

A STUDY OF THE INTESTINAL FLORA UNDER NORMAL AND ABNORMAL CONDITIONS.

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IN this communication an attempt has been made to study the bacterial flora of the human intestinal tract in health, and when under the influence of, errors of diet, active purgation, and chronic constipation; in septic infections of the throat, respiratory and urinary tracts, and in infective processes of the intestinal walls. It was thought that a wider knowledge of the faecal flora apart from gross intestinal lesions might be of considerable advantage, more especially when full appreciation is given to the fact that so many conditions are attributed to so-called "intestinal infections."

This work has been carried out on the lines indicated especially by American investigators, who have introduced an elaborate technique for the bacteriological examination of the faecal flora, and by the method introduced by Dudgeon which has been adopted as a routine procedure. The Americans have insisted on the importance of the individual's diet whenever the bacteriology of the faecal flora is being investigated, or, in other words, the output must be controlled by the intake. It is also necessary to avoid active purgation before such examinations are undertaken.

In my Presidential Address to the Section of Tropical Diseases and Parasitology of the Royal Society of Medicine in 1924, I referred to these facts and to the importance of examining the patient's mouth, throat, respiratory and urinary tracts, so as to determine whether abnormal bacteriological findings in the faecal flora were dependent upon infection in these areas.

Method of examination. Fresh samples of faeces, free from urinary contamination, were employed for all these investigations. In some instances sterile glass receptacles were used, in others clean glass pots which had not been sterilised, but no special advantage occurred from the use of the former.

The general appearance of each specimen was recorded and a microscopical examination was made for undigested food and mucus. Red cells, leucocytes, epithelial cells and parasites were searched for in film preparations of the fresh specimens fixed by Schaudinn's method and stained with haemalum and Bordeaux red.

The ratio of Gram-negative and Gram-positive bacteria, and of cocci to bacilli. American workers especially have laid considerable stress on the value of film preparations of faeces so as to determine the Gram-negative and positive

ratio. Film preparations of the faeces, which were made a routine procedure, were stained by Jensen's method for Gram preparations, except that as Gram-negative bacilli in faeces may stain so faintly as to be readily overlooked, it was found necessary to employ carbol fuchsin as a counter-stain instead of neutral-red. Certain points on the selection of the material for making these film preparations must be referred to. Thin films are essential. If a large amount of undigested food, especially muscle, is present, films should be made from such material, and from mucus, as well as from the rest of the faeces, otherwise divergent results may be obtained. Unless attention is paid to these particulars any information likely to be derived from this method of examination can be disregarded. Additional films can also be made from a watery emulsion of the faeces centrifuged sufficiently to remove solid matter so that a uniform preparation is obtained. This method of examination should only be regarded as a superficial preliminary test, as, not infrequently, even when an elaborate technique has been employed, the cultural results are not in agreement with the results of the film preparations.

I think there has been an undue tendency of late to place too much reliance on the evidence obtained from film preparations, although this method is of great value when inflammatory changes are present in the bowel wall. In films of the faeces mixed with iodine bacilli have been seen which contained blue or blue-black or red-brown granules, sometimes in large numbers. Henneberg has discussed the importance of these bacilli in detail.

The system of recording the results of the investigations on each case is shown in the following headings to charts which I employed throughout the work.

No. and ref. no.	Diet and purgatives.	Films of faeces.	Naked eye appearance of faeces, etc.	Aerobic cultures				Anaerobic				Diasc.
				Plates		Other media.	Liquid media.	Liquid media			Blood reactions.	
				Blood agar.	Litmus lactose agar.			Robertson.	Milk.	Kim.		

Spirochaetes. These were found in film preparations of the faeces in 7 per cent. of the cases and generally in large numbers. They were short and thick with 2, 3 or 4 turns in the majority of instances, but delicate spirochaetes showing many spiral turns occurred. The presence of spirochaetes was not found to be in relation to any special variety of intestinal lesion, as they occurred among the normal, post-dysenterics and patients suffering from chronic constipation who were passing mucus in the stools. Both the coarse and fine spirochaetes stained well by Fontana's method, and with strong carbol fuchsin followed by washing in weak acetic acid. There was no direct evidence to show whether they were pathogenic or merely "passengers" in the faeces. Some of the patients—the dysenterics—had *been abroad, but the majority had not been out of England.*

It is now well known that spirochaetes have been demonstrated in water supplies and Hindle has recently shown that if a small quantity of faeces, human or animal, is mixed with ordinary tap water in a Petri dish, and the mixture incubated at 25° C. for about ten days, large numbers of *Leptospira*

appear in the cultures and persist for some weeks. They are present often in large numbers in certain common affections of the mouth and pharynx, and in three of the patients investigated numerous spirochaetes were seen in the oral lesions, and in one patient who had large numbers of spirochaetes in the faeces, widespread Vincent's angina was present. I have found in several cases that sections prepared from carcinomatous ulceration of the tongue by Levaditi's method have shown short coarse, and fine long spirochaetes with many delicate spiral turns in very large numbers in the ulcerated lesions.

Hudson and Parr found intestinal spirochaetes in large numbers among rats fed on a protein diet, more especially in films prepared from the caecum.

