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THE INTESTINAL FLORA OF INFANTS AND YOUNG CHILDREN.[1]

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(Plates XLV.–XLVII.)

In attempting to discover in what way the intestinal flora in disease differed from that of normal conditions, it was first necessary to be familiar with the range of variety possible in health. To commence with the simplest and proceed to the more complex meant, in this case, to commence with infants and advance to adults. In this first paper I propose, therefore, to deal with organisms met with in children who were free from intestinal disorder, including such organisms met with in cases of diarrhœa as were clear of suspicion of causal connection or secondary implication.

To get an unbiassed view of morbid changes in a flora it is necessary to include the entire flora in one's analyses, with the attendant risk of becoming so involved in ramifications as to arrive nowhere. To overcome as far as possible this risk, I dealt with the intestinal flora in groups, and by comparison of a group from one case with, on the one hand, the corresponding group from another, and with, on the other hand, the remaining groups from the same case, I was able to proceed with the research on more or less mathematical lines.

A study of the literature, and preliminary experimentation, showed that the flora as seen in Gram-stained films of the fæces might be divided into (1) Gram-positive bacilli of the acid-tolerant type, (2) Gram-negative bacilli and coccobacilli, (3) Gram-positive cocci, and (4) spore-bearing bacilli; and that the Gram-negative bacilli might be divided by cultural methods into (*a*) the lactose-fermenting group (type *B. coli*), and (*b*) the non-lactose-fermenting group (type *B. paratyphosus* or *B. dysenteriæ*).

With the flora subdivided in this manner I first studied the appropriate means of isolation of each subdivision, and then combined these methods into the scheme of routine examination given below.

Of the total number of cases put through this routine examination I propose to deal in this paper mainly with twenty-one, all completely free from diarrhœa, of whom six were fed on the breast alone, five on the breast and bottle, seven on the bottle only, while three were getting

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cornflour, fish, meat, etc., in addition to cow's milk. The consideration of eighteen cases of diarrhœa is reserved for a second paper, though it is necessary to touch on a few points concerning these cases in the present paper also. Three cases of intestinal indigestion are kept for a subsequent paper, as they have been incompletely studied. Part I. deals with the method of examination, Part II. with the isolated organisms, Part III. with the flora under different conditions of feeding.

PART I.

Scheme of Routine Examination.

Details concerning the child
- Age and address.
- Method of feeding.
- Previous health.
- Present condition.

Details concerning the motion
- Colour.
- Consistence.
- Odour.
- Blood.
- Mucus.

Preliminary Steps.

(A) Gram-stained films of fresh fæces made. Organisms classified as—
1. Gram-positive bacilli of the acid-tolerant group.
2. and 3. Gram-negative bacilli and coccobacilli, including lactose fermenters and non-lactose fermenters.
4. Coccal forms.
5. Spore-bearing bacilli.

(B) Emulsions of fæces made
- Emulsion 1. Ordinary broth aerobic.
- Emulsion 2. Glucose broth anaerobic.

Methods of Isolation.

(C) Three plates of MacConkey's medium spread from emulsion I. — Lactose-fermenters and non-lactose fermenters.

(D) One ordinary agar-plate, aerobic — Coccal forms.

(E) Primary anaerobic media (fluid)
- Tube 1. Glucose broth.
- ,, 2. Glucose broth + 0·4 per cent. of acetic acid.
- ,, 3. Glucose broth + white of egg, pasteurised.
- ,, 4. Milk pasteurised at 80° C.

(F) Secondary anaerobic media
- Plate 1. Glucose agar spread from Tube 1 after four days' growth.
- ,, 2. Glucose agar spread from Tube 2.
- ,, 3. Glucose agar spread from Tube 3.
- ,, 4. Glucose agar spread from Tube 4.

Methods of Purification.

All organisms isolated by the above procedure to be repurified by re-plating, and isolated colonies once again picked off.

Methods of Identification.

Lactose-fermenting bacilli by MacConkey's tests
- Lactose fermentation.
- Saccharose ,,
- Dulcit ,,
- Adonit ,,
- Inulin ,,
- Inosit ,,
- Motility.
- Vosges and Proskauer reaction.
- Production of indol.
- Liquefaction of gelatin, and also by pathogenicity.

METHODS OF IDENTIFICATION (*continued*)—

Non-lactose-fermenting bacilli by Morgan's and Lewis' tests . .	Lactose fermentation. Glucose ,, Mannit ,, Dulcit ,, Saccharose ,, Salicin ,, Motility. Production of indol. Liquefaction of gelatin. Agglutination and absorption. Pathogenicity.
Acid-tolerant bacilli, coccal forms, and spore bearers . . .	Tests described under group headings.

The fresh motion was preserved for me on the child's napkin. Two or three representative portions were then collected in a sterile tube by means of a throat swab, and taken at once to the laboratory. The specimen thus obtained was naturally contaminated to a certain extent by its surroundings. This can be avoided in older children by cutting the solid motion in half with a sterile knife and selecting the uncontaminated centre. It is not usually possible to obtain a perfectly uncontaminated specimen from an infant, especially if suffering from diarrhœa.

It is necessary to have one of the emulsions (B) under anaerobic conditions, as sometimes several hours elapse before one is ready to inoculate the primary anaerobic media.

The medium used in C was MacConkey's bile salt lactose neutral red agar. A different amount of emulsion 1 was transferred to each plate with a platinum loop, and spread over the surface by means of a glass rod bent at a right angle, and sterilised by flaming. If well spread, one can with certainty distinguish lactose-fermenting colonies from non-lactose fermenters within forty-eight hours. If too thickly spread, or if an insufficient thickness of medium has been employed, many colonies which are apparently non-lactose fermenters will, when transferred to fluid media, be found to ferment lactose. When this occurs one can either spread fresh plates from emulsion 1 or keep the old plates in the incubator for four or five days, at the end of which time one can usually distinguish true non-lactose fermenters at the edge of the plate from fictitious.

After some time I gave up the use of D, as, owing to the original contamination of the specimen, aerobic organisms which were obviously not of intestinal origin grew on the ordinary agar plates.

The primary anaerobic media, as soon as inoculated, were incubated in Buchner's tubes, the pyrogallic-caustic soda method being used to secure anaerobiosis.

The secondary anaerobic media were inoculated from the first after about four to six days' growth. The opportunity was at the same time taken of making a Gram-stained film of the growth in the primary tube.

For securing anaerobiosis for plates, several methods were tried. A Bulloch's bell jar holding eight plates was at first used; hydrogen obtained from a cylinder, and the pyrogallic acid-caustic soda method, were used in combination. The disadvantage is, that when one has to spread several plates, those first spread are exposed to the air for a considerable time before all the operations are complete. The method most used, and which was found to be satisfactory, was that of Lentz. A composite ring impregnated with pyrogallic acid is placed on a glass plate; caustic soda is poured into the centre of the ring, the inoculated half of a Petri capsule inverted over the whole, and luted down to the plate with plasticine. Inoculated plates need to be exposed to the air for only a fraction of a second, and after growth colonies can be examined under

the low power before admitting oxygen. The method of McLeod, which does not fulfil the latter condition, was not found to be satisfactory.

Colonies from these plates were usually transferred to deep glucose agar stabs, about twenty being usually selected from each case. As a rule many were found to be the same organism and were discarded.

Gram's method of staining was used throughout, dilute fuchsin being used as a counter-stain.

PART II.

The Acid-tolerant Group of Organisms.

Such an extremely small amount of research has been done upon this group by British workers that I propose to deal with it at considerable length.

The first member isolated was obtained by Tissier in 1899 [1], and called by him later the *Bacillus bifidus*. He described it as a strict anaerobe, and he considered that the intestinal flora of breast-fed infants consisted of this organism in almost pure culture (1900 [3]). Tissier was, however, not aware of the acid-tolerant properties of his organism, and at first strongly denied this characteristic, attributed to the *B. bifidus* by other workers.

Moro (1900 [2]) first discovered 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, in artificial culture, was able to resist an acidity fatal to *B. coli* and the enterococcus. This bacillus he called the *B. acidophilus*, which is a misnomer, as the 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*.

Tissier (1900 [3]) also described another variety, a facultative aerobe, which he claimed to be distinct from the *B. acidophilus* of Moro, and which he named the *B. exilis*.

Rodella (1901 [4]) was able to isolate acid-tolerant bacilli from the stools of both breast-fed and bottle-fed children, but did not isolate the strictly anaerobic *B. bifidus*. He described one variety which he thought might be distinct, as it grew constantly on gelatin, while other varieties did not.

Cahn (1901 [5]) always found the *B. acidophilus* in the stools of children fed on the breast, though in smaller numbers than in children fed on cow's milk. He thought that the *B. exilis* of Tissier was not a distinct variety. He obtained the *B. bifidus* from breast-fed children, and, to a lesser extent, from bottle-fed, but was unable to get it in pure culture, as it was always mixed with *B. acidophilus*. In these circumstances it is doubtful whether he was justified in claiming to have obtained the *B. bifidus*.

