bacilli, indol positive, Vosges and Proskauer negative, which did not liquefy gelatin and produced acid and clot in litmus milk. All produced acid and gas in lactose peptone water but no change in saccharose, adonit, inulin or inosit. XL. 1 and 2 produced acid and gas in dulcit, whereas XL. 3 and 4 produced no change.

XL./

XL. 1 and 2 had thus the reactions of No.34, group II McConkey (B.Coli.communis), while XL. 3 and 4 were No.4 group I McConkey (B.Grünthal)

NON-LACTOSE FERMENTING BACILLI.

None were present on the Plates.

BACILLI OF THE ACID-TOLERANT GROUP.

5 strains were studied in this case, and they were of two types.

XL.II. isolated from Plate B. Was a moderately plump gram-positive bacillus of the type which was so prevalent in the original films of faeces.

In glucose broth the bacilli were more slender and were of the usual straight or slightly curved acidophilus type.

GLUCOSE AGAR PLATE.

The colonies were of the somewhat dense, smooth edged variety.

GLUCOSE BROTH ANAEROBIC.

Cloudiness and deposit in 2 days.

GLUCOSE BROTH +·4% ACETIC ACID.

Cloudiness and deposit.

GLUCOSE/

GLUCOSE AGAR STAB.

Good growth all down, extending up to the surface.

GLUCOSE BROTH AEROBIC.

Cloudiness with deposit. The reaction in 2 days was strongly acid.

BLOOD SERUM ANAEROBIC.

No visible growth.

ORDINARY AGAR ANAEROBIC.

Growth of minute colonies in 2 days.

BLOOD AGAR ANAEROBIC.

Growth of minute colonies in 2 days.

ORDINARY AGAR SLOPE AEROBIC.

No visible growth.

ORDINARY AGAR PLATE AEROBIC.

No growth.

GLYCERIN AGAR SLOPE AEROBIC.

No visible growth.

POTATO AEROBIC.

Whitish growth.

PEPTONE WATER AEROBIC.

No growth.

GELATIN/

GELATIN STAB.(room temperature)

No growth.

Was thus the usual type of acidophilus which grew better in the absence of oxygen than in the presence of it, but in the growth of which the presence or absence of sugar was the chief factor.

The other type of acid-tolerant bacillus, to which XL. 9, 12, 16, and 17 seemed to belong, differed from the XL.II. type chiefly in being more delicate in every way. Morphologically it was more slender and tended to grow in chains.

Colonies on glucose agar plates were very delicate and smooth-edged.

Growth in glucose agar stab culture was much more delicate and was slower, not being visible for 3 or 4 days.

This type also was acid-tolerant.

COCCAL FORMS.

Two types of cocci were isolated in this case also: one to which XL.15 belonged was a diplococcus of the ordinary enterococcus type morphologically/

morphologically XL.15.

MORPHOLOGY.

(Glucose agar stab 8 days old). Small gram-positive cocci in clusters and diplos;

(Glucose broth anaerobic 5 days old) rather plump gram-positive diplococci; (blood agar aerobic 24 hours old). small cocci occurring in diplos and clusters. This organism was grown on glucose agar plate, ordinary agar plate, glucose agar plate anaerobic, blood serum, agar slope, blood agar slope, and glycerin agar slope, in all of which produced small colonies of the usual streptococcus type visible in 24 hours. In glucose broth marked cloudiness and deposit was produced. In ordinary broth a slight turbidity; on potato no visible growth, and in gelatin stab at room temperature a delicate growth without liquefaction.

This organism was isolated from Plate C. and was therefore strongly heat-resisting before isolation.

The second type of coccus isolated was the oval gram-positive cocco bacillus of which XXXVIII.

11/

11 and 17 are types. Three strains of this organism were isolated in the present case, called XL. 7, 13, and 14. XL. 7 was isolated from Plate A, XL. 13 and 14 from PlateD. on which they seemed to be present in pure culture.

XL. 14 was studied on various media on which it gave appearances identical with those of XXXVIII 11 and 17.

SPORE-BEARERS.

No Spore-bearers seen or isolated.

GENERAL RESULTS.

Gram-positive bacilli of the acidophilus type were present in an enormous majority in this case. The type seen in the fields was isolated and studied: a second more slender type was also isolated.

Gram-negative bacilli were relatively scanty. Those isolated were intestinal B.Coli.

No non-lactose fermenters were present on the plates.

Coccal/

Coccal Forms were scanty. Those isolated were of the enterococcus type, and of the oval cocco-bacillus type.

Spore-bearers were absent.

SPECIMEN XLI.

M.H. aged 10 months.

Charteris Ward, Royal Hospital for Sick Children.

Examined November 3rd 1912.

STATE.

Suffering from acute diarrhoea.

METHOD OF FEEDING.

Was on the breast till 8 months, after that with milk, boiled bread, porridge. Is at present on albumen water.

HISTORY.

Had no diarrhoea till 10 days ago. During the last 10 days has had increasingly severe diarrhoea, & during the last 2 or 3 days, blood and mucus in the stools.

MOTION.

Greenish, very loose, containing mucus and a little bright red blood.

THE BACTERIOLOGICAL EXAMINATION.

GRAM-STAINED FIELDS.

i./

i. Gram-positive bacilli:-

Are extremely scanty, only about half a dozen being found after much search: these are of the ordinary moderately slender acidophilus type.

ii. Gram-negative bacilli and coccobacilli:-

Constitute by far the greatest number of the organisms present. They are rather plump bacilli and coccobacilli; very few slender forms are present.

iii. Coccal forms:-

Are fairly numerous and occur mainly in short chains of 4 - 6 rather large cocci; some rather large oval diplococci also present.

iv. Sporebearers:-

Are absent.

McCONKEY PLATES.

Showed after 3 days growth several thousand lactose fermenting colonies, along with one non-lactose-fermenting colony. This turned out in fluid medium to be a lactose-fermenter.

The broth emulsion was left in the incubation/

incubator for 3 weeks, and fresh McConkey Plates then spread from it. Numerous non-lactose-fermenting colonies grew.

PRIMARY ANAEROBIC MEDIA.

Tube A. 15 days old showed cloudiness with deposit. Gram-stained contained gram-negative coliform bacilli. No gram-positive bacilli seen.

Tube B. showed slight cloudiness with deposit. Gram stained it showed some oval cocci and diplos, most of them gram-negative, along with some very degenerate looking gram-negative bacilli.

Tube C. showed cloudiness with deposit. The film contained mainly coliform gram-negative bacilli: other organisms are mainly rather large oval gram-positive cocci and diplococci.

Tube D. showed some clotting. Gram stained it showed some coliform gram-negative bacilli and a few gram-positive diplococci of enterococcus type

SECONDARY ANAEROBIC MEDIA.

Plate A. showed (12 days old) dense whitish colonies/

colonies mainly of pin-head size. Subculture from these show coliform bacilli.

Plate B. had only a few rather large white colonies, which on examination were found to contain rather large streptococci. These were regarded as contaminations.

Plate C. showed some small and very small colonies. From these the oval gram-positive coccus, (see XXXVIII. 11 and 17) and a peculiar type of gram-negative bacilli (XLI. 15.) were obtained.

Plate D. Showed some small and very small colonies. From these also the oval gram-positive coccus was obtained.

LACTOSE-FERMENTING-BACILLI.

Two colonies selected from first series of McConkey Plates were as usual repurified before using.

XLI.1,was a gram-negative bacillus which produced indol, was Vosges and Proskauer negative, did not liquefy gelatin, and produced acid and clot from litmus milk. It produced acid and gas in lactose,/

lactose, saccharose, glucose, mannit, salicin, sorbit, raffinose, galactose, maltose, and dextrin peptone water, but no change in dulcit, adonit, inulin, or inosit.

XLI. 2 was a motile gram-negative bacillus which was indol positive, Vosges and Proskauer negative did not liquefy gelatin, and produced acid and clot in litmus milk. It produced acid and gas in lactose peptone water, but no change in saccharose, dulcit, inulin, adonit or inosit.

XLI. 1. was therefore No. 106 or 107 group IV. McConkey, while XLI. 2. was No. 4 group I. McConkey.

NON-LACTOSE-FERMENTING BACILLI.

None were isolated from the primary plates. Two called XLI. 10 and 11 were isolated from the Plates made from the old broth emulsion.

After repurification these were found to differ in one point only.

They were slightly motile gram-negative bacilli which produced indol, were negative to Vosges and Proskauer's reaction, liquefied gelatin and/

and produced a very strong fermentation of milk with an alkaline reaction in the whey. They produced acid and gas in glucose but no change in lactose, mannit, dulcit and salicin.

XLI. 10 produced no change in saccharose peptone water, while XLI. 11 produced acid and gas.

Growth in the saccharose tube was repeated twice, and in all the tubes once, and their reactions were found to be stable.

Two ~~c.c.~~ cc. of a 24 hours broth culture of XLI. 11 introduced intra-peritoneally to a guinea pig produced no effect.

Agglutination experiments were carried out.

BACILLI OF THE ACID-TOLERANT GROUP.

None were isolated.

COCCAL FORMS.

Two strains XLI. 14 and 17 were particularly studied, both being isolated from Plate C. XLI. 14 was found to be identical with the usual type of enterococcus while XLI. 17 was the oval gram-positive coccus or coccobacillus fully described under/

under XXXVIII. 11 and 17.

SPOREBEARERS.

None were isolated.

From Plate C. in this case a peculiar type of gram-negative bacillus was isolated which has been met with in 2 other cases, both of these being normal.

XLI. 15.

MORPHOLOGY. (Glucose agar stab 7 days old.)

Gram-negative bacilli rather large, almost all with an unstained portion at the end resembling a spore.

(Glucose broth 4 days old.) Large long gram-negative bacilli many spindle shaped, practically all apparently spore-bearing.

(Ordinary agar slope anaerobic.) Small gram-negative bacilli coliform, none showing spores or spindle shape.

This bacillus was isolated from Plate C. and had therefore resisted a heat of 80° c. for 10 minutes before isolation. After isolation, however, it was killed by pasteurisation.

Tested/

Tested on a large number of media it was found to resemble the ordinary intestinal B.coli in growth characteristics.

Was not tested in sugars.

GENERAL RESULTS.

Gram-positive bacilli of the acid-tolerant type were very scanty.

Gram-negative bacilli of the coli type were extremely numerous and were found to be almost all lactose fermenters.

Non-lactose-fermenters were only isolated by the use of the old broth emulsion and were of an un-named type.

Coccal forms were numerous and those isolated were the enterococcus and the oval gram-positive coccus.

Spore-bearers were absent.

SPECIMEN XLII.

I.A. aged 1 year 1 month.

Female.

Charteris Ward, Sick Children's Hospital.

Examined November 19th,1912.

STATE.

Suffering from acute diarrhoea.

METHOD OF FEEDING.

Was breast-fed till 5 weeks ago. Since then has been getting cow's milk.

HISTORY.

Patient developed lobar pneumonia a fortnight ago for which she was admitted to the ward. Had no diarrhoea up to that time.

Yesterday she developed acute diarrhoea, contracted in the ward. The cases from which specimens XLI. XLIII. and XLIV. were obtained are also at present in the ward.

AFTER HISTORY.

Patient died on November 23rd, having suffered from severe ileo-colitis.

MOTION.

Obtained November 19th, was a stringy brownish motion containing red blood and mucus.

THE/

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FILMS.

i. Gram-positive Bacilli: -

Are extremely scanty; about half a dozen small and slender bacilli are to be seen, one rather large and plump bacillus.

ii. Gram-negative Bacilli and Coccobacilli: -

Are very numerous. Vary considerably in morphology, some being very small and delicate bacilli, others larger and plumper.

iii. Coccal Forms: -

Are also very numerous. They are mainly gram-positive cocci occurring in diplos and short chains. Some of the larger oval isolated cocci are also present.

iv. Spore-bearers: -

A very small number of gram-positive bacilli bearing spores are to be seen.