Material employed for investigation. The samples of faeces which form the basis of this communication were obtained from 200 cases, which included typhoid fever, post-dysentery, "colitis," patients suffering from septic infections of the mouth or respiratory tracts, healthy human subjects who were suffering from dietetic errors and from individuals who were apparently perfectly healthy. The faeces of all these patients were subjected to an elaborate aerobic and anaerobic investigation so as to ascertain whether numerous solid and liquid media are necessary in each individual case. The number of cases (200) submitted to this procedure is not large, but the amount of work entailed on each case was very considerable. It was thought that greater information would be obtained by this means than by less elaborate methods on a large number of cases. This bacteriological procedure proved of considerable value in the selection of the most appropriate media for such purposes, and among the much larger numbers of cases investigated with less detail, various points of special importance, learnt from the study of the selected cases, were confirmed, or otherwise, or elaborated. The bacterial flora of the faeces of healthy subjects, or of healthy individuals who were suffering temporarily from the effects of dietetic errors, was very fully investigated because of the importance of the subject. So much stress is often given to abnormal findings in the bacteriology of the faecal flora in various conditions believed to be due to "intestinal infections," that it is essential to be acquainted with the range of limit in health or among healthy people whose diet has been abnormal. It was also necessary to study the effect of purgatives on the bacteriology of the faecal flora, and if possible to learn the value, if any, of intestinal antiseptics.

Cultivation experiments. In every instance, Dudgeon's method—as described in detail by Wordley—was employed for the preliminary treatment of the faeces, which were thickly smeared over the surface of sterile, unglazed, porous tiles. When the faeces were partially dry the material was transferred to a fresh tile to complete the drying process. The powder was scraped off with a sterile knife, *well mixed* so as to obtain a true sample of the faeces, and then sufficient of the powder to cover the pointed end of a scalpel was used for plating on suitable solid media. Further portions were added to various tubes of liquid media. It is important to adopt this technique without

such modifications as result in the faeces being more rapidly prepared, but inefficiently dried, as unsatisfactory and unreliable results occur. Faeces which contain a large amount of "fats" do not "powder." In such cases the best preparation that can be obtained by drying is employed. A very large number of media have been used for plating the powdered faeces, but experience has led me to regard litmus lactose agar, agar to which fresh human blood has been added at 50° C. in the proportion of 1 c.c. of whole blood to 10 c.c. of melted agar, and blood agar similarly prepared, but heated to 90° C., to be the most valuable of all media for faecal examinations.

The dry faecal powder is suitably spread on these plates and thus accurate information is obtained of the relative proportions of the aerobic bacteria. The presence of haemolytic strains is shown, and abundant growths of streptococci and staphylococci are readily obtained. The chief advantage of this method of isolating bacteria from the faeces is that the micro-organisms are cultivated on artificial media without the addition of any substance which retards the growth of one organism at the expense of another. No special advantage occurred from the employment of kim oleate agar and whey agar as recommended by American workers, although these media were given a very thorough trial. The dry powder was added to liquid media and incubated anaerobically. Here again a very large number of media have been tried which have been recommended from time to time by various workers on this subject, and other media which I have experimented with myself, but the most useful were found to be Robertson's heart muscle, milk, and Deyell's 50 per cent. ox bile. These are now used by me as a routine. In the preparation of Robertson's heart muscle medium, I allow the fat to remain and add a further portion, which forms an excellent solid cap to assist in the production of an anaerobic atmosphere. Media used anaerobically were boiled before being inoculated with the faecal powder. No advantage would be gained by detailing all the solid and liquid media which have been employed, but my experience of faecal bacteriology has led me to believe that "drying" the faeces has so simplified matters that elaborate artificial media are not required. In some cases the dried faecal powder was added to liquid serum media and incubated aerobically. Acid broth was used to cultivate the acidophilus group, as will be referred to later.

The streptococci. It is not intended to enter into a lengthy discussion on the streptococci isolated from human faeces, as this subject has been dealt with by so many workers on intestinal bacteriology and on the streptococci in general, but more especially in the valuable contribution to the subject made by Dible in 1921. In the present communication 50 specimens were taken for examination from 50 patients suffering from typhoid fever, dysentery, and other inflammatory processes affecting the intestinal tract, from normal individuals, and those suffering from constipation and from dietetic errors. By this means the sampling was unrestricted and devoid of limitations. From these 50 cases, 115 cultures of streptococci isolated from the dried

faeces by Dudgeon's method, from plates of blood agar, agar, litmus lactose agar, and kim oleate agar have been subjected to full detailed investigation.

On morphological grounds 82 out of the 115 strains of streptococci isolated were found to be diplococci not unlike the pneumococcus when grown in liquid media, and are referred to as enterococci.

Heat resistance. Every strain was grown for 24 hours aerobically in dextrose (1 per cent.) broth and beef broth, and then exposed to a temperature of 60° C. for 15 minutes, as recommended by Dible. Dextrose, however, was found to be unsatisfactory for the purpose as the combination of acid production by the growth of the organism in dextrose, and heat, produced a far greater population of thermolabile cocci than occurred when these cocci were grown in beef broth. This strength of dextrose broth was tested with every strain of streptococcus and proved unsatisfactory for this reason.

Haemolysis. This was tested for by incubating the organisms on fresh human blood agar, and in two tubes of 5 c.c. of peptone water, one of which contained 0.5 per cent. and the other 0.85 per cent. of sodium chloride and 0.1 c.c. of solid human red cells. The results were read at the end of 24 hours' incubation at 37° C.

The enterococci. The 82 strains of cocci included under the name enterococci were found to grow as Gram-positive diplococci in liquid media such as lactose-glucose-mannite and salicin broth with or without the addition of sterile blood serum, on blood agar, and in milk, and these cocci morphologically somewhat resembled the pneumococcus, although definitely of a coarser type. Chain formation apart from a few individuals did not occur with the cocci included in this group; in all media they were definitely diplococci. On agar, lactose agar, kim oleate agar, and blood agar the colonies varied in size, as observed with various strains of streptococci. It was found that a much higher percentage (69.5) of these 82 strains were heat-resistant than with the long-chain streptococci (21.0), or with the medium-chain (42.8). Dible considers heat resistance to be a characteristic feature of the enterococci, and that the typical enterococcus ferments mannite. In this series, acidification of mannite occurred in 47 or 57.3 per cent. out of the 82 strains which were investigated. This was not as common as Dible's results show, but on the other hand only 1 strain out of the 19 long-chained streptococci fermented it. Lactose, dextrose, and salicin were acidified by most of the enterococci. Only 3 strains acidified inulin, but all acidified milk and 77 out of the 82 strains clotted it.