Mereshkowsky (1905 [6-9]), 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, including man. They did not in any case isolate the anaerobic *B. bifidus* described by Tissier. Those which they isolated they divided into two classes, according to the appearance of colonies on glucose agar plates. Type 1 was a smooth-edged variety, and this Mereshkowsky considered to be the same as the *B. bifidus* of Tissier; but it was neither bifid nor anaerobic. Type 2 showed colonies with a toothed border, and this variety Mereshkowsky considered to be the *B. acidophilus* of Moro. Type 2 grew better on gelatin than Type 1.

Obraszow found one type or the other or both in the fæces of all of a very large number of animals ranging from molluscs to man, and including a great number of varieties, cold and warm blooded.

Podgaetzky examined the fæces of eight human beings from 1 month old to 34 years, and found acid-tolerant bacilli in all.

These workers fed puppies on these organisms for long periods, and were able to transform the intestinal flora so that the fæces contained Gram-positive acid-tolerant bacilli 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. I wish to draw particular attention to this very careful and complete research, and to the deductions drawn therefrom.

Tissier stated (1905 [10]) that he found the *B. bifidus* and the *B. acidophilus* in different parts of the intestine of infants, from the stomach to the anus; the former predominated in breast-fed children, the latter in bottle-fed.

He also published a paper (1906 [23]) 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 the group on sugars, whereby acid was produced and harmful forms killed. In cases of infantile diarrhœa treated by means of cultures of *B. acidiparalactici*, alone or mixed with *B. bifidus*, and large amounts of lactose, he obtained good results.

Jacobson (1908 [11]) was, I believe, the first to point out that *B. acidophilus* grows with great difficulty on acidified solid media, unless it is first grown in acidified fluid media. He considered that *B. bifidus*, when perfectly pure, needed a complete absence of oxygen for growth.

Noguchi (1910 [12]) studied the growth characteristics of *B. bifidus*, and was able to transform it in the laboratory from the strictly anaerobic type (*B. bifidus* of Tissier) to the facultatively aerobic type (*B. acidophilus* of Moro), and back again to the anaerobic type.

Distaso (1911 [13]) was the first to call the acid-tolerant group by this name. He divided the group into three classes according to the kind of acid produced by the fermentation of sugars by these organisms—(1) Acetic acid, (2) lactic acid, (3) butyric acid.

The acetic-acid-producing group, to which both the *B. acidophilus* and the *B. 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 considered that *B. bifidus* was distinct from the *B. acidophilus* in being a strict anaerobe, in showing true bifurcation, in never producing formic acid, and in being 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 *B. welchii* and one or two others.

Distaso found that the *B. bifidus* outgrew the *B. welchii* and others in an acid medium.

His main conclusion from his observations was, that the acetogenes group, by the production of acid, inhibits the growth of other organisms.

Other varieties of the acid-tolerant group have been described by other workers. The differences between these so-called varieties were mainly morphological. As these bacilli are extremely polymorph, such a classification is valueless.

One variety perhaps worthy of mention is that described by Herter (1907 [14]). This bacillus he called *B. infantilis*, because of its frequent occurrence in the stools of cases of intestinal infantilism,—proof, however, was wanting that the bacillus had a causal connection with the condition, or that it differed from members of the group met with in healthy infants.

In commencing my study of the acid-tolerant group of organisms I was therefore able to regard as proved its ubiquity in the animal intestine; its innocuousness; its ability to resist a strength of acid sufficient to kill other known intestinal organisms; and the power of at least some of its members to ferment at least some sugars, with the production of acid. There was also strong evidence that the relative proportion of this group to other groups was greater in breast-fed children than in those fed in other ways. Further, there was the assumption that members of this group were able to act as regulators of the intestinal flora. The number of varieties contained in the group was undecided, but the majority of opinion pointed to there being two main sub-groups.

For some time my attempts to grow the acid-tolerant organisms in artificial culture were unsuccessful, due, I now believe, to endeavouring to grow them directly on solid acidified media without the preliminary use of fluid media. When I used a half per cent. glucose broth, with 0·4 per cent. of acetic acid added to it, as a preliminary anaerobic medium, and plated the resultant growth on glucose agar, acidified or not, I was able to constantly isolate these organisms in all the normal bottle-fed cases where the fæcal films had shown them to be present. Yet in the breast-fed cases, where the films showed very large numbers of Gram-positive bacilli, morphologically resembling the punctate form of *B. bifidus*, I found it extremely difficult to isolate these organisms, and in three cases was unsuccessful.

There is, therefore, an important difference between the Gram-positive bacilli of bottle-fed cases and those of purely breast-fed cases, in that the former, even though present in small numbers and mixed with other organisms in considerable variety, can be isolated with ease; while the latter, seen in the films in almost pure culture, can only be induced to grow on artificial media by the exercise of great patience.

My personal belief is that Tissier was correct in his statement that the bacillus seen in the films from breast-fed cases is the strictly anaerobic bacillus called by him the *B. bifidus*; and I believe that the peculiarity of staining from which the punctate form gets its name is due, not to any idiosyncrasy of the living bacterial protoplasm, but to the fact that the bacilli are all degenerate or dead.

It must be remembered that in the lower part of the intestine these organisms meet with oxygen, and, after passage of the motion, are exposed entirely to the air; as they have not the advantage of living in symbiosis with facultatively aerobic forms, as is the case in children where the flora is more mixed, they are probably in a dead or dying condition before their implantation on artificial media.

In bottle-fed children, on the other hand, the majority of the acid-tolerant bacilli isolated were of the hardier facultatively aerobic type called by Moro the *B. acidophilus*.

As to whether these organisms are temporary phases of one and the same bacillus, or whether they are quite separate, I have not as yet come to a definite conclusion. Some of my strict anaerobes, after some weeks artificial culture, grew in glucose agar exposed to the air, and could not be distinguished from the facultatively aerobic type. Also some of my facultatively aerobic types, when grown upon unsuitable media such as ordinary broth or glycerin agar, developed into "formes de souffrance," generally claimed to be distinctive of the strictly anaerobic type.

Differences between various strains of acid-tolerant bacilli were observed in: (1) Growth in glucose agar stabs; (2) growth in gelatin at room temperature; (3) growth on potato; (4) appearance of colonies on glucose agar plates; and (5) in power of fermenting carbohydrates. It does not seem to me justifiable to found a classification on the strength of these distinguishing characteristics, which have not been proved to be sufficiently stable.

For these reasons I describe the fifty-two strains isolated and studied under one group.

Cultural Characteristics.

All are Gram-positive in young culture. In old culture, 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. The facultatively aerobic type (*B. acidophilus*) is a moderately regular, moderately slender, straight or slightly curved bacillus, which usually tends to collect in clusters side by side. The strictly anaerobic type (*B. bifidus*) is, as a rule, very irregular in shape and size. In pure culture, spirilla, comma forms, long threads, coccal forms, straight and curved bacilli may be seen. In no case, either in films made from fæces or in those made from cultures, did I see the bifid forms from which the type gets its name.

Another very common form is the straight, slender, delicate bacillus described by Tissier as the *B. 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 coccobacillary forms were also met with.

Bodies resembling spores may sometimes be seen in bacilli of the acid-tolerant group, and occasionally they resist pasteurisation at 80° C. before isolation from the fæces, but in pure culture, in my experience, these have all been killed by pasteurisation.

Fifteen varieties examined for motility were found to be non-motile. These were all upwards of 4 days old when tested, and growing in glucose broth.

On glucose-agar.—All members grew well in the absence of oxygen, and the majority grew well in its presence.

The colonies are minute and are first visible, as a rule, between the second and third day; about the fourth day they have attained the size of a small streptococcus colony.

Three types of colonies were seen—(*a*) A smooth-edged rather dense white

type, (*b*) a smooth-edged rather delicate type, and (*c*) a peculiar type, that under the low power of the microscope resembles a little collection of tangled threads. Types (*a*) and (*b*) under the low power are brownish and delicately granular.

In glucose-agar stab.—Growth as a rule was visible before forty-eight hours. In most cases it extended right up to the surface, though frequently heavier below than above, but in a few the upper half inch of the medium remained sterile. On repeated sub-culture, however, all these organisms grew to the top of the tube.

Three main types of growth were observed—(1) A massive growth with heavy leaf-like outgrowths; (2) a delicate straight growth with some minute outgrowing colonies to be observed, some of them on stalks; and (3) a vague ill-defined pale growth.

There is another type occasionally met with, in which 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, what Cahn was describing when he spoke of having mixed growths of *B. bifidus* and *B. acidophilus*. If, however, one breaks the tube and makes sub-cultures 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 two days with a white, tenacious deposit. After about ten days the supernatant fluid is fairly clear, with a heavy deposit.