McÇONKEY PLATES.

2 days' old showed lactose-fermenting and non-lactose-fermenting colonies. The latter formed over/

over 5 per cent of the total.

PRIMARY ANAEROBIC MEDIA.

Tube A. 4 days' old showed cloudiness with deposit, and contained chiefly gram-positive cocci. These were of moderate size, oval, and occurred in diplos and short chains. Gram-negative bacilli in fair numbers. Gram-positive bacilli - a very few.

Tube B. showed cloudiness with deposit, and contained some very small slender degenerate-looking bacilli, taking the stain badly.

Tube C. showed cloudiness and deposit. The film showed gram-positive cocci as streptos and diplos. A few gram-negative bacilli also.

Tube D. showed clotting with abundant whey formation. The whey gram-stained showed rather small gram-positive diplococci.

SECONDARY ANAEROBIC MEDIA.

Plate A. 9 days' old, showed colonies varying in size from coli type to minute. From the smaller colonies B.acidophilus was obtained.

Plate B. showed no growth.

Plate C. showed some small colonies, from which some very irregular gram-positive bacilli were/

were obtained.

Plate D. showed rather dense small colonies and from these rather large gram-positive diplococci were obtained.

LACTOSE-FERMENTING BACILLI.

2 strains studied XLII. 1 and 2, which were identical in their reactions.

They were motile gram-negative bacilli, indol positive, Vosges and Proskauer negative, did not liquefy gelatin and produced acid and clot in litmus milk. They produced acid and gas in lactose peptone water but no change in saccharose, dulcit, adonit, inulin or inosit.

They were therefore B.Grünthal (No.4 McConkey).

A guineapig inoculated intra-peritoneally with 2cc. of a 24 hours' broth culture of XLII. 1, showed no sign of illness.

On repeating the reactions of XLII. 1 and 2, some months later these were found to have changed. This is discussed elsewhere in the thesis.

NON-LACTOSE-FERMENTING BACILLI.

One/

One colony XLII. 5, was studied.

It was a motile gram-negative bacillus, which was indol positive, Vosges and Proskauer negative, did not liquefy gelatin and produced an alkaline reaction in litmus milk.

It produced acid and gas in glucose peptone water, but no change in lactose, mannit, dulcit, saccharose and salicin.

It was therefore Morgan's No.1. Bacillus. It was tested as to its agglutinability by serum XLIII. 6, with negative results.

2cc. of a 24 hours' broth culture introduced intra-peritoneally to a guineapig, produced death within 3 days.

BACILLI OF THE ACID-TOLERANT GROUP.

Three strains studied, XLII. 11, 12, and 13. These were all isolated from Plate A.

MORPHOLOGICALLY, these were similar. They were all rather plump gram-positive bacilli, stouter than bacilli of the acid-tolerant group usually are. Varied somewhat in length, but were otherwise uniform in appearance. In old cultures involution forms were produced. All grew under anaerobic conditions on agar slope.

ON/

ON GLUCOSE AGAR PLATE, they were all smooth-edged colonies.

GLUCOSE AGAR STAB.

XLII. 11 grew as a very heavy growth with leafy out-growths.

XLII. 12 grew as feathery isolated colonies.

XLII. 13 grew as a rather delicate growth all down.

GELATIN STAB.

No growth from any.

POTATO.

White shining growth.

GLUCOSE BROTH + ·4 PER CENT ACETIC ACID.

Cloudiness and deposit.

ORDINARY BROTH.

Very poor growth.

No action was produced by XLII. 11 on sugars in peptone water after a week's incubation.

This action on sugars in broth was as follows: -

LACTOSE/

	XLII. 11.		XLII. 12.	
	3 days	14 days	3 days	14 days
LACTOSE.	-	A.	A.	A.
SACCHAROSE.	-	A.	A.	A.
DULCIT.	-	-	A.	A.
INULIN.	-	A.	A.	A.
GLUCOSE.	-	A.	A.	A.
MANNIT.	-	-	A.	A.
SALICIN.	-	-	A.	A.
MALTOSE.	-	A.	A.	A.

IN LITMUS MILK.

XLII. 11 produced no change in 3 days, but acid without clot in a fortnight.

XLII. 12 produced acid and clot in 3 days.

COCCAL FORMS.

A diplococcus XLII. 15, isolated from Plate D. was like the enterococcus morphologically, but considerably larger.

In culture, its growth characteristics were indistinguishable from the usual enterococcus.

SPORE-BEARERS/

SPORE-BEARERS: -

None were isolated from Plates C or D.

GENERAL RESULTS.

Gram-positive bacilli of the acid-tolerant group were very scant, in the original films.

Gram-negative bacilli were numerous.

Non-lactose-fermenting bacilli formed about 5 per cent of the colonies on the McConkey plates. The strain studied was Morgan's No. 1. Bacillus. Coccal Forms were very numerous, some occurring in chains.

Spore-bearers were present in very small numbers.

SPECIMEN XLIII. (From same CASE as XXXI.)

M.L. aged 8 months.

Female.

Charteris Ward Royal Hospital for Sick Children.

Examined November 19, 1912.

STATE.

Acute diarrhoea, with blood and mucus in stool.

METHOD of FEEDING.

See under Specimen XXXI. On albumen water at present.

HISTORY.

Patient seemed to be doing well and had no gastro-intestinal disorder till 3 days ago, when severe diarrhoea started. Has been having 6 or 7 motions a day, some with blood and mucus present. There are 3 other cases at present in the ward, suffering from severe ileo-colitis, from whom specimens XLI., XLII., and XLIV. have been obtained. (M.M. from whom specimen XXIX., XXXIX., and XLV. have been obtained, was also in the ward at the same time).

MOTION.

Small slimy motion with much blood and mucus present.

BACTERIOLOGICAL/

BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FIELDS.

i. Gram-positive Bacilli.

None are present in the films. Compare this with specimen XXXI. in which they formed a considerable proportion of the organisms present.

ii. Gram-negative Bacilli and Cocco-bacilli.

These are present in almost pure culture. They are rather plump bacilli, some of fair length.

iii. Coccal Forms.

Only one variety is present and that in small numbers. It is a rather plump gram-positive coccus in short chains.

iv. Sporebearers.

Absent.

In the films there are also very large numbers of uninucleated cells, probably epithelial.

McCONKEY PLATES.

After 2 days incubation show lactose fermenting colonies and also numerous non-lactose fermenters. The latter amount to over 20% of the total.

PRIMARY/

PRIMARY ANAEROBIC MEDIA

Tube A. after 3 days' incubation, showed cloudiness and deposit, gram stained showed small, slender, gram-positive bacilli of acidophilus type, some gram-negative coliform bacilli, and a few gram-positive diplococci.

Tube B. showed slight cloudiness with deposit. Gram-stained some rather coarse gram-positive cocci and diplococci were seen.

Tube C. showed no growth.

Tube D. showed stormy fermentation with much whey formation. 2 cc. of the whey introduced subcutaneously to a guineapig, produced superficial ulceration, but no other effect.

This whey gram-stained showed some small gram-positive diplococci.

SECONDARY ANAEROBIC MEDIA.

Plate A. 9 days old showed colonies of coli type and numerous very small and minute colonies.

Plate B. showed very small colonies of two varieties, one very delicate, the other denser. Both have irregular edges.

XLIII. 11, was isolated from the former type, XLIII. 12 from the latter).

LACTOSE/

LACTOSE-FERMENTING BACILLI.

Two lactose-fermenting colonies XLIII.1 & 2 selected for study.

They were found to be identical in their reactions. They were non-motile gram-negative bacilli, which were indol-positive, Vosges and Proskauer negative, and produced acid and clot in litmus milk. They produced acid and gas in lactose and dulcit, but no change in saccharose, adonit, inulin and inosit. They did not liquefy gelatin. Belonged, therefore, to Group II. McConkey.

NON-LACTOSE-FERMENTING BACILLI.

Two colonies were selected for study. They were found to be identical in their reactions.

They were motile gram-negative bacilli, which produced indol, did not liquefy gelatin, and produced an alkaline reaction in litmus milk. They produced acid and gas in glucose peptone water, no change in lactose, mannit, dulcit, saccharose, and salicin.

They had thus all the cultural characteristics of Morgan's No.1 Bacillus.

A rabbit was immunised with repeated doses of/

of vaccines of XLIII.6, and the serum was found to agglutinate 2 Strains from other cases as highly as it did the homologous strain, but failed to agglutinate some others.

2 cc. of a 24 hours' broth culture of XLIII 4, introduced intraperitoneally to a guineapig, produced death within 24 hours.

5 mice were inoculated subcutaneously with doses of XLIII. 6, varying from 1/150th to 1/20th of a 24 hours' agar slope culture, with no apparent effect.

1 Mouse fed for 5 days on bread soaked in broth cultures of XLIII. 6, - showed signs of illness, but did not die till 3 weeks later, and the organism was not recovered from the heart blood.

BACILLI of the ACID-TOLERANT GROUP.

Two strains were isolated and studied. XLIII. 8 amd 11. XLIII. 8, was isolated from Plate A., XLIII.11 from Plate B.

Both were moderately slender, gram-positive bacilli, both resisted the addition of ·4% of acetic acid to glucose broth. In glucose agar stab both grew up to the surface, and in glucose broth produced cloudiness and deposit.

XLIII./

XLIII. 11, colonies on glucose agar plate were of the very irregular variety.

Were thus acid-tolerant bacilli of the acidophilus type.

COCCAL FORMS.

XLIII. 12, isolated from Plate B. turned out to be the enterococcus of the usual type. In fluid media some chains of fair length were produced. It will be observed that in this case it resisted the addition of ·4% of acetic acid to the glucose broth.

XLIII. 14 and 15 - isolated from Plate D. were the oval gram-positive cocco-bacillus of which a more full description is given under XXXVIII. 11. This egg-shaped coccus was in this case also, heat-resistant, before isolation. Pasteurisation at 80'c for 10 minutes when in pure culture, however, killed it.

SPORE-BEARERS.

Were absent.

GENERAL RESULTS.

Happily in this case, one is able to compare/

compare this specimen of a diarrhoea stool directly with specimen XXXI. obtained from the same case before diarrhoea started. The striking difference is that bacilli of the acid-tolerant type had completely disappeared from the field, - though 2 strains were isolated. That gram-negative bacilli were present in almost pure culture, and that Morgan's No. 1 Bacillus formed a large proportion of these. That coarse gram-positive strepto-cocci were also present in the field, those isolated being of the enterococcus type and of the oval cocco-bacillary type.

SPECIMEN XLIV.

R.M. aged 5 months.

Male.

Charteris Ward, Sick Children's Hospital.

Examined November 24th, 1912.

STATE.

Suffering from acute diarrhoea.

METHOD OF FEEDING.

Was a bottle-fed child, cow's milk and water.

HISTORY.

Patient was admitted to the Ward on Nov. 13th. On November 19th he started a very severe attack of diarrhoea, and has been having 6 or 7 motions in the 24 hours. No blood has been seen.

AFTER HISTORY.

Patient died on November 25th, the day after the examination of the specimen.

MOTION.

Very slimy greenish motion containing mucus, but no blood.

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FIELDS.

i./

i. Gram-positive Bacilli: -

Are practically absent. Only one or two are to be found after some search. These are of the usual acidophilus type.

ii. Gram-negative Bacilli and Coccobacilli: -

Are very numerous, and are coliform.

iii. Coccal Forms: -

These are present in considerable numbers, many as diplos, a few in chains. They vary in size some being coarse, others smaller and more delicate. Practically all are gram-positive.

iv. Spore-bearers: -

Are absent.