Haemolysis. The results of the haemolytic test with the enterococci is of considerable interest owing to the high percentage of haemolytic types. These are shown in the accompanying table together with the heat resistance and mannite reactions as follows:

	Heat resistant.	Acidification of mannite.	Total.
Haemolytic 21	Stable 14	13	82
Non-haemolytic 61	„ 42	38	

Dible found that only 2 of his strains which were obtained from the same sample of faeces were of the haemolytic type; 1 strain was haemolytic, the other formed methaemoglobin.

Wordley, in 1922, examined 76 samples of faeces by the method introduced by Dudgeon from 76 cases of scarlet fever in the first week of the disease, and obtained haemolytic streptococci from 24, or 27·9 per cent., and in 33 stools from 33 cases of acute nephritis haemolytic streptococci were obtained in 16, or 48 per cent. He states that in the majority of cases examined the haemolytic streptococci were numerous and scattered over the surface of the blood agar plates. Wordley studied 41 strains along the lines laid down by Dible, with the result that 19 were found to be haemolytic, diplococcal in form, with coarse colony formation, acidified mannite, and were resistant to heat; while 17 strains were haemolytic, formed long chains, did not affect mannite and were thermolabile. Five strains which formed diplococci did not affect mannite and were thermolabile.

Of all the individual characteristics of the enterococci, in my opinion, the morphological appearances are the most important. The coarser growth on solid media as compared with the chain-forming streptococci and the large number of mannite fermenters and of thermo-stable forms are of undoubted importance, but the Gram-positive diplococcal arrangement on solid and in various liquid media is so striking as to be the central basis around which the other features are linked. There is no better example of the importance of morphology than in the case of the enterococcus.

It is an important fact that haemolytic organisms isolated from the faeces which resemble streptococci, when fully investigated, frequently prove to be enterococci.

The long-chained streptococci. Nineteen strains of Gram-positive long-chain streptococci were investigated of which 18 acidified lactose and the growth was precipitated leaving a clear supernatant fluid, while only 1 strain acidified mannite and this also fermented inulin. Fifteen strains acidified salicin, all acidified milk and 15 of these strains clotted milk, which would be a very high figure for long-chained streptococci isolated from acute inflammatory processes. Long-chain formation occurred with all these streptococci in the various liquid media employed, and some of them produced bacillary formation in the chains, as so commonly occurs among the mouth streptococci. Six of the long-chained streptococci were haemolytic and all these strains were thermolabile—a direct contrast to the enterococci.

The short and medium-chained streptococci. These cocci were all Gram-positive, formed short or medium chains when grown in liquid media and all grew anaerobically.

The short and medium-chained streptococci fermented lactose or salicin in every instance, and formed a granular deposit in 10 out of the 14 strains. With mannite, however, there was a great difference from the long-chained varieties as 8 out of the 14 acidified it, and inulin was acidified by 2 strains.

Milk was acidified in every instance and clotting occurred with 12 out of the 14 strains tested. Five strains were haemolytic and 2 of them thermolabile.

The following table shows the results of the streptococcal contents of the faeces obtained from 3 cases—(A) Dilatation of the colon; (B) Post-dysenteric diarrhoea; (C) "Normal"—and illustrates the varieties of streptococci which occur under widely divergent clinical conditions. We learn from intestinal bacteriology that all varieties of streptococci may occur in the human faeces, which is in support of a widespread belief that the human subject may be infected from his intestinal tract, but the proof positive evidence that streptococci have originated from this source is of course very difficult to obtain. It is well to realise that the types of streptococci met with in normal faeces may exactly correspond with those found in the faeces in pathological processes affecting the intestinal tract.

Diagnosis	No.	Haemolysis	Heat resistance	Morphology	Mannite
A. Dilatation of colon (chronic constipation)	1	H.	Labile	Long-chained Streptococcus	-
	2	N.H.	Stable	Diplococcus	-
	3	H.	Labile	Long-chained Streptococcus	-
	4	H.	"	" " "	-
B. Post-dysenteric diarrhoea	1	N.H.	Labile	Short-chained Streptococcus	-
	2	N.H.	Stable	Diplococcus	+
	3	H.	"	Short-chained Streptococcus	-
	4	H.	Labile	Long-chained Streptococcus	-
C. Normal	1	N.H.	Stable	Diplococcus	+
	2	H.	Labile	Long-chained Streptococcus	-
	3	N.H.	Stable	" "	-

In several cases of infection of the throat and respiratory tract haemolytic streptococci were found to be abundant in cultures from the tonsillar exudate or sputum, while haemolytic streptococci with similar reactions in milk, carbohydrate media, and heat resistance were isolated from the faeces.

Cases have occurred from which haemolytic streptococci have been isolated from the faeces in large numbers, but when an alteration in the diet alone has been introduced, it was found to be no longer possible to cultivate them.

Many instances occurred in which streptococci were grown in almost pure cultures on blood agar from the dried faecal powder. The colonies of streptococci covered the whole surface of the medium.