Glucose broth + 0·4 per cent. acetic acid.—Growth in this is similar to growth in glucose broth. Some were tested in glucose broth + 0·6 per cent. acetic acid in which all grew well, and in glucose broth + 1 per cent. 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 a marked tendency to remain discrete, as they do in glucose agar, cultures showed a faint film of coalesced colonies. It was usually difficult even under the low power to decide whether growth was present.

In ordinary broth.—Growth was in all cases extremely poor or absent, aerobically and anaerobically.

On glycerin agar.—A growth of the same nature as on ordinary agar took place.

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, either aerobically or anaerobically.

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, with or without oxygen.

In ordinary agar stab.—A heavy growth occurred in some; in most a weak growth took place; in some none.

In litmus milk.—Of eight strains studied in this medium, six produced acid and clot within a fortnight, two produced acid only.

In peptone water containing carbohydrates.—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 table shows the reactions of six strains tested on sugars in broth.

TABLE I.

Organism.	Lactose.		Saccharose.		Dulcit.		Inulin.		Glucose.		Mannit.		Salicin.		Maltose.		Case.
	3 Days.	14 Days.	3 Days.	14 Days.	3 Days.	14 Days.	3 Days.	14 Days.	3 Days.	14 Days.	3 Days.	14 Days.	3 Days.	14 Days.	3 Days.	14 Days.	
XLII. 12.	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	Diarrhœa.
XLII. 11.	–	A	–	A	–	–	–	A	–	A	–	–	–	–	–	A	Diarrhœa.
XXXV. 14.	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	Normal Breast-fed.
XXXV. 17	–	A	–	–	–	–	–	A	–	A	–	–	–	A	–	–	Normal Breast-fed.
XXIX. 11.	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	Diarrhœa.
XXXI. 8.	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	Normal Breast-fed.

It will be observed that of the two organisms, the fermenting powers of which are slower and less universal than those of the others, one was isolated from a case of diarrhœa, the other from a normal breast-fed infant.

Pathogenicity.

A guinea-pig inoculated intraperitoneally with 4 c.c. of a 4-days' old glucose-broth culture of XXXV. 17 (breast-fed) showed no ill effects.

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

Two mice fed for five days on bread soaked in glucose-broth cultures of XXIX. 11 (from a diarrhœa case) showed no signs of illness.

I give here a series of experiments carried out with a member of this group of the *B. acidophilus* type, along with two other intestinal organisms; one of these, bacillus No. 71 (MacConkey), being one of the most common varieties of the colon group met with in the human intestine, both adult and infantile; the other, Morgan's No. 1 bacillus, being the organism so frequently met with in cases of infantile diarrhœa.

The object of the experiments was to determine the influence, retarding or otherwise, which these organisms might have upon one another when grown in combination in media containing sugar; from which one might gain an idea of their influence upon one another in an intestine containing sugar.

For the first series four tubes containing equal amounts of peptone + 2 per cent. of lactose were taken. Tube A was inoculated with *B. coli* (one loopful from an agar-slope culture); tube B, with Morgan's No. 1 bacillus (same amount); tube C, with *B. coli* and Morgan's No. 1 bacillus in equal amounts; tube D, with *B. coli*, Morgan's No. 1 bacillus, and *B. acidophilus* in, roughly speaking, equal amounts. These tubes were then placed in the incubator at 37° C., and from day to day an estimation was made of the numbers of each variety present in each tube. This was done in tubes A, B, and C, by taking one small loopful from the tube and spreading it over a MacConkey's bile salt lactose neutral red agar plate. In twenty-four to seventy-two hours a count of the lactose-fermenting and non-lactose-fermenting colonies was made, and one thus got an idea of the relative numbers of the *B. coli* and Morgan's No. 1 present in the tube at the time of spreading the plate. A similar process was gone through for tube D, but in addition a glucose agar plate was spread at each examination. One was thus able to estimate the change from day to day in the relative numbers of the three varieties present, the relative numbers of the *B. coli* and Morgan's No. 1 on the one hand, to the *B. acidophilus*

on the other being estimated from the glucose agar plate, the proportions of the first two, one to another, from the MacConkey plate.

The results of these experiments are at best only rough estimations, and one can place little value on them so far as the influence of *B. coli* on Morgan's No. 1 is concerned. But they are sufficiently conclusive as to the effect of *B. acidophilus* on the other two.

The following table shows the results of the first series of experiments :—

TABLE II.—*Growth in Lactose Peptone Water.*

	Organisms.	1 Day.	2 Days.	3 Days.	4 Days.	5 Days.	7 Days.	9 Days.	11 Days.	14 Days.	18 Days.
A	*B. coli*	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+ +	+ +	+	+
B	Morgan's No. 1	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
C	*B. coli* + Morgan's No. 1	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+ +	+ +	+	+
		+ + +	+ + +	+ +	+ +	+ +	+	+	+	V.F.	V.F.
D	*B. coli* + Morgan's No. 1 + *B. acidophilus*	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+	O		
		+ + +	+ + +	+ +	+ +	+ +	+	V.F.	O		
		O	O	O	O	V.F.	+	+ +	+ + +		

+ + + = Thick growth on plate. + + = Moderate growth. + = Thin growth.
V.F. = Very few colonies. O = No colonies.

Experiment A shows that *B. coli* in a lactose peptone water medium, are alive after eighteen days, although much diminished in number as compared with the second day.

Experiment B shows that Morgan's No. 1 bacillus, which does not ferment lactose, is at least as numerous at the end of eighteen days' incubation as on the second day.

In Experiment C, the medium was inoculated with *B. coli* and Morgan's No. 1 bacillus in equal amounts. The result shows *B. coli* to be unaffected by the addition of Morgan's No. 1 bacillus; the Morgan's No. 1, on the other hand, is so affected by the acid produced through the sugar-splitting qualities of the *B. coli* as to be nearly extinct at the end of eighteen days' incubation.

Experiment D shows the effect of inoculation of the medium with *B. acidophilus* in addition to the other two. During the first four days of incubation no effect was observed; *B. coli* were present in as large numbers and in the same proportions as in Experiment C; *B. acidophilus* was so outnumbered by the other two that no minute colonies were present on the glucose-agar plate. The effect of the acidity of the medium by the *B. coli* became quickly evident; on the fifth day, a few *acidophilus* colonies were present, and the multiplication of *B. acidophilus* and death of the other two now took place with great rapidity, so that on the eleventh day no *B. coli* nor Morgan's No. 1 bacilli were present on the plates, a pure culture of *B. acidophilus* in large amount being obtained. That is to say, the effect of the addition of *B. acidophilus* to the other two, was to shorten the lives of the latter by upwards of a week, the Morgan's bacillus being slightly more susceptible to acid than *B. coli.*

Four further experiments on exactly similar lines were then carried out, glucose being substituted for lactose. The results are tabulated in Table III.

TABLE III.—*Growth in Glucose Peptone Water.*

	Organisms.	1 Day.	2 Days.	3 Days.	4 Days.	5 Days.	7 Days.	9 Days.	11 Days.	14 Days.	18 Days.
E	*B. coli*	+ + +	+ + +	+ + +	+ + +	+ +	+	V.F.	0		
F	Morgan's No. 1	+ + +	+ + +	+ + +	+ + +	+ +	+ +	+ +	+	+	0
G	*B. coli* + Morgan's No. 1	+ + +	+ + +	+ + +	+ + +	+ +	+ +	+	0		
		+ + +	+ + +	+ + +	+ +	+	+	V.F.	0		
H	*B. coli* + Morgan's No. 1 + *B. acidophilus*	+ + +	+ + +	+ + +	+ + +	+ +	V.F.	0			
		+ + +	+ + +	+ + +	+ +	+	0	0			
		0	0	0	0	V.F.	+ + +	+ + +			

Experiment E shows the effect of growing *B. coli* in glucose water. A larger amount of acid is produced by the splitting of this sugar than by the splitting of lactose, and the medium is sterile in eleven days.

In Experiment F, Morgan's No. 1, which produces acid and gas in glucose-peptone water, is killed in eighteen days.

When the two organisms are sown together (Experiment G) *B. coli* is unaffected by the presence of Morgan's No. 1 bacillus, and dies as in Experiment E in eleven days. Morgan's bacillus, on the other hand, has its life shortened by the acid produced by the *B. coli* from eighteen to eleven days.

In Experiment H, where *B. acidophilus* was also used, up to the fourth day the *B. coli* and Morgan's bacillus were as in Experiment G, the *B. acidophilus* not being present on the plates. From this point a very rapid change took place, and, by the seventh day, all the Morgan's No. 1 had been destroyed. By the ninth day, all the *B. coli* also were destroyed, *B. acidophilus* remaining in pure culture.

The dominance of *B. acidophilus* over *B. coli* and Morgan's No. 1 bacillus in a peptone water solution containing sugar, marked where lactose was used, was even more notable where glucose was the sugar employed. With both sugars also, there was evidence of a slighter inhibitory influence of *B. coli* over the non-lactose-fermenting organism.