McCONKEY PLATES.

Plates showed lactose-fermenting colonies and also non-lactose-fermenters, the latter forming about 5 per cent of the total.

PRIMARY ANAEROBIC MEDIA.

Tube A. 8 days' old showed deposit and cloudiness, and contained gram-negative bacilli and coccobacilli, rather plump. No gram-positive organisms were found in the film.

Tube/

Tube B. showed some deposit, no cloudiness. No organisms were found in the film.

Tube C. showed very slight deposit with no cloudiness. No organisms were found in the film.

Tube D. showed clotting. The whey, gram-stained, showed some degenerate-looking, rather large gram-negative bacilli.

SECONDARY ANAEROBIC MEDIA.

Plate A. six days' old, showed only a few colonies, of moderate size. From these were obtained gram-negative bacilli of the type XLI. 15, oval gram-positive cocco-bacilli, and the enterococcus.

Plate B. showed a very few small colonies. From these also were obtained the large gram-negative bacilli of type XLI. 15.

Plate C. showed no growth.

Plate D. showed small and minute colonies. From these all were obtained gram-negative bacilli of type XLI. 15.

LACTOSE-FERMENTING BACILLI.

One colony XLIV. 6, selected for study.

This was a motile gram-negative bacillus, indol-positive, which did not liquefy gelatin, gave a/

a negative Vosges and Proskauer reaction, and produced acid and clot in litmus milk.

It produced acid and gas in lactose and dulcit peptone water, but no change in saccharose, adonit inulin or inosit.

It was therefore B.coli Communis, (No. 34 McConkey).

NON-LACTOSE-FERMENTING BACILLI.

Two studied, called XLIV. 1 and 4.

These were non-motile gram-negative bacilli which did not liquefy gelatin and produced an alkaline reaction in litmus milk. XLIV. 1, gave a negative.

They produced acid in glucose and mannit peptone water, but no change in lactose, dulcit, saccharose, salicin, inulin, or raffinose.

Morphologically they bore no resemblance to the large gram-negative bacillus isolated from the anaerobic plates, being small and delicate bacilli and coccobacilli.

They were thus Dysentery Bacillus Type "Y" of Hiss and Russell.

A rabbit was immunised with killed culture of XLIV. 1, and its serum is being used for agglutination/

agglutination tests.

2 cc. of a 24 hours' broth culture of XLIV. 1, introduced intraperitoneally to a guinea-pig produced no immediate result. The guineapig died 17 days later. The organism was not recovered from the heart blood.

BACILLI OF THE ACID-TOLERANT GROUP.

None were isolated in this case.

COCCAL FORMS.

Two forms were isolated from Plates A.

One was the enterococcus.

The other was the large gram-positive egg-shaped coccus.

SPORE-BEARERS.

None were seen in films or isolated.

The other organism isolated was the large gram-negative bacillus with unstained portion at the end like a spore (See XLI. 15). It will be noted that this was obtained both from acid medium and from pasteurised culture, before isolation from the faeces.

GENERAL/

GENERAL RESULTS.

Gram-positive bacilli of the acid-tolerant group were extremely scanty.

Gram-negative bacilli were very numerous.

The lactose-fermenting bacillus studied was B.coli Communis.

Non-lactose-fermenting bacilli were present on the McConkey Plates forming about 5 per cent of total colonies.

Those isolated were culturally Dysentery Bacilli; agglutination experiments are being carried.

Coccal forms, some in chains, were numerous.

Sporebearers were absent.

SPECIMEN XLV. (from same case as SPECIMENS XXIX. and XXXIX.)

M.M. aged 6 months.

Female.

Charteris Ward, Royal Hospital for Sick Children.

Examined January 6th, 1913.

STATE.

Now quite healthy and strong.

METHOD of FEEDING.

Milk and water now.

HISTORY SINCE LAST EXAMINATION.

Patient had another very severe attack of diarrhoea during November and December, but is now much improved, has put on weight and is being sent home.

MOTION.

Fairly solid, pale rather fatty motion.

BACTERIOLOGICAL EXAMINATION.

GRAM-STAINED FIELDS.

i. Gram positive Bacilli.

These are dominant in the fields and are of the acid-tolerant type, being mainly moderately slender, slightly curved bacilli.

ii. Gram-negative Bacilli and Coccobacilli.

These are scanty and this is the great difference between fields of this specimen on the

one/

one hand and those of XXIX, and to a lesser extent of XXXIX on the other.

iii. Coccal Forms.

These are numerous in this case and varied. Some are slender diplococci, others rather plump, some of fair size. Are almost all gram-positive.

iv. Spore Bearers are absent.

Compared with XXIX the chief difference is a great diminution of the gram-negative bacilli.

MCCONKEY PLATES.

Showed after 2 days incubation a small number of non-lactose-fermenters in addition to numerous lactose-fermenting colonies of the ordinary type. The non-lactose-fermenters formed about 2-3% of the total colonies.

PRIMARY ANAEROBIC MEDIA.

Tube A. 4 days old showed marked cloudiness and deposit. Gram-stained showed mainly coccal forms, most as diplos but short chains and also clusters were fairly numerous. Gram-negative bacilli were also present in moderate numbers. No gram-positive bacilli seen.

Tube/

Tube B. showed no cloudiness and a very slight deposit after 4 days growth. Gram stained no organisms were found in film.

Tube C. showed slight cloudiness with deposit. Gram stained showed coliform gram-negative bacilli, rather plump along with some coarse gram positive streptococci.

Tube D. showed no clotting. No organisms found.

SECONDARY ANAEROBIC MEDIA.

Not used in this case.

LACTOSE-FERMENTING-BACILLI.

Two colonies were selected for study. XLV. 3 and 4.

They were found to be identical in their reactions. They were motile gram negative bacilli, which produced indol and were negative to Vosges and Proskauers test. Neither liquefied gelatin and both produced acid and clot in litmus milk. They produced acid and gas in Lactose and saccharose peptone water, no change in dulcit, adonit, inulin or inosit.

Belonged/

Belonged, therefore, to group IV. McConkey

NON-LACTOSE-FERMENTING BACILLI.

Two colonies were selected for study XLV. 1 and 2. Both were replated before using.

XLV., 1, was found to ferment lactose in fluid medium, 1 though somewhat feebly and slowly, and was discarded.

XLV.2, was a non-motile gram-negative bacillus, which produced a slightly alkaline reaction in litmus milk in a fortnight, did not produce indol, nor liquefy gelatin. It produced acid but no gas in glucose and mannit, and no change in lactose dulcit, saccharose and salicin.

Corresponded culturally, therefore, to B.Dysenteriae "Y" of "Hiss and Russell".

1 cc. of a 24 hours broth culture introduced intraperitoneally to a guineapig produced death within 24 hours.

GENERAL RESULTS.

Judging by the films the main difference between this specimen and XXIX. and also XXXIX. was an increase in the gram-positive bacilli of the acid/

acid-tolerant type, and a decrease of the gram negative bacilli.

The decrease in lactose-fermenters noticed in Specimen XXXIX. was also noticeable in this specimen. It will be further observed that the lactose-fermenting-bacilli studied in this specimen were of a different type from those studied in Specimen XXIX.

Further non-lactose-fermenters were still present in this case, though these were less numerous relatively to the lactose-fermenters than in XXXIX, and consequently <u>absolutely</u> still less numerous. The one studied was of the Dysentery group, compared with Morgan's No.1 type, isolated from XXXIX. and XXIX.

Coccal forms were still numerous.

Spore bearers as before - absent.

SPECIMEN XLVI.

J.B. aged 1 year 4 months.

Charteris Ward, Royal Hospital for Sick Children.

STATE.

Acute Diarrhoea.

METHOD OF FEEDING.

Was a bottle-fed child. Lately more solid food.

HISTORY.

Has always had a tendency to diarrhoea. Never very bad till lately when it has become very acute. For the last month has had several motions a day and for the last few days there has been red blood in the motions.

Had whooping cough lately.

Had an ischiorectal abscess which was opened on December 30th.

MOTION.

Obtained January 6th, 1913, contains red blood and mucus. (Patient died on January 9th.)

THE BACTERIOLOGICAL EXAMINATION.

GRAM/

GRAM STAINED FILMS.

i. Gram-positive Bacilli: -

Gram-positive bacilli are present in very small numbers. They are of the usual moderately slender, acidophilus type.

ii. Gram-negative bacilli and cocco-bacilli: -

These are present in extremely large numbers and are of the usual coliform type.

iii. Coccal Forms: -

These are also present in large numbers, though greatly out-numbered by the gram-negative bacilli.

iv. Spore-bearers: -

Are absent.

There are present in the films numerous red blood corpuscles, epithelial cells and pus cells.

McCONKEY PLATES.

Showed after two days incubation lactose fermenting colonies of the ordinary type along with numerous non-lactose-fermenters. The latter form about ten per cent of the total colonies.

PRIMARY/

PRIMARY ANAEROBIC MEDIA.

Tube A. four days old showed cloudiness with deposit. The film contained diplococci of the enterococcus type and some gram-negative coliform bacilli. No gram-positive bacilli were present.

Tube B. showed no growth and no organisms were found in the film.

Tube C. showed slight cloudiness with deposit. The film contained gram-positive enterococci, and some gram-negative coliform bacilli.

Tube D. showed some clotting. The whey gram-stained contained some gram-positive diplococci of the enterococcus type.

SECONDARY ANAEROBIC MEDIA.

None were made in this case.

LACTOSE-FERMENTING BACILLI.

Two colonies were selected re-purified and tested.

XLVI. 3, was a non-motile gram-negative bacillus, indol-positive, Vosges and Proskauer negative, which did not liquefy gelatin and produced acid and clot in litmus milk. It produced acid and gas/

gas in lactose and dulcit peptone water, but no change in saccharose, adonit, inulin or inosit.

XLVI. 4, was a doubtfully motile gram-negative bacillus which was indol negative, Vosges and Proskauer positive, did not liquefy gelatin and produced acid and clot in litmus milk. It produced acid and gas in lactose and saccharose, no change in dulcit, adonit, inulin or inosit.

NON-LACTOSE-FERMENTING BACILLI.

Two colonies were selected for study.

XLVI. 1 and 2.

These were non-motile gram-negative Bacilli which were indol-positive, Vosges and Proskauer negative, did not liquefy gelatine and produced an alkaline reaction in litmus milk after a preliminary acidity. They produced acid and gas in glucose and mannit but no change in lactose, dulcit, saccharose and salicin.

They had thus all the cultural charteristics of the dysentery bacillus type "Y" of Hiss and Russell.

Their agglutination reactions for final confirmation of their identity are not yet completed and will be included in another part of the thesis/

thesis. 1 cc. of a twenty-four hours'broth culture of XLVI. 1, introduced intra-peritoneally to a guinea-pig produced death within twenty-four hours.

BACILLI OF THE ACID-TOLERANT GROUP.

None were isolated.

COCCAL FORMS.

Two strains were isolated called XLVI. 5 and 6, which proved to be diplococci of the enterococcus type.

SPORE-BEARERS.

Were absent.

GENERAL RESULTS.

Gram-positive bacilli of the acid-tolerant group were extremely scanty in the films.

Gram-negative bacilli were present in very large numbers and a large proportion of these were non-lactose-fermenting bacilli. These strains of the latter which were studied were of the dysentery group.

Coccal forms were numerous and an unusual number were present in films in chains.

Spore-bearers were absent.

SPECIMEN XLVII.

D.D. aged 2 months.

Charteris Ward, Royal Hospital for Sick Children.