Haemolytic colon bacilli. In 6 per cent. of this series of cases haemolytic colon bacilli were isolated from the faeces, and in most instances when these bacilli were present the bacterial content of the faeces was excessive, haemolytic colon bacilli were cultivated in very large numbers and no non-haemolytic colon bacilli were isolated. The clinical conditions associated with the presence of these organisms in the faeces were multiple, as they were found in cases of infection of the urinary tract due to the same bacillus, diarrhoea, paratyphoid fever (B) and from patients with enteroptosis and the symptoms commonly considered to be due to intestinal toxæmia. The evidence that these bacilli were haemolytic was determined at the outset on human blood

agar plates and subsequently in the salt-peptone tubes which contained 2 per cent. of fresh human red cells. It is, however, necessary to realise that colon bacilli may be actively haemolytic in liquid media, although non-haemolytic on blood agar plates, or the haemolysis may be indefinite. The chief cultural differences among haemolytic colon bacilli occurred with cane sugar and dulcitol, but they were all agglutinated with the appropriate anti-sera prepared from the haemolytic strains isolated from the urinary tract and faeces by Dudgeon, Wordley and Bawtree. The number of haemolytic strains isolated from the faeces in this series is, however, distinctly less than obtained by these observers. In my experience, when an abundant growth of haemolytic colon bacilli is obtained from the faeces there is a general toxæmia together with symptoms relevant to the intestinal tract, which may be controlled by specific haemolytic colon bacillary vaccines. This line of treatment in my hands has given better results than I have obtained with vaccine therapy in any other form of intestinal infection. The above-mentioned authors have already drawn attention to this fact, and considerable experience has led me to believe that this view is correct. The diarrhoea and the toxæmia may subside, although resistant to other forms of treatment, and certainly vaccine therapy should be employed when these organisms are isolated from the faeces and the clinical condition demands it. Patients with this form of intestinal infection may show the presence of specific agglutinins in the blood. Further instances have occurred of infection of the urinary tract with haemolytic colon bacilli and the presence of the same organism in the faeces as shown by serological tests.

Non-haemolytic colon bacilli occur in the faeces as judged by the methods of examination referred to in this communication either in very small numbers, or in greatly increased numbers or approximately the normal average. Instances occur when no colon bacilli will grow in one medium and only in a limited degree in others, although all such media are known to be suitable. Greatly increased numbers may occur in the faeces of patients with bowel infections, among the population believed to be suffering from intestinal toxæmia, which can sometimes be explained by over-eating especially of protein food, from excess of alcohol, and in cases of intestinal toxæmia not associated with dietetic excesses. It is essential to employ a routine technique with which the observer has had ample experience before deciding that the bacterial growth is increased or diminished, as even colon bacilli may grow at the outset much better on one medium than another. A case under the care of Mr Cyril Nitch, of infection of the intestinal tract which presented exceptional features, will be briefly referred to. The patient, an adult man, had numerous large infective granulomata in the wall of the small intestine which necessitated re-section of the infected area. A special strain of a non-haemolytic colon bacillus was isolated from the interior of several of these granulomata, and the same bacillus was grown from a specimen of his urine. This bacillus was fully agglutinated by one of the standard anti-sera

prepared by Dudgeon and his co-workers from a non-haemolytic colon bacillus (4869) isolated from the urinary tract and which has been found to agglutinate numerous non-haemolytic colon bacilli isolated from the urine. If the colon bacillus was the cause of the granulomata the condition is unique as far as I am aware.

The mucus capsulatus group occurred in 5.5 per cent. of these cases. These organisms were most commonly found in association with an abnormal condition of the intestinal tract such as diarrhoea, colitis, and in the later stages of typhoid fever.

B. faecalis alkaligenes has been described as a normal inhabitant of the intestinal tract, but this idea is quite contrary from my experience of cases examined in this country. During the Great War I endeavoured to obtain cultures of the bacillus from the faeces, and although contaminated water supplies were so common, yet, this bacillus was seldom isolated.

B. proteus was cultivated from the faeces of patients with infections of the urinary tract due to this bacillus, and very occasionally from cases of diarrhoea.

The slow lactose-fermenting bacilli. These bacilli occur in the intestinal tract and may give rise to intestinal disorders. They were obtained in 2 per cent. of the cases in this series. They have been mistaken for the paratyphoid group although the indol test readily distinguishes them. During the Great War slow lactose-fermenters were cultivated from the faeces of soldiers suffering from enterica and dysentery, not infrequently. In 1924, Dudgeon drew attention to the importance of haemolytic bacilli among this group as the cause of acute infection of the urinary tract, although at this period these bacilli had not been isolated from the faeces of patients suffering from this form of acute urinary fever. During the last year a more complete attempt has been made to investigate in detail, in conjunction with Dr Pulvertaft, slow lactose-fermenting bacilli isolated from the faeces and urine. It would appear as far as our investigations have advanced that several subgroups exist. Slow lactose-fermenters have been cultivated from the faeces, chiefly from cases of diarrhoea, and occasionally pure cultures have been obtained, but apart from diarrhoea cases they are uncommon in the faeces of patients in this country. In my experience slow lactose-fermenters are present in cultures from the faeces obtained from the region of the caecum more frequently than from the lower bowel, at the few opportunities which have occurred during life.

The staphylococci. These organisms are of regular occurrence in the faeces of breast-fed infants and may be the predominant, or even the only, organism on the surface of blood agar and litmus lactose agar plates. *Staphylococcus albus*, or *aureus*, or both, were found in the faeces of adults in this series in about 35 per cent. of the cases, sometimes in large numbers. The faeces of patients suffering from diarrhoea may give an abundant growth of staphylococci, but it is especially in pulmonary cases that *S. aureus* or *albus* are cultivated in large numbers. The presence of *S. aureus* in the faeces of patients

suffering from boils is by no means uncommon, and sometimes patients whose faeces contain *S. aureus* will subsequently develop boils.

The presence of *S. aureus* or *albus* in the faeces in the later stages of typhoid fever, often in large numbers, is important, more especially of *S. aureus*. I have been aware of this for many years, and have found that the presence of *S. aureus* in the faeces during the course of typhoid fever may be the precursor of an inflammatory process—due to this coccus—elsewhere in the body. In the present series only three cases of typhoid fever occurred, but colonies of *S. aureus* were found in the faeces in each instance; and in two of the cases in great abundance. One of these patients died from an inflammatory process due to this organism. It is probable that the prolonged milk diet is the cause of the abundance of staphylococci occurring in the faeces in typhoid fever which may be associated with a fermentative type of intestinal flora. Lockhart, working in my laboratories, studied the intestinal flora of 4 cases of typhoid fever over a period of one month and in 2 of these cases boils developed. In one of the patients *S. aureus* had been cultivated from the faeces subsequent to the onset of boils, and in the other *S. albus*; and in the former case *S. aureus* was the cause of the boils and in the latter *S. albus*.