The mutual effect of the three organisms growing in lactose broth are shown in Table IV.

TABLE IV.—*Growth in Lactose Broth.*

Experiment.	Organisms.	1 Day.	2 Days.	3 Days.	4 Days.	5 Days.	6 Days.
K	*B. coli* + Morgan's No. 1 bacillus + *B. acidophilus*	+ + +	+ + +	+ + +	+ +	0	...
		+ + +	+ + +	+	0	0	...
		0	0	0	+	+ + +	...

In glucose broth the dominance of *B. acidophilus* over the other two is more marked than when peptone water is employed as a vehicle, Morgan's No. 1 being dead within four days, *B. coli* within five, leaving the acid-tolerant organisms in pure culture.

The final experiment of this series is tabulated in Table V. Glucose broth was inoculated with all three organisms.

TABLE V.—*Growth in Glucose Broth.*

Experiment.	Organisms.	1 Day.	2 Days.	3 Days.	4 Days.	5 Days.	6 Days.
L	*B. coli* + Morgan's No. 1 bacillus + *B. acidophilus*	+ + +	+ + +	+ + +	+ +	0	...
		+ + +	+ + +	+ +	V.F.	0	...
		0	0	0	+	+ + +	...

The results are seen to be very similar to those of Experiment L, the first two organisms being dead within five days.

One may therefore make the broad statement that in an artificial medium containing glucose or lactose, *B. acidophilus* is much better able to resist the acidity produced by the fermentation of that sugar than is either *B. coli* or Morgan's No. 1 bacillus; thus the addition of *B. acidophilus* notably shortens the life of the other two organisms in such a medium, more particularly that of Morgan's No. 1; that this effect is especially marked where broth is the vehicle; and that, certainly where lactose is used, and probably where glucose is used, *B. coli* has similar though feebler inhibiting action on Morgan's No. 1 bacillus.

THE LACTOSE-FERMENTING GROUP OF GRAM-NEGATIVE BACILLI.

MacConkey's work in the differentiation of intestinal *B. coli* by means of the sugar and other tests has shown that there are numerous varieties of this group. Other workers, notably Twort (1907 [15]), have denied the value of MacConkey's tests in the differentiation of organisms of this group, on the ground that the reactions were not sufficiently stable. MacConkey 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 (1905 [16]) and Horrocks among others.

MacConkey divided the lactose-fermenting bacilli into four 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 MacConkey only met with thirty-six varieties.

Out of 178 lactose-fermenters isolated by MacConkey (1909 [17]) from human fæces, forty-two were organisms called by MacConkey "No. 71," thirty-seven were the *B. coli communis* ("No. 34"), thirty-three were *B. vesiculosus* ("No. 5"), while *B. neapolitanus* ("No. 72"), *B. schafferi* ("No. 35"), an unnamed organism ("No. 1"), *B. grünthal* ("No. 4"), and *B. lactis aerogenes*

("No. 103"), occurred with frequency. The other twenty-eight varieties were rare.

In a previous work MacConkey (1905 [18]) examined the fæces of animals and human beings, and out of 480 coli-like organisms, only four were the *B. lactis aerogenes*. He was unable to find any notable difference between the *B. coli* of normal cases and those from six cases of diarrhœa.

In the present work I have tried to decide whether by the use of MacConkey's tests one can differentiate between the coliform organisms isolated from breast-fed cases and those isolated from bottle-fed and other cases; whether there is any difference between the coli-like organisms isolated from normal cases, and those isolated from cases of diarrhœa; whether the reactions are stable as judged by a repetition of the tests after some months' cultivation on artificial media; whether there is any difference in virulence to animals of the organisms isolated from diarrhœa cases and those from normal.

I give in the form of tables the varieties met with in my cases. I include the varieties met with in the cases of diarrhœa, as, with one possible exception, there was no reason to believe them to have any connection with the diarrhœa.

All organisms were plated twice over before testing in order to ensure purity. For inoculation of the test media, agar-slope cultures not exceeding three days in age were used.

TABLE VI.—*Reactions of Lactose-fermenting Bacilli isolated from Normal Infants on the Breast only.*

Organisms.	Motility (4 to 6 hours).	Indol (7 days).	Gelatin Liquefaction (2 months).	Lactose (7 days).	Saccharose (7 days).	Dulcit.	Adonit.	Inulin.	Inosit.	Voges and Proskauer Reaction.	Litmus Milk (14 days).	Name.	Number of MacConkey's Classification.
XXXV. 2.	–	+	–	AG	–	–	–	–	–	–	AC	*B. vesiculosus*	5
XXXV. 3.	+	+	–	AG	AG	AG	–	–	–	–	AC	–	71
XXXVII. 14A.	+	+	–	AG	–	AG	–	–	–	–	AC	*B. coli communis*	34
LI. 1.	+	+	–	AG	–	–	–	–	–	–	AC	*B. grünthal*	4
LI. 2.	+	+	–	AG	–	–	–	–	–	–	AC	,,	4
LI. 3.	+	+	–	AG	–	–	–	–	–	–	AC	,,	4
LIV. 1.	–	–	–	AG	AG	–	AG	–	AG	+	AC	*B. lactis aerogenes*	103
LIV. 2A.	–	–	–	AG	AG	–	AG	–	AG	+	AC	,,	103
LIV. 2B.	–	–	–	AG	AG	AG	AG	–	AG	+	AC	–	67
LV. 1.	–	+	–	AG	AG	–	–	–	–	–	AC	*B. coscoroba*	107
LV. 2.	–	+	–	AG	AG	–	–	–	–	–	AC	,,	107
LVI. 1.	+	+	–	AG	–	AG	–	–	–	–	AC	*B. coli communis*	34
LVI. 2.	+	+	–	AG	–	AG	–	–	–	–	AC	,,	34

TABLE VII.—*Reactions of Lactose-fermenting Bacilli isolated from Normal Infants on Breast and Bottle.*

Organisms.	Motility.	Indol.	Gelatin.	Lactose.	Saccharose.	Dulcit.	Adonit.	Inulin.	Inosit.	Vosges and Proskauer.	Litmus Milk.	Name.	No. in Classification.
XXXVI. 1	–	+	–	AG	–	–	AG	–	–	–	AC	*B. acidi lactici* (Hüppe)	2
XXXVI. 2	–	+	–	AG	–	–	AG	–	–	–	AC	*B. acidi lactici*	2
XXXVI. 3	–	+	–	AG	–	–	AG	–	–	–	AC	,,	2
XXXVI. 9	–	+	–	AG	–	–	AG	–	–	–	AC	,,	2
XXXVIII. 3	–	+	–	AG	AG	AG	–	–	–	–	AC	*B. neapolitanus*	72
XXXVIII. 4	–	+	–	AG	AG	AG	–	–	–	–	AC	,,	72
XXXVIII. 5	–	+	–	AG	AG	AG	–	–	–	–	AC	,,	72
XXXVIII. 6	–	+	–	AG	AG	AG	–	–	–	–	AC	,,	72
LII. 1	–	+	–	AG	–	AG	–	–	–	–	AC	*B. schafferi*	35
LII. 2	+	+	–	AG	AG	AG	–	–	–	–	AC	–	71

TABLE VIII.—*Reactions of Lactose-fermenting Bacilli isolated from "Normal" Infants on the Bottle only.* (*Cow's Milk.*)

Organisms.	Motility.	Indol.	Gelatin.	Lactose.	Saccharose.	Dulcit.	Adonit.	Inulin.	Inosit.	Vosges and Proskauer.	Litmus Milk.	Name.	No. in Classification.
XI. 1.	–	+	–	AG	AG	–	–	–	–	–	AC	*B. coscoroba*	107
XI. 2.	+	+	–	AG	AG	–	–	–	–	–	AC	–	106
XI. 4.	+	+	–	AG	AG	AG	–	–	–	–	AC	–	71
XI. 5.	+	+	–	AG	AG	AG	–	–	–	–	AC	–	71
XI. 6.	+?	–	–	AG	AG	–	AG	–	AG	+	AC	*B. lactis aerogenes*	103
XVI. 2.	–	+	–	AG	–	AG	–	–	–	–	AC	*B. schafferi*	35
XXXI. 2.	–	+	–	AG	AG	AG	–	–	–	–	AC	*B. neapolitanus*	72
XL. 1.	+	+	–	AG	–	AG	–	–	–	–	AC	*B. coli communis*	34
XL. 2.	–	+	–	AG	–	AG	–	–	–	–	AC	*B. schafferi*	35
XL. 3.	+	+	–	AG	–	–	–	–	–	–	AC	*B. grünthal*	4
XL. 4.	+	+	–	AG	–	–	–	–	–	–	AC	,,	4
XLVII. 2.	–	+	–	AG	AG	AG	–	–	–	–	AC	*B. neapolitanus*	72
XLVII. 3.	–	+	–	AG	AG	AG	–	–	–	–	AC	,,	72
XLVII. 4.	–	+	–	AG	AG	AG	–	–	–	–	AC	,,	72
XLVIII. 3.	+	+	–	AG	–	AG	–	–	–	–	AC	*B. coli communis*	34
XLVIII. 4.	+	+	–	AG	–	AG	–	–	–	–	AC	,,	34
L. 3.	–	+	–	AG	–	AG	–	–	–	–	AC	*B. schafferi*	35
L. 4.	–	+	–	AG	AG	AG	–	–	–	–	AC	*B. neapolitanus*	72