Examined Jan.6th 1913.

STATE.

Suffers from pyloric spasm.

METHOD OF FEEDING.

Breast Fed for 2 days. Since then milk and water.

HISTORY.

Has had a good deal of vomiting since birth, but has had no diarrhoea to speak of. Bowels as a rule rather constipated.

MOTION.

Pale rather fatty looking stool.

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FILMS.

i. Gram positive bacilli:-

Gram positive bacilli of the acidophilus type constitute the dominant variety present/.

present. These are straight or slightly curved bacilli moderately slender, taking the stain well. No punctate or bifid forms seen.

ii. Gram-negative bacilli and cocco bacilli.

These are very scanty, those present being of coliform type.

iii. Coccal forms.

Gram-positive cocci are scanty, being mainly of large oval type.

Diplococci of the enterococci are very scanty, some larger diplococci being present in greater numbers.

iv. Spore-bearers.

Are absent.

McCONKEY'S BILE SALT LACTOSE NEUTRAL RED AGAR. PLATES.

These after two days incubation showed lactose fermenting colonies only.

PRIMARY ANAEROBIC MEDIA.

Tube A. After four days anaerobic growth showed marked cloudiness and deposit. Gram-stained showed/

showed numerous gram-positive bacilli of the acidophilus type; a few coarse oval cocci in short chains; a few enterococci both gram-positive and gram-negative; a very few gram-negative coliform bacilli.

Tube B. showed slight cloudiness and deposit. Gram-stained, this consisted of moderately slender gram-positive bacilli of the usual acid-tolerant type.

Tube C. showed no growth.

Tube D. showed slight clotting. No organisms were found in films from the whey.

LACTOSE FERMENTING BACILLI.

These lactose fermenting colonies were selected for study, named XLVII.1, 2 and 3. XLVII.1 was a non-motile gram-negative bacillus which produced indol, did not liquefy gelatin, produced acid and clot in litmus milk and acid and gas in lactose dulcit, glucose, mannit and salicin; no change in saccharose peptone water.

2cc. of a twenty four hours' broth culture introduced interperitoneally to a guinea pig produced death within eighteen hours.

Organisms/

Organisms XLVII. 2 and 3 were found to be identical in all reactions. They were non-motile gram-negative bacilli which produced indol, were negative to Vosges and Proskauer's test and did not liquefy gelatin in two and a half months. Both produced acid and clot in litmus milk and acid and gas in lactose saccharose and dulcit with no change in adonit, inulin and inosit.

NON-LACTOSE FERMENTING BACILLI.

None were present on the McConkey plates.

BACILLI OF THE ACID-TOLERANT GROUP.

No bacilli of the acid-tolerant group were specially studied though present in large numbers.

SPORE-BEARERS.

Absent.

GENERAL RESULTS.

Gram-positive bacilli of the acid-tolerant type were the dominant variety in this case.

Lactose Fermenting Bacilli formed only a small part of the flora. No non-lactose fermenting bacilli were found. Coccal forms also formed only a small part of the flora. Spore-bearers were absent.

SPECIMEN XLVIII.

S. aged 1 year 9 months.

Charteris Ward, Royal Hospital for Sick Children.

Examined January 6th 1913.

STATE.

Bronchitis. Healthy gastro-intestinally.

METHOD OF FEEDING.

On breast till 11 months, now gets mixed diet.

HISTORY.

Was admitted on December 14th for his lung condition which is doing well. Has never had diarrhoea.

AFTER HISTORY.

Kept free from diarrhoea during his stay in the ward, which was up till January 17th.

MOTION.

Yellowish fairly solid motion.

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FILMS.

i. Gram-positive bacilli:-

These are present in considerable numbers. Most of these are of the usual moder-ately/

moderately slender acidophilus type but there are also larger and plumper bacilli in fair numbers.

ii. Gram-negative bacilli and cocco bacilli.

These also are present in considerable numbers, and are of varied coli-form type.

iii. Coccal Forms.

These are fairly numerous, some are of the enterococcus type; many of the larger egg-shaped variety.

iv. Spore-bearers.

A fair number of gram-positive and gram-negative spore-bearing bacilli morphologically resembling B.enteritidis sporogenes or B.aerogenes capsulatus are present.

McCONKEY PLATE

Showed a large number of lactose fermenting colonies with in addition a small number of non-lactose-fermenting colonies.

PRIMARY ANAEROBIC MEDIA.

TUBE A. four days old showed marked cloudiness and deposit. Gram-stained showed almost/

almost pure culture of lanceolate gram-positive diplococci of enterococcus type. A few gram-negative coliform bacilli also present. No gram-positive bacilli.

Tube B. showed no growth.

Tube C. showed no growth.

Tube D. showed stormy fermentation with a considerable amount of whey formation but no organisms could be found in the film.

LACTOSE FERMENTING BACILLI.

Two lactose fermenting colonies XLVIII 3 and 4 were selected for further study. These were found to be identical in their reactions. Both were motile.

Gram-negative bacilli which produced indol, did not liquify gelatine, were negative to Vosges and Proskauer's test and produced acid and clot in litmus milk. Both produced acid and gas in lactose, saccharose and dulcit but no change in adonit, inulin or inosit.

NON-LACTOSE FERMENTING BACILLI.

Two/

Two colonies were selected for further study XLVIII i and 2. XLVIII. i. was a non-motile gram-negative bacillus which produced indol, did not liquify gelatine and was negative to Vosges and Proskauer's test. It produced acid and gas in glucose, mannit and dulcit, but no change in lactose, saccharose or salicin. In Litmus milk it produced acid but no clot in a fortnight. Its agglutination reactions were tested with sera of para A., para B and Gaertner with negative results.

1 cc. of a twenty-four hours broth culture inoculated interperitoneally into a guinea-pig produced death within twenty-four hours.

GENERAL/

GENERAL RESULTS.

Gram-positive bacilli - of the acetogenes type, were at least as numerous as other varieties in this specimen. Gram-negative bacilli were also numerous. The lactose fermenting organisms isolated were found to belong to group 3. McConkey. Non-lactose fermenting bacilli were numerous, and the strain studied was found to be culturally indistinguishable from B. paratyphosis A. but by the serum reactions was found to be entirely different. Coccal forms were inconspicuous, but spore-bearing bacilli of the bacillus welchii type were present in fairly large numbers.

SPECIMEN XLIX.

T.T. aged 5 weeks.

Charteris Ward, Royal Hospital for Sick Children.

Examined Jan. 6th, 1913.

STATE.

Suffering from pyloric stenosis.

METHOD OF FEEDING.

Breast-fed for first 3 weeks of life. Since then has been on Allenbury.

HISTORY.

Vomiting and constipation since birth.

AFTER HISTORY.

Pylorus was stretched by Mr. Stiles, a few days after date of examination of the specimen. Severe diarrhoea started after this and continued till death 10 days later.

MOTION.

Greenish rather loose stool.

BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FIELDS.

i./

i. Gram-positive bacilli: -

These are present in considerable numbers but are out-numbered by the gram-negative bacilli. They are mainly short, moderately slender bacilli of the acidophilus type.

ii. Gram-negative bacilli and cocco-bacilli: -

These are present in large numbers being the dominant organism present. They are of coliform type, but vary considerably in size and shape.

iii. Coccal Forms: -

These are not very numerous. Most are small oval coccal bacilli; a fair number are rather small diplococci of the enterococcus type. Nearly all are gram-positive. The larger coccal forms as seen in some of the older cases are absent.

iv. Spore-bearers: -

No spore-bearers or large bacilli present.

McCONKEY PLATES.

These after two days incubation showed lactose-fermenting colonies with a considerable number of non-lactose-fermenters. The latter comprised/

comprised more than ten per cent of the total colonies.

PRIMARY ANAEROBIC MEDIA.

Tube A. four days old showed marked cloudiness and deposit. Gram-stained showed gram-positive cocco-bacilli alone and in chains. It was not easy to determine whether these were cocci or the cocco-bacillary form of the acidophilus.

Tube B. showed no cloudiness but slight deposit. Gram-stained, no organisms could be found in the films.

Tube C. showed cloudiness and deposit. Gram-stained showed coliform gram-negative bacilli and slender gram-positive bacilli of the acidophilus type. No spores.

Tube D. showed no change. Gram-stained, no organisms were found in films.

SECONDARY ANAEROBIC MEDIA.

None were made from this case.

LACTOSE-FERMENTING BACILLI.

Two lactose-fermenting colonies were selected from the McConkey Plates for study, called XLIX. 3 and 4. These were found to be identical in their reactions. They were doubtfully motile gram/

gram-negative bacilli which produced indol were negative to Vosges and Proskauer's test and did not liquefy gelatin. They produced acid and clot in litmus milk, and acid and gas in lactose, saccharose, and dulcit peptone water, but no change in adonit, inulin or inosit.

NON-LACTOSE-FERMENTING BACILLI.

Two non-lactose fermenting colonies were selected for further study from McConkey Plates called XLIX. 1 and 2. Both were gram-negative bacilli which did not liquefy gelatin, produced indol and were negative to Vosges and Proskauer's test. XLIX. was non-motile, produced acid and gas in glucose, mannit and dulcit. No change in lactose, saccharose or salicin. In litmus milk it produced an acid reaction in a fortnight, but no clot. XLIX. 2, produced acid and gas in glucose, mannit, dulcit and saccharose peptone water, no change in lactose or salicin. In litmus milk it produced no change.

2 cc. of a twenty-four hours' broth culture of XLIX. 1, introduced intra-peritoneally to a guineapig, produced death within twenty-four hours.

XLIX. 1,/

XLIX. 1, was tested against high agglutinating sera of para A, para B, and Gaertner, with negative results.

GENERAL RESULTS.

Gram-negative bacilli were the dominant variety in this case though the gram-positive were fairly numerous in the original fields. Of the lactose-fermenting bacilli studied, both were members of group 3, of McConkey's classification. Non-lactose-fermenting bacilli were present in large numbers, two varieties being found, one having the cultural characteristics of the bacillus paratyphosus A, but the agglutination reactions proved it to be a different organism. Coccal forms were inconspicuous and spore bearers were absent.

SPECIMEN L.

A.G. aged 10 months.

Charteris Ward. Royal Hospital for Sick Children.

Examined Jan. 6th 1913.

STATE.

Suffering from rickets.

METHOD OF FEEDING.

Was a bottle fed child. lately has been getting porridge etc.

HISTORY.

Has never suffered from diarrhoea.

AFTER HISTORY.

Had no diarrhoea after the date of taking the specimen while in the ward.

MOTION.

Rather dark brownish, fairly solid.

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FILMS.

i. Gram-positive bacilli:-

These are present in large numbers. Some are small and slender of the straight acidophilus/

acidophilus type, others are a little larger and stouter.

ii. Gram-negative Bacilli & Cocco-bacilli: -

These are of varying size and are present in fairly large numbers though not so numerous as the gram-positive.

iii. Coccal Forms: -

These are numerous, mainly rather large oval cocci, isolated or in pairs; small cocci of thè ènterococcus type are scanty but small oval isolated cocci are numerous.

iv. Spore-bearers: -

None seen in films.

McCONKEY'S PLATES.

These show after two days' incubation large numbers of lactose-fermenting colonies, amounting to, roughly speaking, about 5 per cent of the total.

PRIMARY ANAEROBIC MEDIA.

Tube A. four days' old showed cloudiness and deposit. Gram-stained showed rather plump gram-negative bacilli, short and squat as the chief organisms, fairly numerous, very small slender gram-negative bacilli, gram-positive diplococci, lanceo-late/

late but large in fair numbers ; straight slender gram-positive bacilli in fair numbers.