Dudgeon and Bamforth refer to an interesting point concerning the relationship of *S. aureus* in the faeces with boils and typhoid fever, and with the presence of precipitins in the blood serum. A patient towards the end of the third week of typhoid fever developed septic foci in various parts of the body due to *S. aureus*, and numerous colonies of this organism were isolated from the faeces. This septic state persisted for more than a fortnight. The patient's serum gave a very strong precipitin reaction with their *B. typhosus* filtered antigen, and with one of their *S. aureus* filtered antigens.

The presence of *S. aureus*, in particular, in the faeces of adults in large numbers, or still more so its persistence as proved by multiple examinations, is in my experience important, as an inflammatory focus may be present and without question should always be looked for. In babies fed on breast milk this particular view does not hold, as *S. aureus* may enter with the milk and leave with the faeces without ill-effects on the baby. On the other hand, the presence of masses of *S. aureus* in the faeces of babies may be due to an inflammatory focus in the mother's breast, and they may be found in the faeces for prolonged periods and in large numbers. Dudgeon and Jewsbury have suggested that this might serve to explain the origin of inflammatory processes due to *S. aureus* in the tissues of babies and young children.

Diphtheria-like bacilli. In 6 per cent. of the cases diphtheria-like bacilli were present in cultures obtained from the faeces. These bacilli were larger and coarser than true diphtheria bacilli, very granular, and every strain showed a strong "dot reaction" by one of the modifications of Neisser's method. These diphtheria-like bacilli grew especially well on blood agar plates forming colonies which were dry, of a greyish colour, and were

non-haemolytic. They were tested in the 1 per cent. sugar media without the addition of blood serum, with the following results: dextrose was acidified by all the strains and mannite was unaffected; lactose and salicin were acidified by a few of the strains. With some samples of faeces large numbers of colonies of these bacilli were cultivated on fresh blood agar plates. In no instance was any ill effect observed.

All these diphtheria-like bacilli isolated from the faeces were injected into guinea-pigs, but were found to be non-pathogenic.

The presence of bacilli in the faeces with morphological resemblances to true diphtheria bacilli is important, especially in children. In two cases from this series the patients were children with enlarged tonsils, and although true diphtheria bacilli had not been recovered from the tonsils, yet, finding diphtheria-like bacilli in the faeces had complicated matters until the investigations were completed. Schoedel, in 1900, quoted by Nuttall and Graham-Smith, is stated to have observed diphtheria bacilli in the lower ileum and in the freshly passed faeces of patients suffering from diphtheria.

Anaerobes. Attempts to cultivate anaerobic bacteria were made in the media already referred to. In 35 per cent. of the total cases Gram-positive bacilli were isolated which produced typical storm fermentation in milk and did not liquefy blood serum. It was found that Robertson's heart muscle medium was the most effective for primary culture, as in some of the cases the original milk tubes did not show the stormy fermentation, but *B. welchii* was obtained in subcultures from the Robertson's tubes. The usual practice was to heat the blown Robertson's tubes to 80° C. for 20 minutes and then subculture into milk, 50 per cent. bile, pure human serum, or other media. Guinea-pigs inoculated from the milk tubes died within 36 hours and showed a widespread serous exudation separating the skin from the underlying muscles. A patient suffering from typhoid fever, whose faeces gave a very abundant growth of *B. welchii* just before death was found to have a gangrenous area in the intestine and foaming viscera at the autopsy.

Bacilli were frequently present in the faeces, which were Gram-positive, showed both central or terminal spaces, clotted and digested milk without producing stormy fermentation, blackened Robertson's medium with much gas formation and gave it a very putrid odour, liquefied blood serum and gelatine, and were non-pathogenic to guinea-pigs. This organism I have regarded as *B. enteritidis sporogenes*. It was more frequently found in normal faeces than *B. welchii* and grew in milk and meat media much more slowly than *B. welchii*.

In many instances other anaerobes were grown on the media primarily inoculated from the faeces, but no investigation of these organisms was attempted. Bacilli with terminal spores were seen in Robertson's cultures occasionally which may correspond to the organism described by Weinberg, who isolated, out of 122 samples of faeces, 4 strains of *C. putrificum* which had terminal spores. This organism digests meat protein and egg albumen

in 7 to 8 days, but has no action on carbohydrates. *C. tertium*, another terminal spore anaerobe, was also isolated. Aerobic bacilli with terminal spores identified by Weinberg as *B. reptans* were isolated by him from the intestines of man on four occasions out of 40 samples of faeces investigated.

The important question which requires careful investigation is whether *B. welchii* gives rise to acute gastro-enteritis or acute colitis in the human subject. Morris, Porter and Meyer consider that *B. welchii* plays a subordinate part in summer diarrhoea, but may be responsible for some of the grave symptoms of intoxication in infantile dysentery. Sir Alexander Houston (1925) devotes several pages to discussing this subject, but he concludes his remarks by emphasising the difficulties that arise as follows:

“At the same time he (Houston) cannot help feeling that, in the present state of our knowledge the importance of the ‘stormy fermenters’ in relation to gastro-intestinal disorders and in connection with water supply has been unduly magnified.” A definite increase of large Gram-positive bacilli in film preparations of the faeces cannot be accepted as positive evidence of an increased growth of *B. welchii*, and still more so that it is responsible for the intestinal infection. An increase in the number of large Gram-positive bacilli in films of the faeces may occur among patients suffering from the effects of dietetic errors, diarrhoea, and chronic constipation, while intestinal infections due to other organisms may also be accompanied by an increase of large Gram-positive bacilli in film preparations. The whole subject is beset with difficulties which hinder the expression of a positive opinion as to whether cases of acute gastro-enteritis and colitis due to *B. welchii* occur. I have not met with any proof positive case of diarrhoea, or entero-colitis due to this organism, but in my opinion this subject requires still further investigation. *B. welchii* may cause occasionally gas gangrene of the peritoneal tissues during the life of the patient, or it may produce necrosis and gangrene of the abdominal wall following abdominal section, and very occasionally diffuse gangrene in mother and infant following childbirth, of which I have seen five cases. Such cases are presumably due to an autogenous infection from the intestinal tract.