TABLE IX.—*Reactions of Lactose-fermenting Bacilli isolated from "Normal" Young Children on a Mixed Diet.*

Organisms.	Motility.	Indol.	Gelatin.	Lactose.	Saccharose.	Dulcit.	Adonit.	Inulin.	Inosit.	Vosges and Proskauer.	Litmus Milk.	Name.	No. in Classification.
XIV. 1.	+	+	–	AG	–	–	–	–	–	–	AC	*B. grünthal*	4
XIV. 2.	+	+	–	AG	–	–	–	–	–	–	AC	,,	4
XIV. 3.	+	+	–	AG	–	–	–	–	–	–	AC	,,	4
XXX. 8.	+	+	–	AG	AG	AG	–	–	–	–	AC	–	71
XXXII. 2.	+	–	–	AG	–	–	–	–	–	–	AC	–	7
XXXII. 3.	+	–	–	AG	–	–	–	–	–	–	AC	–	7
XXXIV. 7.	+	+	–	AG	–	AG	–	–	–	–	AC	*B. coli communis*	34
XXXIV. 8.	+	+	–	AG	–	AG	–	–	–	–	AC	,,	34

TABLE X.—*Reactions of Lactose-fermenting Bacilli isolated from Cases of Diarrhœa.*

Organisms.	Motility.	Indol.	Gelatin.	Lactose.	Saccharose.	Dulcit.	Adonit.	Inulin.	Inosit.	Vosges and Proskauer.	Litmus Milk.	Name.	No. in 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	,,	72
XIX. 1	+	+	–	AG	–	AG	AG	–	–	–	AC	–	33
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. 1A	–	+	–	AG	–	AG	–	–	–	–	AC	*B. schafferi*	35
XXXIII. 1B	–	+	–	AG	–	AG	–	–	–	–	AC	,,	35
XXXIII. 2	+	+	–	AG	–	AG	–	–	–	–	AC	*B. coli communis*	34
XLI. 1	+	+	–	AG	AG	–	–	–	–	–	AC	–	106
XLI. 2	+	+	–	AG	–	–	–	–	–	–	AC	*B. grünthal*	4
XLII. 1	+	+	–	AG	–	–	–	–	–	–	AC	,,	4
XLII. 2	+	+	–	AG	–	–	–	–	–	–	AC	,,	4
XLIII. 1	–	+	–	AG	–	AG	–	–	–	–	AC	*B. schafferi*	35
XLIII. 2	–	+	–	AG	–	AG	–	–	–	–	AC	,,	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. schafferi*	35
XLVI. 4	+	–	–	AG	AG	–	–	–	–	–	AC	–	–

Before drawing conclusions from the results above tabulated, it was necessary to satisfy oneself that the behaviour of these bacilli towards MacConkey's tests was sufficiently innate. Thirty-two strains, therefore, some isolated from children suffering from diarrhœa, others from healthy subjects, were put through the series of tests for a second time, after an interval ranging from three to six months. During this interval they were kept at room temperature, growing on ordinary agar slopes or in broth; each was subcultured every six weeks or two months. The second series of test media was, like the first, inoculated from agar-slope cultures not exceeding three days in age.

Out of thirty-two strains examined, nine showed change, twenty-three remaining unchanged. In eight of the nine the second reading differed from the first in one particular only; on the ninth two changes were observed. Change in saccharose fermentation occurred five times, change in dulcit fermentation once; three times a strain labelled motile at the first testing was found to be non-motile at the second; while two strains lost the power of producing gas.

TABLE XI.

Organisms.	Mobility (4 to 6 hours in broth).	Indol.	Gelatin.	Lactose.	Saccharose.	Dulcit.	Adonit.	Inulin.	Inosit.	Voges and Proskauer.	Litmus Milk.	Period between first and second Testings.	No. of Changes.
XX. 1	+	+	–	AG	–	AG	–	–	–	–	AC	6 months	0
XX. 2	+	+	–	AG	–	–	–	–	–	–	AC	,,	0
XIX. 1	+	+	–	AG	–	AG (–)	AG	–	–	–	AC	,,	1
XIX. 2	+	+	–	AG (A)	–	–	AG (A)	–	–	–	AC	,,	1
XXI. 12	–	+	–	AG	–	AG	–	–	–	–	AC	,,	0
XXII. 1	–	+	–	A	–	A	–	–	–	–	AC	,,	0
XXXVIII. 3	–	+	–	AG	AG	AG	–	–	–	–	AC	4 months	0
XXXVIII. 4	–	+	–	AG	AG	AG	–	–	–	–	AC	,,	0
XXXVIII. 5	–	+	–	AG	AG	AG	–	–	–	–	AC	,,	0
XXXVIII. 6	–	+	–	AG	AG	AG	–	–	–	–	AC	,,	0
XL. 1	+	+	–	AG	–	AG	–	–	–	–	AC	3 months	0
XL. 2	–	+	–	AG	–	AG	–	–	–	–	AC	,,	0
XL. 3	+	+	–	AG	–	–	–	–	–	–	AC	,,	0
XL. 4	+	+	–	AG	–	–	–	–	–	–	AC	,,	0
XI. 1	–	+	–	AG	AG	–	–	–	–	–	AC	,,	0
XI. 4	+	+	–	AG	AG	AG	–	–	–	–	AC	,,	0
XI. 5	+	+	–	AG	AG (–)	AG	–	–	–	–	AC	,,	1
XLII. 1	+ (–)	+	–	AG	–	–	–	–	–	–	AC	,,	1
XLII. 2	+	+	–	AG	– (AG)	AG	–	–	–	–	AC	,,	1
XLIII. 1	–	+	–	AG	–	AG	–	–	–	–	AC	,,	0
XLIII. 2	–	+	–	AG	–	AG	–	–	–	–	AC	,,	0
XLIV. 6	+	+	–	AG	– (AG)	AG	–	–	–	–	AC	,,	1
XXXVI. 1	–	+	–	AG	–	–	AG	–	–	–	AC	4 months	0
XXXVI. 9	–	+	–	AG	–	–	AG	–	–	–	AC	,,	0
XIV. 3	+	+	–	AG	–	–	–	–	–	–	AC	6 months	0
XVIII. 1	–	+	–	AG	AG	AG	–	–	–	–	AC	,,	0
XXX. 8	+ (–)	+	–	AG (A)	AG (A)	AG (A)	–	–	–	–	AC	4 months	2
XXXI. 2	–	+	–	AG	AG	AG	–	–	–	–	AC	,,	0
XXXIII. 2	+ (–)	+	–	AG	–	AG	–	–	–	–	AC	,,	1
XXXIV. 7	+	+	–	AG	– (AG)	AG	–	–	–	–	AC	,,	1
XXXIV. 8	+	+	–	AG	–	AG	–	–	–	–	AC	,,	0
XLI. 1	–	+	–	AG	AG	–	–	–	–	–	AC	3 months	0

The signs in brackets show the results at the second testing where they differed from those obtained at the first. Loss of gas-production was regarded as one change, even when affecting more than one sugar.

Of ten varieties tested, five showed change. Six strains of *B. coli communis* (No. 34 MacConkey) were re-tested; three showed change at the second reading, two now giving the reactions of No. 71, while the third had now to be regarded as *B. schafferi* (No. 35). Of three strains of No. 71 re-tested, two showed change, one to *B. coli communis*, the other to a non-gas-forming variety of No. 72. Six strains of *B. grünthal* (No. 4) were tested and two showed change, one to *B. vesiculosus* (No. 5), the other to the unnamed No. 106. A strain of No. 33 changed to No. 1, while a strain of No. 1 lost the power of gas-formation. On the other hand, six strains of *B. neapolitanus* (No. 72) showed no change; two strains of *B. acidi lactici* (No. 2), five strains of *B. schafferi* (No. 35), and two strains of *B. coscoroba* (No. 107) likewise remained unchanged.

The conclusion arrived at was that the motility of these organisms was not a sufficiently fixed attribute to serve as a method of dis-

tinguishing between different strains. Apart from the failure of this subsidiary test, fermentation of saccharose was judged to be unsatisfactory. I did not satisfy myself as to whether this was due to an instability of the sugar, or to a change in fermenting power on the part of the organism. The solitary discrepancy in the dulcit test does not shake one's faith, and loss of the power of gas-production does not affect the classification.