Tube B. showed slight deposit but no cloudiness. Gram-stained showed a few rather irregular gram-positive bacilli of acid-tolerant type.

Tube C. showed slight cloudiness. Gram-stained, a few gram-positive diplococci were found.

Tube D. showed stormy fermentation with abundant whey formation. The whey, gram-stained, showed some short, thick gram-positive bacilli.

LACTOSE-FERMENTING BACILLI.

Two lactose-fermenting bacilli called L. 3, and L. 4, were selected from McConkey's plates for further study. These were both non-motile, gram-negative, indol-producing, milk-souring lactose bacilli. Both produced acid and gas in lactose and in dulcit peptone water. L. 4, produced acid and gas in saccharose, while L. 3, produced no change. Neither produced any change in adonit, inulin or inosit.

2cc. of a twenty-four hours' broth culture of L. 1, introduced intra-peritoneally to a guinea-pit was non-pathogenic.

L.3,/

L. 3, was B.Schafferi (No. 35 McConkey) while L. 4, was B.Neapolitanus (No. 72.)

NON-LACTOSE-FERMENTING BACILLI.

L. 1, and 2, were motile gram-negative bacilli which produced indol, did not liquefy gelatin, and produced a slightly alkaline reaction in litmus milk. They produced acid and gas in glucose peptone water, but no change in lactose, dulcit, saccharose and salicin.

They were therefore Morgan's No. I. Bacillus.

BACILLI OF THE ACID-TOLERANT GROUP.

Good growth of these in tube B.

GENERAL RESULTS.

Gram-positive bacilli of the acid-tolerant type were present in considerable numbers in this case, though the flora was varied. Gram-negative bacilli were present in fairly large numbers, and of these non-lactose-fermenting bacilli formed about 5 per cent. Those isolated were Morgan's No.I Bacillus. Coccal forms were also fairly numerous and/

and were of the enterococcus type and also of the larger oval variety.

Spore-bearers: were not seen in the original films, though judging from tube D. it is possible they are present.

SPECIMEN LI.

Child aged 10 days.

Maternity Hospital.

Examined February 8th 1913.

STATE.

A healthy full-time child.

METHOD OF FEEDING.

Breast only.

MOTION.

Obtained February 8th 1913, rather green, normal consistence and odour.

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FIELDS.

i. Gram-positive bacilli:-

Practically all the organisms present are of this variety. They are small and slender bacilli, very degenerate looking and taking up the stain badly so as to give rise to "punctate" forms. All are comparatively short, there being no threads; no bifid forms.

Some/

Some are swollen in the middle, some at ends so as to give the impression of possibly being spore-bearing.

ii. Gram-negative bacilli and coccobacilli:-

Are extremely scanty, several fields having to be searched before any can be seen.

iii. Coccal Forms:-

None seen in films.

iv. Spore Bearers:-

None seen in films.

McCONKEY BILE SALT LACTOSE NEUTRAL RED AGAR PLATES.

These after two days incubation showed numerous lactose-fermenting colonies of the usual type, and one feebly-lactose-fermenting colony.

No non-lactose-fermenters present.

(The plates were inoculated with 10 times the usual amount of emulsion.)

PRIMARY ANAEROBIC MEDIA.

Tube A. after a weeks anaerobic growth showed slight cloudiness with considerable deposit.

Gram stained this showed gram-positive bacilli and gram-negative bacilli in about equal numbers./

numbers. The gram-positive bacilli were fairly uniform in appearance being rather slender bacilli, straight or slightly curved; some side by side, some end to end. Most stained equally; some club shaped. No punctate forms, no bifid forms, no long threads. The gram-negative were mainly of coliform type, though some may be forms of the gram-positive bacilli, which have lost the power of taking up the stain.

Tube B., also a week old, showed a clear medium with a coarse granular precipitate at the foot. (This is sometimes produced by the action of the acid on the medium.)

Gram stained, no organisms were seen in the film.

Tube C. after 1 week showed no growth.

Tube D. after one week showed no change.

SECONDARY AEROBIC MEDIA.

A McConkey Bile Salt Lactose Agar Plate was spread from the week old Tube A. with the object of determining the number of living B.coli in this tube at that date.

No/

No growth took place on the plate.

SECONDARY ANAEROBIC MEDIA.

None were used in this case.

LACTOSE-FERMENTING BACILLI.

Two lactose-fermenting, and one feebly-lactose-fermenting colony, were selected from the McConkey Plates for further study. These were replated on McConkey Plates after isolation, and found to be all genuine lactose fermenters.

These were found to be almost identical in their reactions.

They were gram-negative bacilli of coli-form type, all producing indol and all negative to Vosges and Proskauer's reaction. None liquefied gelatin, within 6 weeks. All produced acid and clot in litmus milk, and acid and gas in lactose Peptone water, no change being produced by any of them in saccharose, dulcit, adonit, inulin, or in-osit peptone water. The only difference was in their motility, LI. 3, being non-motile, while a few bacilli of LI, 1, were slightly motile.

2 cc. of a 24 hour's broth culture of LI.

1,/

1, introduced intra-peritoneally to a guineapig produced death within 24 hours.

BACILLI OF THE ACID-TOLERANT GROUP.

None were isolated for the purpose of further study.

COCCAL FORMS.

No coccal forms were present.

SPORE-BEARING BACILLI.

None were present.

GENERAL RESULTS.

Almost the entire faecal flora of this case consisted of punctate gram-positive bacilli of the acid tolerant type.

By means of the special media, non-saccharose-fermenting B.coli were also found to be present.

No coccal forms or spore-bearers were seen in films or isolated.

SPECIMEN LII.

- aged ? days.

Maternity Hospital.

Examined February 8th 1913.

STATE.

A healthy full-time child.

METHOD OF FEEDING.

Breast and Bottle.

MOTION.

Normal in appearance.

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FIELDS.

i. Gram-positive Bacilli:-

Gram-positive Bacilli are present in fairly large numbers but by no means dominate the field as in breast-fed cases. They are for the most part fairly slender, straight, or slighty curved bacilli, taking the stain well. Some very delicate forms and others slightly thicker are to be seen. One or two fairly long threads. No punctate forms; no beadlet forms and no bifid bacilli are to be seen.

ii./

ii. Gram-negative bacilli and Cocco-bacilli: -

These are present in moderate numbers being fully as numerous as the gram-positive forms. They are of ordinary coliform type.

iii. Coccal Forms: -

These constitute the most numerous variety present, but vary considerably in their morphology. Clusters of small gram-positive cocci are numerous. Diplococci are also present in large numbers being of two main varieties, a small lanceolate of the usual enterococcus type and a larger plumper semi-form type with flattened adjacent surfaces. There are also present rather plump oval isolated cocci. Practically all these forms are gram-positive.

iv. Spore-bearers: -

Are absent.

McCONKEY'S PLATES.

These richly inoculated show lactose-fermenting colonies only of the ordinary type. No non-lactose-fermenting colonies present.

PRIMARY ANAEROBIC MEDIA.

Tube/

Tube A. after a week's growth showed marked cloudiness and deposit. Gram-stained showed small gram-positive diplococci of enterococco type with gram-negative coliform bacilli.

No gram-positive bacilli present.

Tube B. showed a fairly large deposit with clear supernatant medium. Gram-stained showed a very few irregular short gram-positive cocco-bacilli.

Tube C. showed slight cloudiness with a slight deposit. Gram-stained showed gram-negative bacilli and coccobacilli of coliform type and a few small slender ènterococci.

Tube D. showed clotting and had a sour odour. The whey gram-stained showed rather irregular gram-positive cocci some as diplos. No spore-bearers seen.

SECONDARY AEROBIC MEDIA.

A McConkey's plate spread from the week-old tube A. remained sterile.

SECONDARY ANAEROBIC MEDIA.

No anaerobic plates were made in this case.

LACTOSE-FERMENTING BACILLI.

Two lactose-fermenting colonies were selected for further study from the McConkey plates. These before study were replated on McConkey plates. In/

In common they were non-motile gram-negative coliform bacilli. Both produced indol; both negative to Vosges and Proskauer's test; neither liquefied gelatin, and both produced acid in Litmus milk. Growth on agar slope and in ordinary broth was characteristic. Both produced acid and gas in lactose and dulcit peptone water. Neither produced any change in adonit, inulin or inosit.

LII. 1, produced no change in saccharose peptone water, whereas LII. 2, produced acid and gas. 2 cc. of a twenty-four hours' broth culture of LII., 2, introduced intra-peritoneally to a guinea-pig produced death within five days. Its insides having been eaten out by other guinea-pigs, no cultures from the heart blood could be made.

No especial study was made of other organisms in this case.

GENERAL RESULTS.

Gram-positive bacilli of the acid-tolerant formed a fair percentage of the organisms seen in the films.

Lactose-fermenting bacilli were also present in moderate numbers and those isolated were found/

found to be saccharose-fermenting strains of B.coli.

No non-lactose-fermenting Bacilli were present.

Coccal forms were numerous, but were not closely studied. No strépto-cocci present.

Spore-bearers absent

SPECIMEN LIII.

- aged 2 days.

Maternity Hospital.

Examined February 8th, 1913.

STATE.

Healthy full-time child.

METHOD OF FEEDING.

Breast only. It is possible that the child had swallowed no milk before the time of passage of the motion.

MOTION.

Is meconium.

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FIELDS.

Show cells and debris but no organisms are to be found.

Two broth tubes were richly inoculated and incubated but both remained sterile.

GENERAL RESULTS.

The meconium of this 2 days' old child was sterile.

SPECIMEN LIV.

___ aged 8 days.

Maternity Hospital.

Examined February 8th 1913.

STATE.

A healthy full time child.

METHOD OF FEEDING.

Breast only.

MOTION.

Greenish semi-solid. Usual odour.

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FIELDS.

i. Gram-positive bacilli:-

All the organisms seen fall into this group, and are of one type. They are small slender delicate bacilli taking the stain badly and irregularly; slope also is irregular and most are degenerate looking; occur singly, and in clusters side by side. No tendency to occur end to end. Are of the type called the "punctate/

"punctate form of bifidus." No long thread. No bifid forms.

ii. Gram-negative bacilli and coccobacilli:-

None seen after much search.

iii. Coccal forms:-

None seen in films.

iv. Spore-bearers:-

None seen in films.

McCONKEY BILE SALT LACTOSE AGAR NEUTRAL RED PLATES.

These, very richly inoculated, showed after 2 days growth lactose-fermenting colonies all of one type.

They were colonies with red centre and pale periphery, smooth edged, rather moist looking, spherical.

No non-lactose-fermenting colonies were present.

PRIMARY ANAEROBIC MEDIA.

Tube A. after 1 weeks growth, showed cloudiness with deposit. Gram stained showed gram-positive bacilli in long threads, some of them staining in clots so as rather to resemble straight chains/

chains of streptococci: some degenerate looking short gram-positive bacilli. Numerous gram-negative bacilli were present, some long, probably degenerate forms of the gram-positive but this is difficult to make out.

Tube B. 1 week old, showed slight cloudiness with deposit. Gram stained, showed straight or slightly curved, fairly slender gram-positive bacilli.

Tube C. no cloudiness or deposit. Gram stained, no organisms.

Tube D. showed marked fermentation, all whey, a little curd. The whey gram stained showed some straight fairly slender gram-negative bacilli, and a very few gram-positive cocci of the enterococcus type.

No spore-bearers in film.

SECONDARY AEROBIC MEDIA.

A McConkey Plate from the week old Tube A. to determine the number of live B.coli in that tube, showed no growth.

SECONDARY ANAEROBIC MEDIA.

Plate/

Plate A. showed in a few days a pure culture of minute colonies of acid-tolerant type.

A glucose agar stab inoculated from the whey of Tube D. showed no growth.