Bassler and Lutz believe that anaerobes are the cause of intestinal toxæmia in man, and for many years Veillon and Zuber, Tavel and Lanz, and others, have considered that the toxæmia of peritonitis is due to anaerobic bacteria, more especially *B. welchii*. Dudgeon and Mitchiner, in their report on the bacteriological examination of the interior of the appendix within the first 30 hours of acute appendicitis, isolated *B. welchii* on five occasions, but no complication followed appendicectomy.

Discussion. The work of Continental and American bacteriologists has opened up a wide field in intestinal bacteriology and has resulted in the classification of the intestinal flora into three or more main groups. On the basis of this classification the treatment of certain intestinal disorders by dietetics has been employed, so as to alter the type of intestinal flora. The

work of Morris, Porter and Meyer has resulted in the classification of three types of intestinal flora: (1) fermentative or saccharolytic; (2) putrefactive or proteolytic, and (3) facultative or normal.

Fermentative stools are usually buff-coloured, foamy, semi-formed, strongly acid and have a sour odour. Putrefactive stools which have a putrid odour may be dry, solid, and dark brown in colour; or pale, and moist, and contain mucus and undigested food; or dirty-green in colour, and semi-formed. Morris, Porter and Meyer have drawn attention to the importance of the constant presence of strongly proteolytic, Gram-positive, spore-bearing aerobes in putrefactive stools, and consider that they are introduced with certain grossly contaminated foods, but up to the present I have not met with bacilli of this type in any sample of faeces examined.

The work on the intestinal flora, by American workers especially, has led to the view that the bacteria present in the faeces are in direct relation to the diet of the host, that the absence of carbohydrates in a diet produces a predominance of proteolytic bacteria resulting in putrefactive stools, and the symptoms of intoxication from intestinal absorption.

It may be as well to quote from Cannon, one of the chief workers on this subject:

It has long been recognised that certain pathological conditions in man are associated with an intestinal flora that is markedly putrefactive in type—headaches, skin disorders, digestive disturbances, non-alcoholic cirrhosis, nervous abnormalities and cardio-vascular-renal disease have been attributed with more or less reason to the effects of excessive intestinal putrefaction.

In chronic diarrhoea it has been observed that the toxæmia is profound when the diarrhoea is of the putrefactive type, whereas in the fermentative type there may be little evidence of toxæmia.

Such extreme views on the importance of intestinal toxæmia require, however, far more practical proof than any worker has yet produced.

Treatment is based on alteration of the intestinal flora by change of diet, especially effected by milk and lactose. Pane's observations are in agreement with those advocated by the Americans, as he found that when a full meat diet is taken proteolytic anaerobic and aerobic bacteria are the chief organisms in the faeces and produce the toxæmia. By means of a milk diet the bacteria which produce acid fermentation proliferate at the expense of the proteolytic organisms. This change, however, may be only of a temporary nature.

The weakness of this classification into proteolytic and fermentative bacteria in faeces, in my opinion, is due to the fact that the term proteolytic is often used without any definite understanding as to which are the proteolytic bacteria. Some authors have even included colon bacilli which I can see no reason to regard as such. The proteolytic anaerobe in human faeces which digests albumen is largely *B. enteritidis sporogenes*, while such proteolytic aerobic bacteria as *B. proteus* and *B. pyocyaneus* are uncommon in the faeces. The chief distinctive feature given to the proteolytic group in the

various published records is that they are largely Gram-negative bacilli—a view with which I cannot agree from my experience of human faeces both in health and disease.

In a communication published in 1917, Dragstedt, Moorhead and Bureky as a result of their experimental observations were led to believe that the toxic material which is responsible for the toxæmia in acute intestinal obstruction is produced chiefly by the activity of the proteolytic group of intestinal bacteria. Stasis of the intestinal contents ultimately led to the development of a proteolytic flora and the consequent toxæmia, in spite of a carbohydrate diet.

In animals, numerous observations have been made of the intestinal flora in closed intestinal loops and of the toxæmia resulting from this surgical operation. The effect of certain diets and of various antiseptics on the contents of the loops has also been investigated.

Dragstedt, Cannon and Dragstedt isolated intestinal segments in dogs, by cutting the small intestine across in two places—about 12 inches apart—and re-establishing union by joining the proximal and distal ends. The isolated segment with intact mesentery was closed at both ends and returned to the abdominal cavity. Before the operations the dogs were fed on bread, milk, and lactose, or boiled rice, beef-heart, and lactose, and in each case the faecal flora was strongly aciduric.

At operation, smears from the jejunum showed Gram-positive organisms chiefly, but at the death of the animals the bacteria in the closed loops were almost entirely Gram-negative and the fluid in the loops gave an alkaline reaction. The isolated closed intestinal loops, previously washed with ether and sterile water, usually showed in 4 or 5 days an accumulation of Gram-negative bacteria in large numbers and the growth was not inhibited. When a saturated solution of tannic acid was used instead of ether, then both the distension and toxæmia were prevented, and the animals might survive indefinitely. The contents of the loops examined after several months to a year still showed large numbers of living Gram-negative organisms.