That there are stable varieties of this group I think there can be no doubt. But a classification which partly depends for its primary division upon the saccharose-fermentation test is, I believe, illegitimate, and strains may be wrongly classified as a result of the inconstancy of this reaction.

This conclusion has unfortunately the result of taking from the value of all my work on this group as tabulated in Tables VI. to X. I can say that, examining the cases fed in differing ways, there was no evidence that a particular kind of feeding favoured the growth of any particular kind of lactose-fermenter. For example, there was no type met with in breast-fed infants which was not also isolated from children on artificial feeding. Further, with one exception, all the varieties met with in diarrhœa cases have also been met with by me in normal children, or by MacConkey in normal adults. The types most frequently met with by MacConkey in man and animals have also been in the main the types isolated most often by myself from the stools of normal children, and from those suffering from diarrhœa. These types are Nos. 71, 34, 72, 35, and 4.

I am also able to state that prolonged growth on a particular medium, *e.g.*, ordinary agar, had no tendency to mould different strains of this group to one particular type.

A further observation of importance is that, contrary to a frequently repeated statement, indol-producing strains are met with in children on the breast only, as well as in those artificially fed.

A point of some interest is that when one selects several strains at random from the MacConkey plates of one case, these are frequently found to be all of one type. It would be necessary to make cultures from several different specimens of fæces from the same case before attaching much importance to this point.

I was satisfied that, with one possible exception, none of the cases of diarrhœa could be considered to owe their illness to the lodgment of an abnormal type of intestinal lactose-fermenter. It has been suggested that habitual inhabitants may at times assume an exalted virulence, and cause diarrhœa or other illness. Tests of the pathogenicity of strains from different cases were therefore carried out. The results are given in Table XII., guinea-pigs being used throughout.

[TABLE XII.

TABLE XII.—*Pathogenicity to Guinea-pigs.*

Strain.	Number in MacConkey's List.	Name in MacConkey's List.	Case from which isolated.	Dose (24 hours' broth culture).	Mode of Inoculation.	Result.
LIV. 1	...	*B. lactis ærogenes*	Normal breast-fed	2 c.c.	Intraperitoneal	Fatal.
LV. 2	...	*B. coscoroba* . .	,,	,,	,,	,,
LI. 1	...	*B. grünthal* . .	,,	,,	,,	,,
LVI. 1	...	*B. coli communis*	,,	,,	,,	Non-fatal.
LII. 2	71	–	Normal breast and bottle	,,	,,	Fatal.
XXXVI. 1	...	*B. acidi lactici* .	,,	,,	,,	,,
XLII. 1	...	*B. grünthal* . .	Diarrhœa	,,	,,	Non-fatal.
XIX. 2	1	–	,,	,,	,,	,,
XX. 1	...	*B. coli communis*	,,	,,	,,	,,
XXI. 12	...	*B. schafferi* . .	,,	,,	,,	,,

One must therefore add to the above conclusions that there was no evidence whatever that coli-like organisms from diarrhœa cases were, at any rate for guinea-pigs, of exalted virulence.

THE NON-LACTOSE-FERMENTING GROUP OF BACILLI.

As the great majority of the organisms falling into this group were obtained from cases of diarrhœa, I leave their discussion to the second paper, which is devoted to these cases.

COCCAL FORMS OF INTESTINAL BACTERIA.

Gram-positive diplococci of somewhat varied morphology form a constant part of the intestinal flora of young children.

The first description of the cultural characteristics of the common intestinal diplococcus was given by Thiercelin (1899 [19]). He considered it potentially pathogenic and capable of producing appendicitis; he thought it was identical with an organism isolated by him from the pus of an appendicular abscess, and also with another which he obtained from a case of cerebro-spinal meningitis. It was pathogenic to mice, slightly to rabbits, and not at all to guinea-pigs.

Much work has been done on the coccal forms since this time. Unfortunately, owing to confusion of nomenclature in different countries, and recent doubt thrown on the value of certain differentiating tests, the subject is in chaos. Whether the enterococcus of Thiercelin is identical with the *Streptococcus enteritidis* of Hirsch-Libmann, whether both or either are the same as the *Streptococcus*

lacticus, what the relation of these is to the *Streptococcus fœcalis*, and whether the streptococci seen in cases of diarrhœa are foreign varieties, is at present unsettled.

The enterococcus has been accused of producing a wide variety of conditions, in which either this organism, or one similar, has been found, such as intestinal catarrh, infectious jaundice, chronic broncho-pneumonia, dysentery, meningitis, post-typhoid suppuration, myelitis, otitis, conjunctivitis, vaginitis, urethritis, etc. (Schmitz, 1912 [20]). 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 diarrhœa in infants, diplococci and streptococci have frequently been noted in large numbers in the stools, and workers, chiefly Booker and Escherich, have supposed streptococcal infections to be the primary cause of the diarrhœa. Lately there has been a tendency to regard streptococci as secondary invaders in these cases.

Gram-positive cocci, which turn out to be the *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, two of the cases of very acute diarrhœa excepted.

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 fed on the breast alone.

Another form, which, I think, is the same organism, resembles the last named but occurs in pairs and short chains. This *third* type has been met with in cases of acute diarrhœa associated with members of the non-lactose-fermenting group. Sometimes they have occurred as diplococci, but frequently also in chains. I came to the conclusion that the occurrence of these cocci in chains in the fæces of diarrhœa 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:—

Type 1.—The organisms which come under this heading showed in the films of fæces a varied morphology. They occur mainly as diplococci resembling somewhat the pneumococcus, but, as a rule, a little larger and plumper. In the fæces they do not occur in chains under normal conditions,

but sometimes do so in fluid media, therefore the chains of cocci seen in the loose stools of diarrhœa cases may quite well belong to this group. On solid media they habitually occur in pairs, with a few clusters, and infrequent short chains. Individual elements show diversity in size and shape; usually lanceolate, they are sometimes round, and occasionally reniform with adjacent flattened surfaces. Frequently the elements of each pair form an acute angle with another. In fluid media, also, they generally are seen as diplococci, but occasionally in long chains.

They are always Gram-positive except in old culture, when they lose the stain.

In almost all the cases an organism has been isolated from the fæces after pasteurisation at 80° C. for ten minutes, which I see no reason to consider a different organism. This diplococcus, when pure, is killed by pasteurisation. This phenomenon has been noticed in the case of several other organisms—that they are able to resist a much greater degree of heat before isolation from the fæces than when pure. All strains were facultative anaerobes. *In ordinary broth*, a not very strong growth, evinced by slight cloudiness and a rather feeble deposit, was usually seen in twenty-four hours. In *glucose broth* there was within twenty-four hours marked cloudiness with a heavy deposit. On *agar slope*, on *blood-serum slope*, and on *blood-agar slope* there was a growth of minute, spherical, smooth-edged, dew-drop-like colonies of the usual streptococcus type. In *peptone water* no growth. In *glucose-agar stab*, a good growth took place all down the stab; in *gelatin stab* (room temperature), a delicate growth, with no liquefaction. In *litmus milk*, some strains produced acid and clot, others acid only. Five 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 five days on bread soaked in broth cultures of enterococci showed no effect. One guinea-pig inoculated subcutaneously with 2 c.c. of the whey of a culture in milk, showed a superficial ulcer with complete recovery.

Type 2.—The second type of coccus, namely, the larger oval Gram-positive coccobacillus, isolated from bottle-fed and older cases, showed in cultures growths identical with those obtained from the enterococcus. Its pathogenicity was not tested. It always tended to grow in clusters, or isolated.

Type 3.—This was like the last, but grew always in pairs; it may have been the same organism.

The streptococci in the diarrhœa 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.

The conclusions arrived at were that the enterococcus of Thiercelin, or an organism indistinguishable from it, was regularly present in the stools of healthy breast-fed infants; that in the motions of artificially fed and older children there was present in addition a larger coarser coccus, also Gram-positive; that in diarrhœa stools both types tended to grow in chains; and that there was no evidence that these last named forms were new varieties.

The Group of Spore-bearing Bacilli.

Tissier has stated that the *B. perfringens* (*B. enteritidis sporogenes*) is a normal constituent of the intestinal flora of infants both breast-

fed and bottle-fed. He has also described a form of sub-acute enteritis in children (1905 [21]) which he considered to be due to infection by this organism. Klein, also (1896 [22]), has described an outbreak of infantile diarrhœa in England in which this organism was isolated in large numbers from the diarrhœic motions.

In the present research, in spite of the provision made for the isolation of these organisms in the scheme of routine examination, the *B. enteritidis sporogenes* was isolated only three times—once from a breast-fed infant, twice from older children on a mixed diet. This organism was never isolated from a case of diarrhœa, nor was any bacillus at all resembling it ever seen in the fæcal films from these cases.

The *B. putrificus* was isolated from no case, though bacilli which might possibly have belonged to this group, judging by morphology alone, were seen in two of the older children on a mixed diet.