LACTOSE-FERMENTING COLONIES.

Two colonies of the type described were selected from the McConkey Plates for further examination, called LIV. 1, and 2.

These were replated on McConkey's medium. LIV. 1, showed colonies all of above type. LIV. 2, showed in addition some small and feebly-lactose-fermenting.

Two colonies were selected from this presumably pure plate of LIV. 2, LIV. 2a, a colony of the red centred type described previously, LIV. 2b a colony of the small and feebly-lactose-fermenting.

When put through the tests these organisms were found to be identical, and the colonies of the type of LIV. 2b were therefore, probably the result of exhaustion of the medium rather than of a change in the organism. All three strains had the following /

following characters.

They were non-motile gram-negative coliform bacilli, a very large number occurring as diplobacilli. They did not liquefy gelatin nor did they produce indol, but gave a positive Vosges and Proskauer reaction. The growth on agar slope and in ordinary broth was of the ordinary coli type. They produced acid and clot in litmus milk.

In lactose, saccharose, adonit, and inosit peptone water they produced acid and gas, the amount of gas produced being considerably greater than that produced by most intestinal B.coli. In dulcit and inulin no change was produced except by LIV. 2b, which produced a slight acid reaction and a very small amount of gas in dulcit peptone water after a weeks growth.

These then had all the characteristics of the Bacillus Lactis Aerogenes.

2 cc. of a 24 hours broth culture introduced intraperitoneally to a guineapig produced death within 24 hours.

NON-LACTOSE-FERMENTING BACILLI.

None/

SPECIMEN LV.

Infant. aged ten days.
Maternity Hospital.

Examined Feb.8th, 1913.

STATE.

A healthy full-time child.

METHOD OF FEEDING.

Breast alone.

MOTION.

Yellowish motion, slight odour.

BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FIELDS.

i. Gram-positive bacilli: -

Most of the organisms present fall into this group. The majority of these are moderately slender, slightly curved bacilli of the common type. There are also fairly numerous stouter straight gram-positive bacilli present. Some degenerate forms seen. Most take the stain well. No long threads and no bifid/

bifid forms present; a few punctate forms are to be seen.

ii. Gram-negative bacilli and cocco-bacilli: -

These are fairly numerous though by no means so numerous as the gram-positive. They are coliform in type.

iii. Coccal Forms: -

These also are present in fair numbers and are almost all in diplos. Some are of the lanceolate enterococcus type, others are semi-form with flattened adjacent surfaces. Some of the latter are very small; all are gram-positive.

iv. Sporebearers: -

None present.

MCCONKEY'S PLATES.

These plates richly inoculated showed after two days' incubation, numerous lactose-fermenting colonies of the ordinary type. No non-lactose-fermenters were present.

PRIMARY ANAEROBIC MEDIA.

Tube A. after one week's incubation showed slight/

slight cloudiness of deposit. This gram-stained showed mainly gram-negative bacilli and cocco-bacilli along with very scanty gram-positive diplococci and a few gram-positive bacilli, most of these very small and slender, some a little larger.

Tube B. also a week old showed very slight cloudiness with deposit; gram-stained showed small slender, slightly curved gram-positive bacilli. There were also some very degenerate-looking gram-negatives which had probably been originally gram-positive.

Tube C. showed no growth.

Tube D. showed some clotting. Gram-stained some small gram-positive cocci mainly in diplo were to be seen.

SECONDARY AEROBIC MEDIA.

A McConkey plate was spread from the week-old Tube A. No growth occurred.

SECONDARY ANAEROBIC MEDIA.

None were used on this case.

LACTOSE-FERMENTING BACILLI.

Two/

Two lactose-fermenting colonies were selected from the McConkey plates for further study. These before use were replated on McConkey's media; named LV. 1 and 2. Their reactions were found to be identical in all respects and are therefore given together.

They were non-motile, gram-negative coliform bacilli; which did not produce indol nor liquefy gelatine, and were negative to Vosges and Proskauer's test. Both produced acid in litmus milk in a few days with clot in a fortnight. Both produced acid and gas in lactose and saccharose peptone water; no change was produced by either on dulcit adonit, inulin or inosit.

2 cc. of a twenty-four hours' broth culture introduced intraperitoneally into a guinea-pig produced death within twenty-four hours.

NON-LACTOSE-FERMENTING BACILLI.

None were present on the McConkey Plates.

ACID-TOLERANT BACILLI AND COCCAL FORMS.

No bacilli of the acid-tolerant group nor coccal forms were studied in this case.

SPORE-BEARERS./

None were present on the McConkey Plates.

BACILLI OF THE ACID-TOLERANT GROUP.

One strain isolated from the Plate A. was studied. Called LIV. 3.

SPORE-BEARERS.

None were seen in the films.

COCCAL FORMS.

None seen in films. A very few of enterococcus type present in Tube D. None isolated.

GENERAL RESULTS.

The films showed the intestinal flora in this case to be nearly a pure culture of bacilli of the acid-tolerant type.

The special media used showed the Bacillus Lactis Aerogenes to be also present.

Coccal forms were present in extremely small numbers and were of the enterococcus type.

Spore-bearing organisms absent.

No B.coli found.

SPECIMEN LVI.

—— aged ? days.

Maternity Hospital.

Examined February 8th 1913.

STATE.

A healthy full-time child.

METHOD OF FEEDING.

Breast only.

MOTION.

Normal in appearance, consistence and odour.

THE BACTERIOLOGICAL EXAMINATION.

GRAM STAINED FIELDS.

i. Gram-positive bacilli:-

Almost all the organisms present are gram-positive bacilli, but a considerable number of these are plump organisms some of them bearing spores, and these are described under the heading "Spore-bearers."

Of the other gram-positive bacilli

a/

a fair number are slender, slightly irregular bacilli of the acid-tolerant type. These are also fairly numerous very long , their degenerate looking threads taking up the stain rather badly.

ii. Gram-negative bacilli and coccobacilli.

These are present in small numbers. Much more numerous than in the usual breast-fed stool.

iii. Coccal forms.

These are present in moderate numbers, being much more numerous than in the usual breast-fed stool. Are of two main types, oval gram-positive cocci or coccobacilli occurring singly, larger than the average enterococcus, and small cocci, many occurring in clusters and some as diplos.

iv. Spore-bearers.

These are present in large numbers. Are plump straight gram-positive bacilli resembling the B. aerogenes capsulatus morphologically. The spores where present are large and oval, and are in many cases nearly as long as/

as the bacillus.

McCONKEY BILE SALT LACTOSE AGAR NEUTRAL RED PLATES.

Were very richly inoculated and showed after 2 days incubation numerous lactose-fermenting colonies of the ordinary type.

No non-lactose-fermenters were present.

PRIMARY ANAEROBIC MEDIA.

Tube A. after 7 days incubation showed slight cloudiness with deposit. Gram stained, showed mainly gram-negative bacilli and coccobacilli.

Gram-positive forms were very scanty and consisted mainly of diplococci oval in shape with a very small and slender diplobacilli and some slightly large gram-positive bacilli of the acid-tolerant type.

Tube B. after a week's incubation showed a slight deposit, but no organisms were found in films.

Tube C. showed slight cloudiness with deposit. This stained showed large straight gram-negative bacilli, none bearing spores. These were probably/

probably forms of the gram-positive spore-bearers of the original films which had lost the power of taking up the stain.

Tube D. Milk showed a strong fermentation, a large amount of whey being present with a very little clot. The whey gram stained showed after much search one plump squat gram-positive bacillus.

SECONDARY AEROBIC MEDIA.

One McConkey Plate was spread from the week-old Tube A. with the object of determining the number of living B.coli present, but no growth took place.

SECONDARY ANAEROBIC MEDIA.

Glucose agar stabs were made from Tubes C. and D. No growth took place.

An anaerobic glucose agar plate was richly inoculated from the whey of Tube D. No growth took place.

LACTOSE-FERMENTING BACILLI.

Two lactose fermenting colonies LVI. 1, and 2, were selected from the McConkey Plates for further/

further study. These were replated on McConkey Plates on which both showed colonies of the usual type.

These were motile gram-negative bacilli, and coccobacilli. Both were indol positive, Vosges and Proskauer negative. Neither liquefied gelatin in 6 weeks. Both produced acid and clot in litmus milk in 1 week, and acid and gas in lactose peptone water; no change was produced by either on saccharose, adonit, inulin or inosit. They differed only in their action on dulcit peptone water, in which LVI. 1, produced acid and gas while LVI. 2, did not.

2 cc. of a 24 hours broth culture of LVI. 1, introduced intraperitoneally to a guineapig failed to produce a fatal result.

NON-LACTOSE-FERMENTING BACILLI.

None were present on McConkey Plates.

BACILLI OF THE ACID-TOLERANT GROUP.

No secondary plate A. was made in this case. It is doubtful whether any would have been obtained as they were so extremely scanty in Tube A.

COCCAL/

<u>COCCAL FORMS</u>.

No attempt was made to study those present.

<u>SPORE-BEARERS</u>.

The attempts to isolate those seen in films proved unsuccessful.

GENERAL RESULTS.

In this case the acid-tolerant bacilli of the acidophilus and bifidus type were largely replaced by spore-bearing bacilli not isolated but resembling morphologically and in their actions on milk, B.aerogenes capsulatus or B.enteritidis sporogenes. These spore-bearing bacilli were possibly to a certain extent acidogenetic and acid-tolerant as is B.aerogenes capsulatus.

Lactose-fermenting bacilli were present in small numbers and those studied were non-saccharose-fermenting strains of B.coli.

No non-lactose-fermenting bacilli were present. Coccal forms were moderately numerous in fields, and were of staphylococcal and enterococcal type.

PLATE I.

SPECIMEN LVI.

Obtained from 8 days old breast-fed child.

All the films are stained by Gram's method, carbol fuchsin being used as a counter stain.

They are drawn as seen under a $\frac{1}{12}$ inch objective, and No. IV. eye-piece.

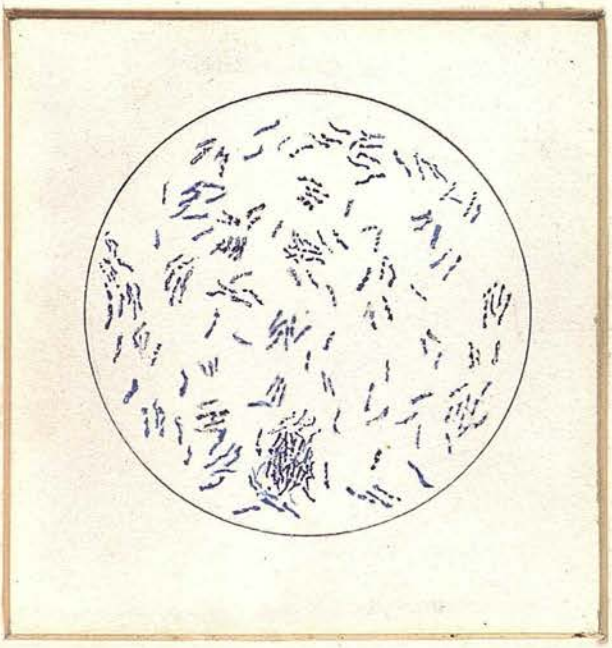


PLATE II.

SPECIMEN XI.

Obtained from 1 month old child on breast and bottle, mainly the former.