Dragstedt and Nisbet from their experimental observations consider that death from acute intestinal obstruction is due to a toxæmia which arises in the intestinal tract from the action of the normal intestinal bacteria on the intestinal contents. The symptoms can be produced in dogs when segments of the small intestine are closed, and the resulting toxæmia disappears when the closed segments are removed.

Isolated segments of the upper jejunum were made by dividing the intestine in two places 8 to 12 inches apart and reuniting the proximal and distal ends around the isolated segments. The blood supply was not interfered with and the mesentery was not injured. The intestinal segments were first washed with water at considerable tension and then with an antiseptic, but in most instances the toxæmia was not diminished and the segment contents were not sterilised.

Ninety per cent. of the animals died within a week, and at the autopsies in the majority of the cases marked distension and perforation of the isolated segments had occurred. In a few instances there was no perforation and no general peritonitis. The material in the loops was very toxic and contained large numbers of bacteria.

Ten per cent. of the animals quickly recovered from the operation and never showed toxic symptoms. Some of these were operated on again after several months. In most cases the loops were intact and contained thick grey offensive toxic fluid in which were masses of proteolytic organisms.

These authors consider that if intestinal antiseptics are required preparations should be selected which will not be absorbed and will not produce an irritating action.

Intestinal antiseptics. My experience, based on a very large number of bacteriological observations with various so-called intestinal antiseptics has led me to believe that they do not influence the bacterial content to any appreciable degree unless the source of the trouble is corrected. Full administration with some drugs which have been employed as intestinal antiseptics has not affected the bacterial flora, as judged by the technique adopted in this communication, in the slightest degree. The only drugs which have reduced and altered the faecal flora have been dimol and occasionally colloidal kaolin, which may produce considerable improvement. The administration of any of the so-called intestinal antiseptics to patients who still continue on a very liberal diet is an error, which is by no means infrequently permitted. My bacterial investigations have led me to believe that a drug such as dimol may reduce the bacterial content of the faeces if the patient is having a correct diet, but if the diet is in error no drug is of any real value. Treatment with intestinal antiseptics to-day is in much the same state as when Harvey Cushing wrote these words: "The advocacy of various so-called intestinal antiseptics, too numerous to mention, is difficult to understand in the face of experimental work undertaken in the vain attempt to establish any efficiency whatever towards the sterilisation of the alimentary canal by any of those drugs ordinarily employed for this purpose."

On the effects of diet on the intestinal flora. In spite of much of the recent work on the effect of diets on the intestinal flora, Harvey Cushing, many years ago, fully considered and discussed in detail, in an admirable paper, several of the points which are being re-investigated to-day. I cannot do better than to give his views on the subject in his own words:

"Confirmatory evidence, therefore, is given of what has been maintained in the foregoing paragraphs, namely, that the number of micro-organisms in the canal depends, in health, *largely upon the number introduced by the mouth*, and not upon the multiplication of the pre-existing bacteria in the medium" (afforded by the intestinal contents). Cushing and Livingood in this paper quote the work of Gilbert and Dominici who showed in 1894 that "a simple milk diet, which presumably greatly lessens the varieties of micro-organisms

introduced, has a remarkable effect in causing a diminution of the number in the faeces, in both man and animals.”

Although all workers on this subject must be agreed as to the importance of the introduction of bacteria in the food, yet, the multiplication of the normal inhabitants is, in my opinion, equally important. Experience has taught me that among the normal population who have committed dietetic errors, bacteria foreign to the faeces may be found, but also the common inhabitants, the enterococci and non-haemolytic colon bacilli, may be greatly increased in numbers. Bacteria, such as haemolytic colon bacilli, which are present in the faeces in a small proportion of normal individuals, and then only sparsely, may as a result of an intestinal disorder appear in greatly increased numbers.

Similar observations have been made on the faeces of infants in whom a detailed study of the faecal flora is much simpler than in adults, because the bacterial intake is so much more under the control of the investigator. Not infrequently an intestinal disorder would appear to excite a very great increase in the normal intestinal flora as shown by observations made before and after the onset of the illness. My observations have shown that pathological processes affecting the colon may not produce a greater or more varied bacterial flora than may occur among healthy subjects whose diet has been at fault.

Experimental observations on the influence of diet on the intestinal flora. Cannon has studied the subject very fully, but especially in relation to the effects of the aciduric group of bacteria which he describes as those that are able to live and multiply in degrees of acidity unfavourable for the development of most micro-organisms. These bacteria are widely distributed in nature, being found in the intestinal tracts of many animals, in milk, silage and other fermentable materials. The principal types found in the animal body are *B. acidophilus*, *B. bifidus* and *B. acidophil-aerogenes*. The term aciduric was applied by Kendall to those bacteria which are capable of withstanding considerable degrees of acidity. In infants, the strain *B. bifidus* is the predominant organism in the lower bowel. It is believed that as these organisms are acidogenic and slightly proteolytic they may serve to control the activity of the proteolytic bacteria which are regarded as the cause of the auto-intoxication arising in the intestinal tract.

These observations of Cannon's are based on the cultural characteristics of 64 strains of *B. acidophilus* and 34 strains of *B. acidophil-aerogenes*, isolated from 93 different sources, mainly sputum and faeces of normal human adults. A few strains have also been secured from the intestinal tracts of the white rat, rabbit, dog, quail and unpasteurised milk. Cannon employed acetic acid dextrose and unneutralised broth infusion for the isolation of these organisms, which were then plated on whey or dextrose yeast agar.

Hunter has directed attention to the value of sodium oleate added to casein media for the cultivation of *B. bulgaricus* and *B. acidophilus*, and he

considered that the best growth occurred with 1 per cent. of sodium oleate.

They are non-motile, Gram-positive rods, somewhat pleomorphic and varying in length from 1–11 μ with palisade grouping in smears from cultures.