The *B. subtilis* was obtained from three cases—two on the bottle, the third on mixed feeding.

Therefore in my series of cases spore-bearing bacilli played an unimportant part.

PART III.

The Intestinal Flora of Infants on the Breast alone.

Six specimens were examined from children ranging in age from 2 to 10 days.

In one from a 2 days' old child, the films showed no organisms, and cultures remained sterile. This was meconium, and it was doubtful whether the child had had the breast even once before the passage of the motion.

In four of the others, the results were nearly identical. The films showed the punctate form of *B. bifidus* in nearly pure culture; in two of these I failed to isolate this organism. Enterococci were seen in the films and isolated in all these four cases, but were very scanty as compared with the *B. bifidus*. Indol-producing lactose-fermenters were present in very small numbers in these cases. Their reactions are given in Table VI.

In the sixth case, lactose-fermenters were present in relatively large numbers, though the *B. bifidus* was still dominant. The enterococcus was fairly numerous, and a spore-bearer was present which on isolation was determined to be the *B. enteritidis sporogenes*.

The Flora of Infants on Bottle and Breast.

Five specimens were examined. They presented a flora very similar to that of breast-fed infants. Gram-positive bacilli of the acid-tolerant type were the dominant form; they, however, consisted mainly of the facultatively aerobic and easily cultivated *B. acidophilus* of Moro instead of the strictly anaerobic *B. bifidus* of Tissier.

Gram-negative bacilli were somewhat more numerous in the films than in the cases fed on the breast only; their reactions are given in Table VII. In one case, a considerable proportion of the Gram-negative bacilli were found to be non-lactose-fermenters; these on further study were found to give the reactions of Morgan's No. 1 bacillus, and by agglutination tests were found to be identical with a strain of Morgan's No 1 isolated from a case of acute diarrhœa, both being completely agglutinated up to a dilution of 1–5000. This first-mentioned case was free from diarrhœa at the time of examination, had never so suffered, and was still free at the time of leaving hospital, a fortnight later. Enterococci of the small type were present in all these cases, but spore-bearers were absent.

The Flora of Infants on the Bottle.

The seven cases examined showed a somewhat more varied flora. The main groups present were the same, but the proportions were altered, and the non-lactose-fermenting group was more widely represented.

In four out of the seven the difference from the breast-fed cases was merely an exaggeration of the difference noticed in children on the breast and bottle. That is, Gram-negative bacilli of the lactose-fermenting group were more numerous, as were also coccal forms. The Gram-positive bacilli of the acid-tolerant group were still the dominant form and were of 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 VIII. The flora of the other three cases showed an addition-difference in the presence of members of the non-lactose-fermenting group. Two of these were bacilli with all the cultural characteristics of the paratyphoid-Gaertner group, but proved by agglutination tests to have no relation to *B. paratyphosus* A., *B. paratyphosus* B., or *B. Gaertner*. The third gave the reactions of Morgan's No. 1 bacillus, but agglutination tests showed it to be distinct from the two strains—the one from the case of diarrhœa, the other from the child on bottle and breast—above mentioned.

The *B. subtilis* was isolated from two of these cases, the *B. enteritidis sporogenes* from none.

The Flora of Children on a Mixed Diet.

Three specimens from children between eighteen months and four years, on a diet more or less approximating to adult in type, were examined.

The acid-tolerant group was present, though relatively diminished, and members were of the *B. acidophilus* type. Coliform bacilli were also numerous, and the characters of the lactose-fermenting bacilli isolated are given in Table X. The coccal forms in these cases were very varied, but in the main consisted of diplococci of the enterococcus type, and of the larger oval cocco-bacillus. Streptococci in long chains were never seen. A spore-bearer which proved on isolation to be the *B. subtilis* was present in one case. *B. enteritidis sporogenes* was present in two, but played an inconspicuous part.

Non-lactose-fermenters were present in two cases—one showing paratyphoid-Gaertner characteristics, but proved distinct by agglutination, the other with characters not possessed by any known variety. These organisms will be discussed along with the diarrhœa cases in a second paper.

Conclusions.

1. That, for purposes of convenience in study, the intestinal flora of infants and young children may be divided into five groups:—
   (1) The Gram-positive bacilli of the acid-tolerant group.
   (2) The Gram-negative lactose-fermenting group.
   (3) The Gram-negative non-lactose-fermenting group.
   (4) The coccal forms.
   (5) The spore-bearing bacilli.

2. That, in the flora of breast-fed infants (the ideal), the acid-tolerant group is immeasurably predominant, and is of the strictly anaerobic type called *B. bifidus*. Coccal forms and lactose-fermenting bacilli are present but scanty; spore-bearers rare. Non-lactose-fermenting bacilli were not seen in my cases.

3. In artificially fed infants the main difference is a decrease of the acid-tolerant group, and an increase of the lactose-fermenting group, along with the appearance of members of the non-lactose-fermenting group. The place of the *B. bifidus* is now largely taken by the facultatively aerobic *B. acidophilus*. The coccal forms show greater variety.

4. In my cases, as in Tissier's, the children on both breast and bottle showed a half-way stage between the two types of flora.

5. The children fed on approximately adult diet differed from the others in that no one variety dominated the others, and that the number of varieties present was greater. Lactose-fermenters were fully as numerous as bacilli of the acid-tolerant group; coccal forms were also numerous, and very varied in type; *B. enteritidis sporogenes* was isolated in two out of three cases.

6. The conclusion was come to that the acid-tolerant group contained at least two main varieties: the strictly anaerobic type, or *B. bifidus*, and the facultatively aerobic type, or *B. acidophilus*. It was found that, in a medium containing lactose or glucose, the *B. acidophilus*

had a restraining influence on the growth of Morgan's No. 1 bacillus, and also on the *B. coli.*

7. A thorough trial was made of MacConkey's tests on the lactose-fermenting group, and the conclusion arrived at that the saccharose test was unreliable, as also the motility test. Allowing for the possible error due to these tests, there was no evidence that the diet resulted in the presence of any particular type. The types met with in diarrhœa cases were, in the main, the types met with in normal children, and there was no increase of virulence in the diarrhœa cases. Prolonged growth on the same medium did not result in moulding strains to the same type. The types met with in my cases were, in the main, the same as those met with by MacConkey in adults.

8. The non-lactose-fermenting group was not represented in the breast-fed infants, but members, including Morgan's No. 1 bacillus, were isolated from the normal artificially fed.

9. The coccal forms showed three main types. The small entero-coccus was present in all normal cases. The coarser types were only met with in the artificially fed. Cocci in long chains were not seen in the films from normal cases.

10. Spore-bearers played a small part, but were represented in both breast-fed and artificially fed infants. They were of the *B. enteritidis sporogenes* type. The more aerobic *B. subtilis* was also met with.

The subject of this research was suggested to me by Professor Ritchie, and I have to acknowledge with gratitude much generous advice given during the progress of the work. The great majority of the specimens and the clinical records of the cases were obtained from Charteris Ward, Royal Hospital for Sick Children, Edinburgh, and I have to thank Dr. Fowler for his kindness in allowing me to make use of them.

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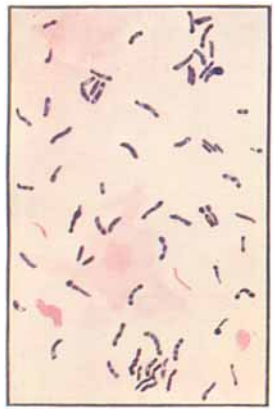


FIG. 1.

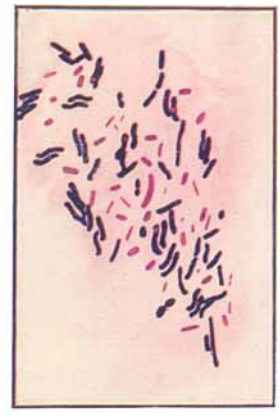
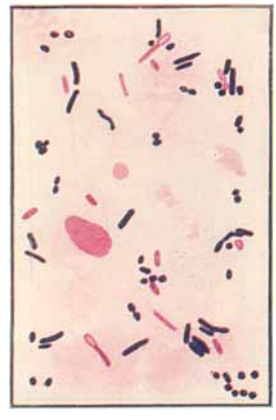


FIG. 2.