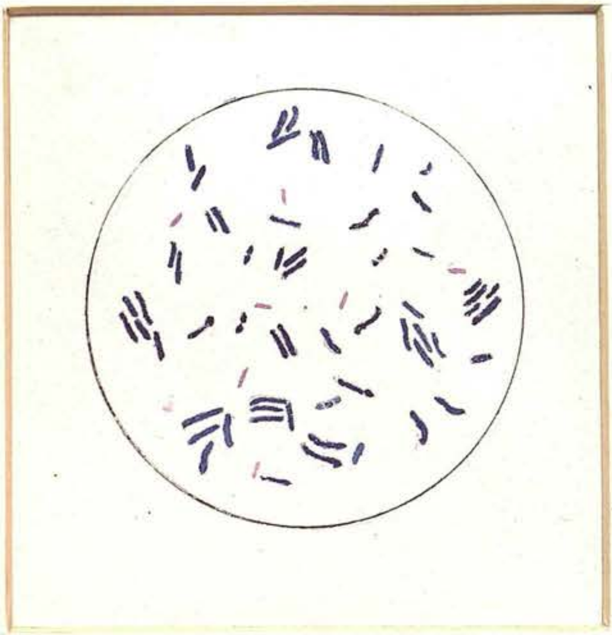


PLATE III.

SPECIMEN XL.

Obtained from 6 weeks old child, bottle-fed since birth.

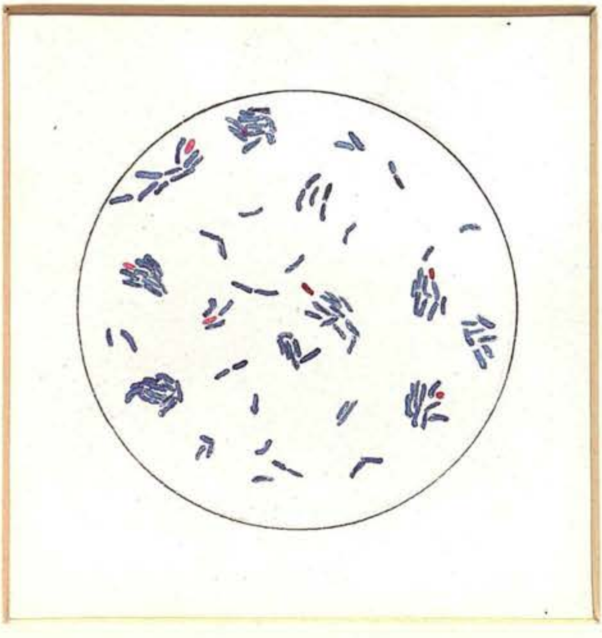


PLATE IV.

SPECIMEN L.

From 10 months old child, mainly bottle fed.

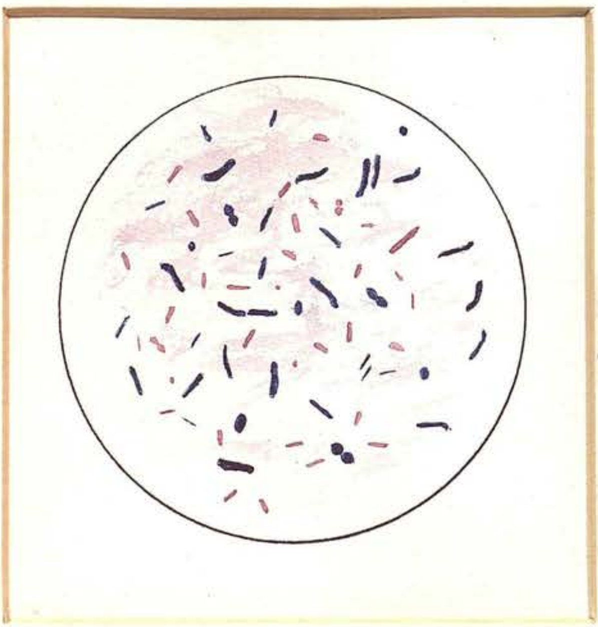


PLATE V.

SPECIMEN XLVIII.

Obtained from 20 months old child on mixed feeding.

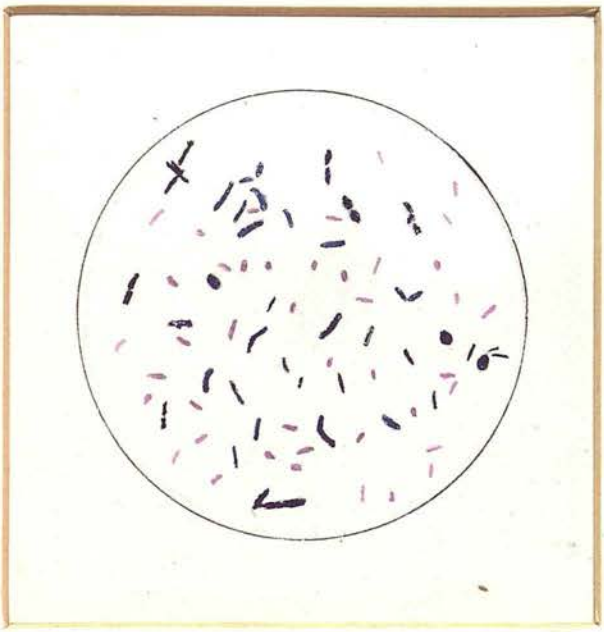


PLATE VII.

SPECIMEN XXXI.

Obtained from a bottle-fed, 7 months old child.

SPECIMEN XLIII.

Obtained from the same child 1 month later when suffering from acute diarrhoea. (Morgan's No. 1)

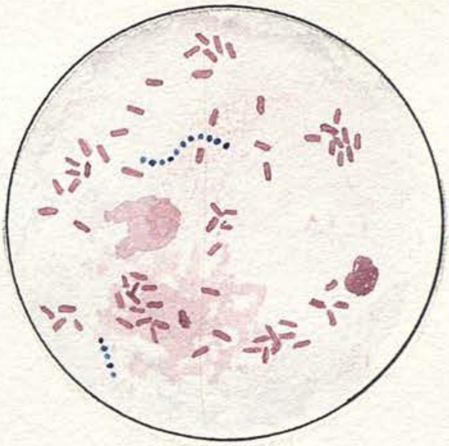
PLATE VI.

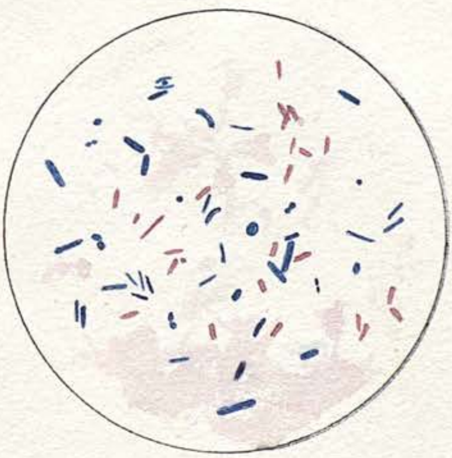
SPECIMEN X.

Obtained from 3 months old bottle-fed child, while suffering from acute diarrhoea. (Proteus)

SPECIMEN XIII.

Obtained from the same child after recovering from diarrhoea.





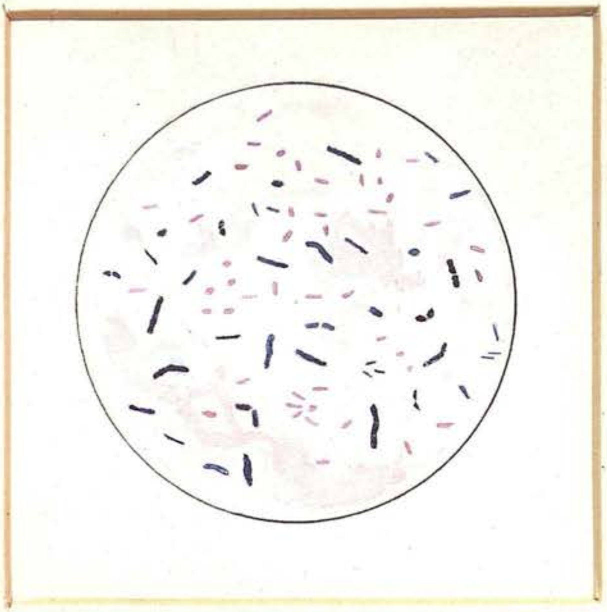


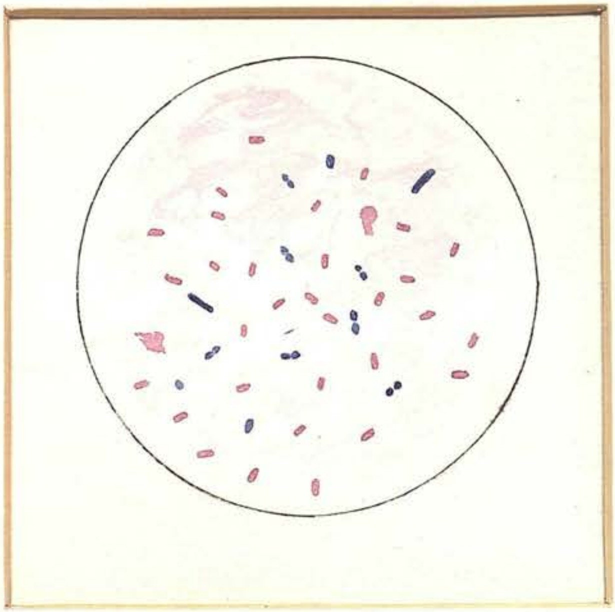
PLATE VIII.

SPECIMEN XXIX.

Obtained from 4 months old child while suffering from acute diarrhoea. Bottle-fed. (Morgan's No. 1 and later Dysentery Bacillus.)

SPECIMEN XLV.

Obtained from the same child after recovery from diarrhoea.



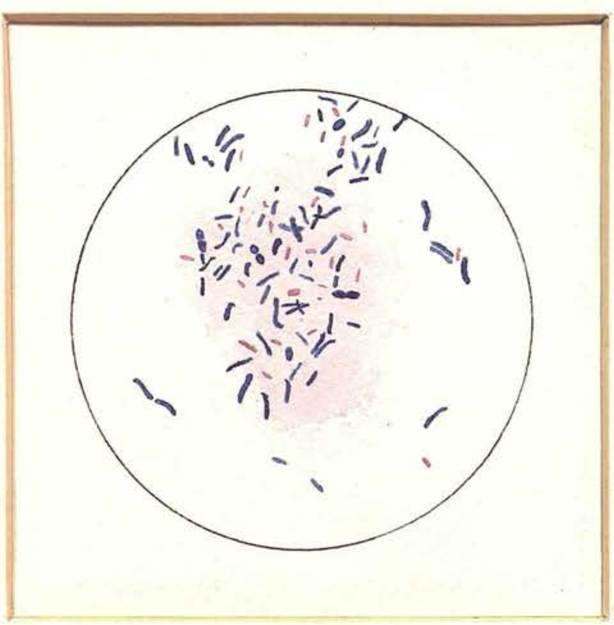


PLATE IX.

SPECIMEN XLIV.

Obtained from a 5 months old bottle-fed child suffering from acute diarrhoea. (Dysentery Bacillus.)

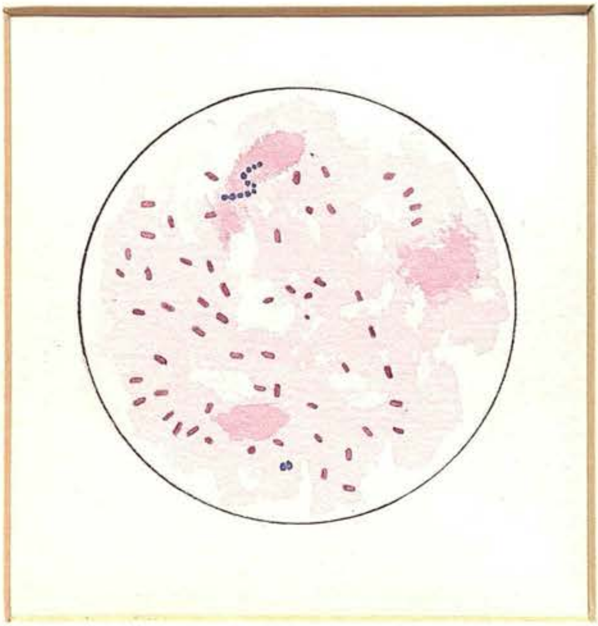


PLATE X.

SPECIMEN XLI.

Obtained from 10 months old child suffering from acute diarrhoea. (Liquefying non-lactose-fermenters.)

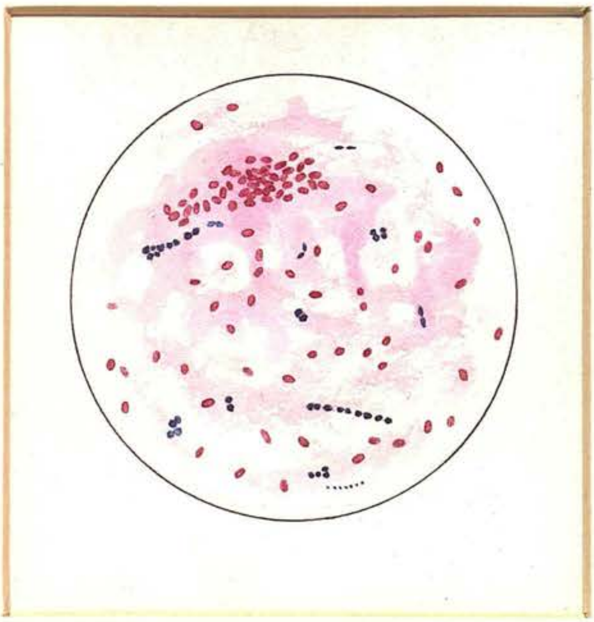


PLATE XI.

SPECIMEN XLVI.

Obtained from a 16 months old child suffering from acute diarrhoea. (Dysentery Bacillus.)

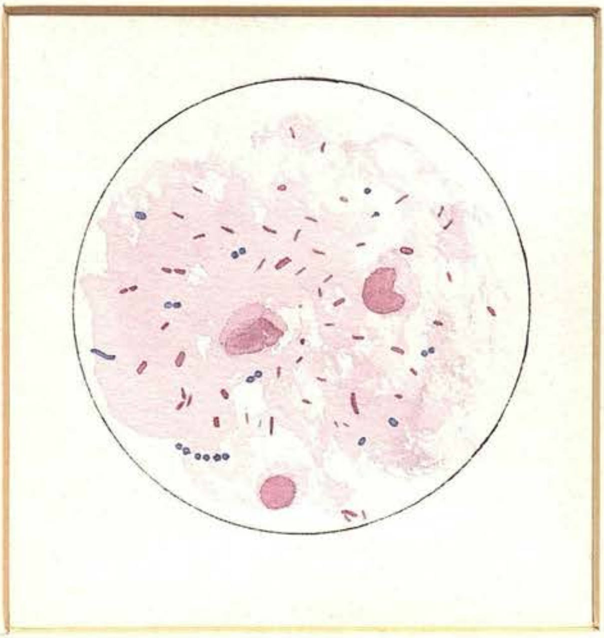


PLATE XII.

BACILLI OF THE ACID-TOLERANT GROUP.

Type B. Acidophilus.

(XL. 11. 4 days glucose agar stab culture.)

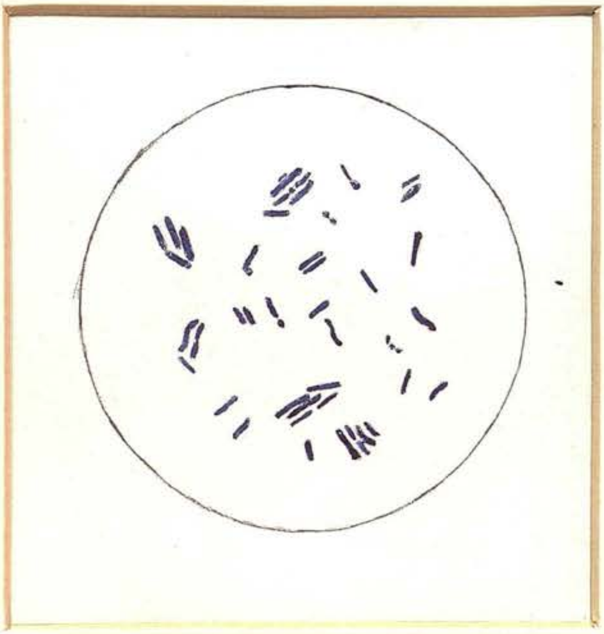


PLATE XIII.

BACILLI OF THE ACID-TOLERANT GROUP.

Type - Bacillus Exilis.

(XXIX. 11. glucose agar stab culture, 4 days old.)

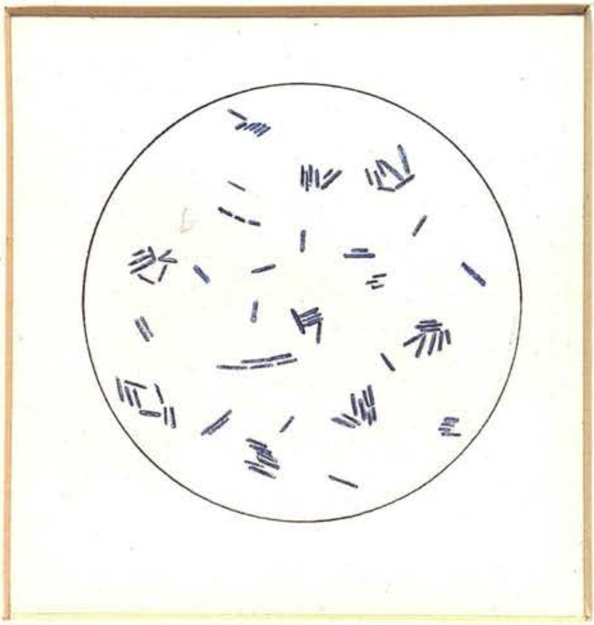


PLATE XIV.

BACILLI OF THE ACID-TOLERANT GROUP.

"Formes de souffrance" of the strictly anaerobic Bacillus Bifidus. Film from a 4 days old deep glucose agar stab culture.

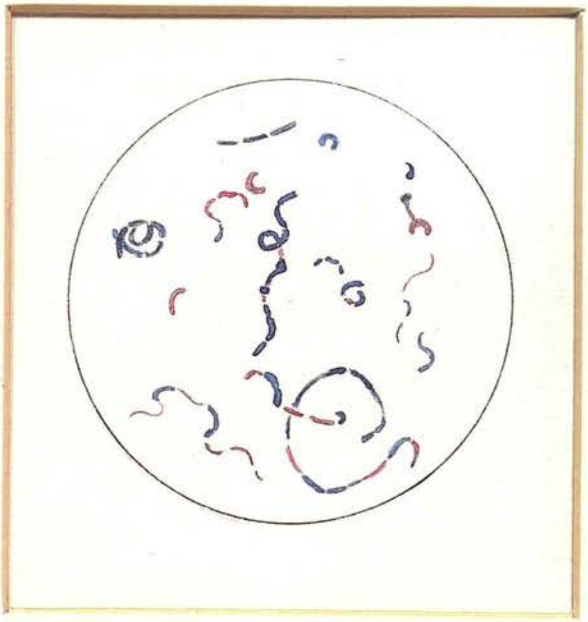


PLATE XV.

THE ENTEROCOCCUS.

(XIV. 5. 3 days agar slope culture.)

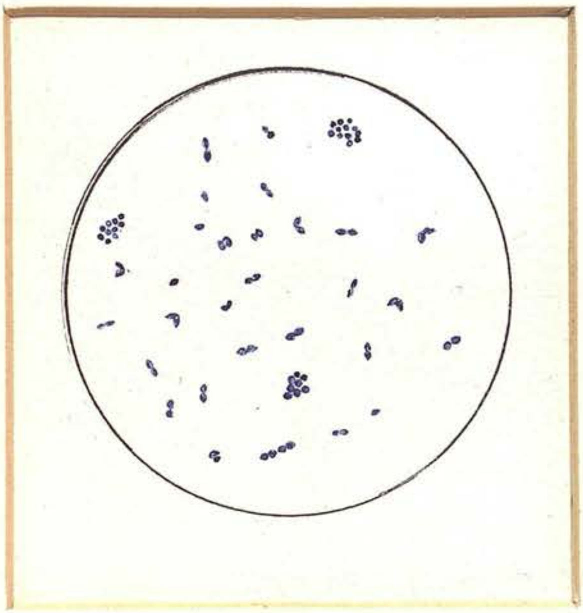


PLATE XVI.

THE EGG-SHAPED TYPE OF COCCOBACILLUS SEEN FREQUENTLY IN BOTTLE-FED AND OTHER CASES.

(XXXVIII. 15. 8 days glucose agar stab.)

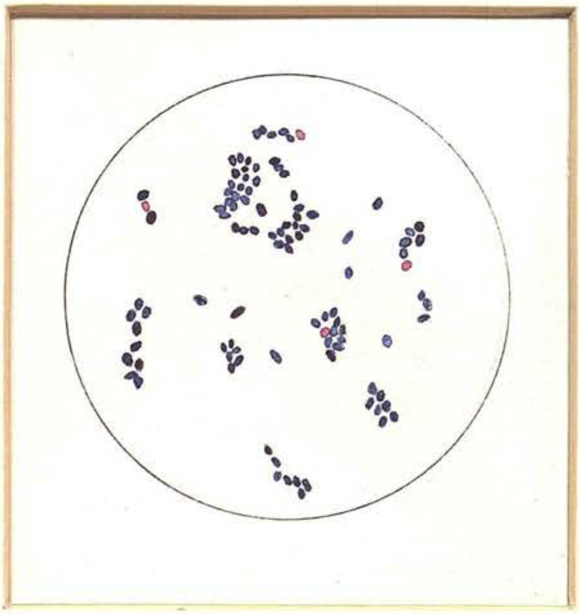


PLATE XVII.

THE LARGER TYPE OF DIPLOCOCCUS SEEN IN BOTTLE-FED AND OLDER CASES.

(XXIX. 6. 4 days old glucose agar stab culture.)

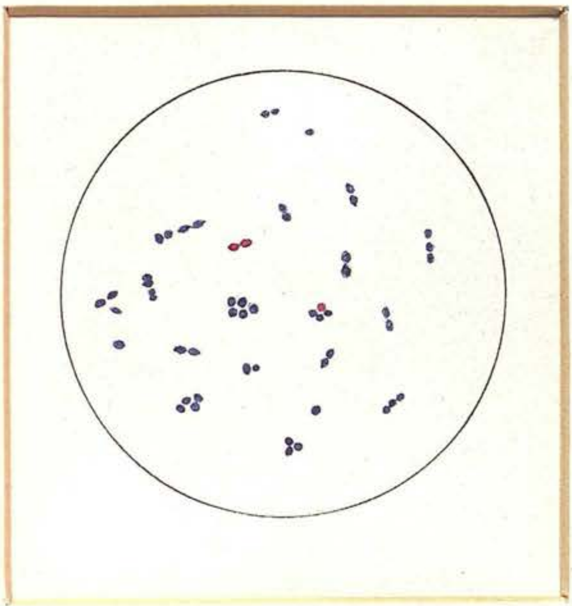


PLATE XVIII.

THE PSEUDO-SPORE-BEARING BACILLUS.

Organism XLI, 15. 4 days old glucose broth culture, anaerobic.