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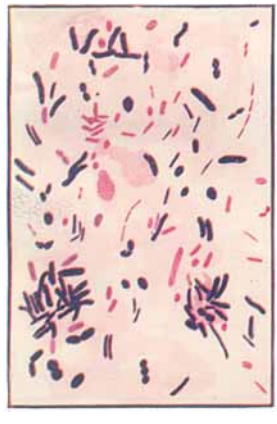

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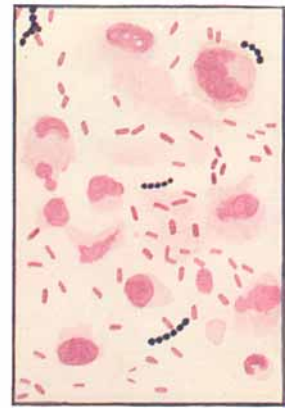

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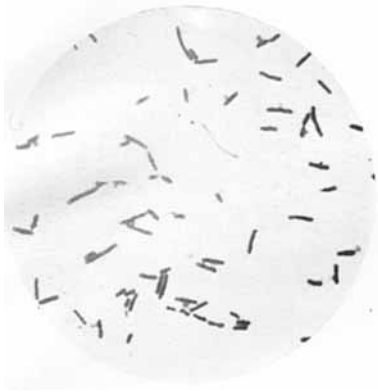

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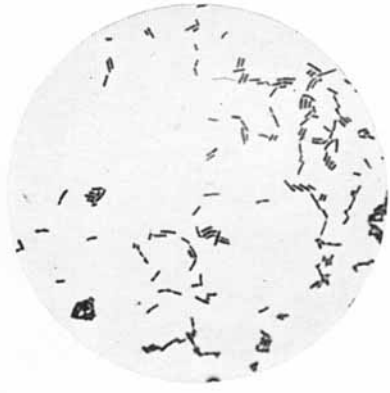

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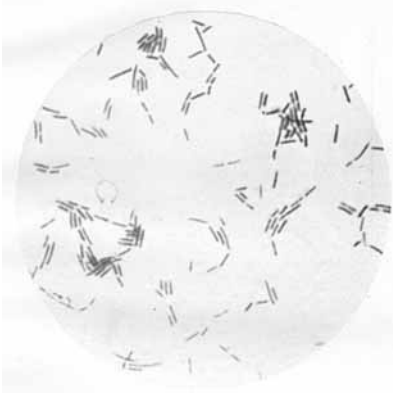

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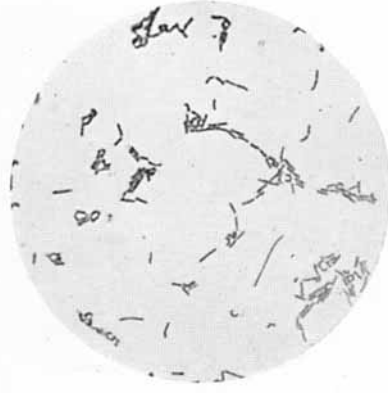

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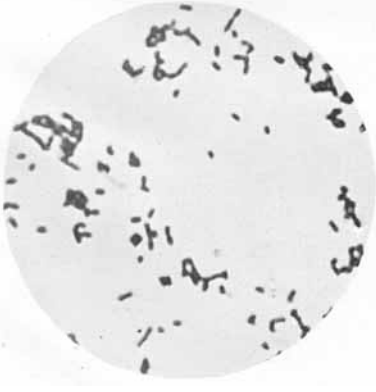


FIG. 10.

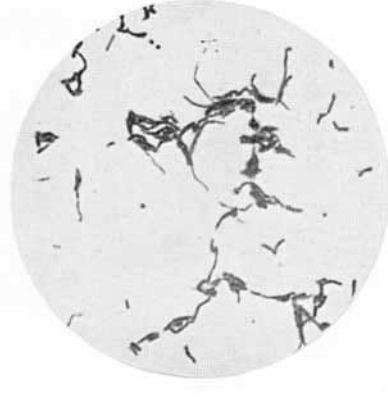


FIG. 11.

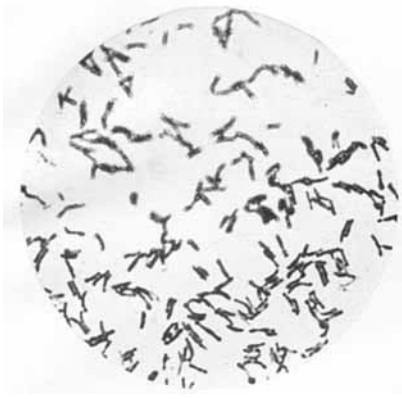
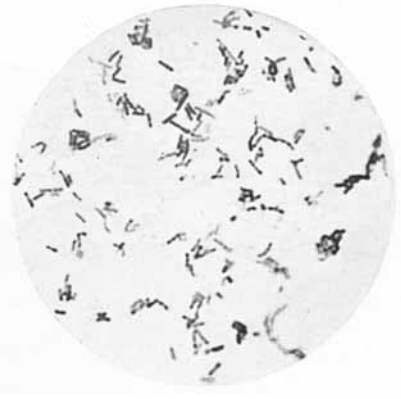


FIG. 12.



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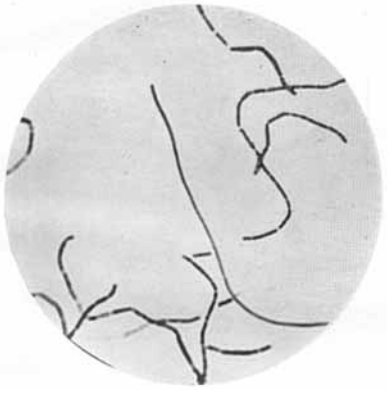

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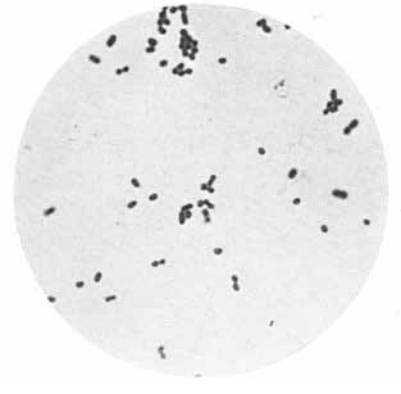

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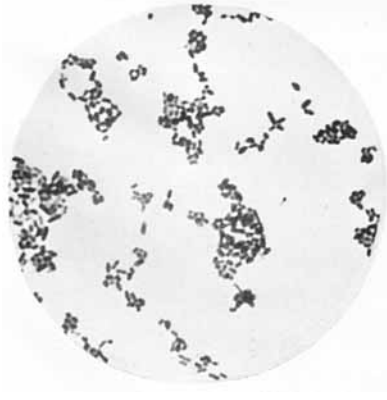

FIG. 16.

FIG. 17.

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DESCRIPTION OF PLATES XLV.–XLVII.

PLATE XLV.

FIG. 1.—Gram-stained smear from fæces of breast-fed child, 5 days old. The punctate form of *B. bifidus* is present in almost pure culture.

FIG. 2.—Gram-stained film from fæces of bottle-fed child (cow's milk), æt. 1 month. Gram-positive bacilli of *B. acidophilus* type are dominant; coliform bacilli and enterococci also present.

FIG. 3.—Gram-stained smear from fæces of breast-fed child, æt. 3 days, during the "second period" of Tissier, before the dominance of *B. bifidus* is established. Note presence of enterococci, *B. acidophilus*, coliform bacilli, and of *B. enteritidis sporogenes*.

FIG. 4.—Gram-stained smear from fæces of 10 months' old child on diet of cow's milk, with porridge, milk puddings, bread, etc. Note the greater morphological variety present.

FIG. 5.—Gram-stained film from fæces of 6 weeks' old bottle-fed child suffering from acute diarrhœa. Gram-positive bacilli of acid-tolerant group have entirely disappeared, and coliform bacilli and streptococci are left. A large proportion of the coliform bacilli were, on isolation, Morgan's No. 1 bacillus.

PLATE XLVI.

FIG. 6.—Glucose agar stab culture, 4 days old, of *B. acidophilus* type of acid-tolerant group.

FIG. 7.—Smaller variety of *B. acidophilus* from 3 days' old glucose agar stab culture.

FIG. 8.—*B. exilis* type of acid-tolerant group. Taken from 4 days' old glucose agar anaerobic plate.

FIG. 9.—Small type of *B. acidophilus*, from 3 days' old anaerobic glucose broth culture.

FIG. 10.—Coccobacillary form of *B. acidophilus*, from 2 days' old glucose agar stab culture.

FIG. 11.—*B. bifidus*; 7 days' old glucose agar stab culture.

PLATE XLVII.

FIG. 12.—*B. bifidus*; 4 days' old anaerobic glucose broth culture. Note irregularity of shape and staining; upper half of field is out of focus.

FIG. 13.—*B. bifidus*; 3 days' old glucose agar stab culture.

FIG. 14.—*B. bifidus*; 5 days' old glucose agar stab culture.

FIG. 15.—The enterococcus; 3 days' old glucose agar stab culture.

FIG. 16.—The oval coccobacillus; 8 days' old glucose agar stab culture.

FIG. 17.—*B. enteritidis sporogenes*; 5 days' old glucose agar stab culture, showing long involution forms, some parts of which have lost their Gram-positive character.

All these microphotographs were taken at a magnification of one thousand. The films were from twice-purified cultures, and were Gram-stained, dilute fuchsin being used as a counter-stain.