B. acidophilus forms a smooth clot in milk with little or no whey formation; the clotting may occur in 48 hours, or take several days. Cannon states that *B. acidophilus* of Finkelstein does not clot milk, and *B. acidophil-aerogenes* grows poorly in milk, while other strains cause partial clotting at the end of 15 to 20 days. The results with 62 strains of *B. acidophilus* and 35 strains of *B. acidophil-aerogenes* tested with litmus milk at 37° C. were as follows:

	<i>B. acidophilus</i>	<i>B. acidophil-aerogenes</i>
No. of strains tested	62	35
No. clotting milk in 12 days	54	6
No. clotting milk in 35 days	56	13
No. not clotting milk in 35 days	6	22

Cannon found from his own observations that the original fermentative powers of some strains of *B. acidophilus* and *B. acidophil-aerogenes* had not altered to any appreciable extent by prolonged cultivation at the end of 9 to 12 months.

The members of this aciduric group are carbohydrophilic and develop feebly or not at all in the absence of a utilisable carbohydrate. Rahe, quoted by Cannon, divided *B. acidophilus* into 4 sub-types and *B. acidophil-aerogenes* into 6, according to the fermentation results in autolysed yeast carbohydrate broth, in which a reaction of pH 5 was necessary. They were divided chiefly into two groups, A and B, the former fermenting the monosaccharides and the latter the disaccharides. The majority of strains of *B. acidophilus* isolated from the intestinal tract belonged to group A, while the majority of the sputum organisms belonged to group B.

The predominant groups of intestinal bacteria are largely determined by the chemical nature of the food consumed—sufficient quantities of lactose or dextrin in the diet will lead to a diminution in the number of *B. coli* and an enormous increase in that of *B. acidophilus*. A diet rich in animal protein brings about an intestinal flora governed by proteolytic and putrefactive organisms. A carbohydrate diet, more particularly one containing definite amounts of lactose or dextrin, produces predominant bacteria which are fermentative or aciduric.

Herter and Kendall, in 1908, demonstrated the effects of diet on the bacteria of the intestinal tract. Kendall noted that in vitro proteolytic organisms are less active in the presence of certain carbohydrates, as fermentation occurs instead of putrefaction. Sittler showed that sucrose and laevulose are unfavourable for the development of *B. bifidus*, while *B. acidophilus* becomes the predominant organism in infants fed on malt soup.

Of the particular carbohydrates tested since that time, lactose and dextrin are considered to be by far the most effective in transforming the intestinal flora; the explanation being that these sugars are unchanged in the intestinal

tract until they reach the area of bacterial activity, when they are fermented. *B. bifidus* especially multiplies in lactose and dextrin.

Others have shown that starchy food led to a simplification of the intestinal flora and especially to the growth of *B. acidophilus*, while mammalian tissues encouraged the growth of putrefactive organisms in the intestines. Fish and casein produced much less deleterious effects, and fats exerted little action on the intestinal flora. Torrey has pointed out that vegetable proteins do not offer the slightest encouragement to the growth of the intestinal putrefactive bacteria.

Bassler and Lutz believe that: "*B. acidophilus* possesses an advantage over *B. bulgaricus* in that its administration by the mouth causes it to occur in large number in the faeces." "This takes approximately about 4 days, and it persists while the administration is kept up." In their opinion the value of lactose is due to its stimulating the growth of acidophilic bacilli (*B. acidophilus* and *B. bifidus*), which alter the reaction of the intestinal contents from alkaline to acid, but *B. acidophilus* accomplishes much more than Metchnikoff expected of *B. bulgaricus*.

Kopeloff and Cheney made certain observations on cases of chronic constipation among asylum patients.

Whole milk was used—1000 c.c. with 300 grams of lactose. Half this amount was administered between dinner and supper and the remainder between supper and bedtime. The milk was incubated at 35° C. for 24 hours. Bacterial counts of the faeces were made weekly by the microscope and occasional platings were made on whey agar. It was found that subjects receiving *acidophilus* milk and lactose obtained relief from constipation and diarrhoea, and in general there was a tendency to an increase in the relative proportion of Gram-positive organisms up to 70 per cent., but with *skimmed milk* this increase occurred to the greatest extent.

The usual daily doses contained from 100 to 200,000,000,000 of living bacilli in the 1000 c.c. The milk should not be incubated at room temperature for more than 3–4 days.

Relief from chronic constipation was obtained by treatment with 1 litre of *B. acidophilus* milk daily, generally reinforced by the addition of lactose. This treatment should be continued for one month. Another noteworthy point may be mentioned: an appreciable percentage of viable *B. acidophilus* organisms was recovered from some patients, months after the ingestion of these bacilli had been discontinued, indicating that a true implantation had taken place.

Chaplin and Rettger have shown that *B. bulgaricus* cannot be acclimatised to the intestinal tract of man and that the bacillus in the stools, which is called *B. bulgaricus*, is really *B. acidophilus*—stimulated by the lactose in the milk. The most effective method of simplifying the intestinal flora is by milk cultures of *B. acidophilus* which they have recommended for the treatment of constipation, diarrhoea, mucus colitis, and sprue.

Kopeloff has shown that relief from chronic constipation has persisted for six months after the ingestion of *B. acidophilus* milk. He recommends 1000 c.c. of milk and 50–100 grams of lactose two to three hours after meals. No special diet is ordered, and no cathartics or laxatives are given.

According to all information *B. acidophilus* does not form toxic or injurious by-products and is non-putrefactive. It is regarded as a suppressor of *B. coli*, *B. welchii*, and *B. proteus*.

The *acidophilus* milk treatment for various intestinal troubles is now carried out on a large scale, but I cannot help thinking that the enthusiasm of the advocates of this line of treatment is overdone and many of the patients could receive as much benefit from far simpler remedies. The effect of this treatment is relief of symptoms and to produce a normal soft, odourless stool, but it may take several weeks to produce this result.

In 1922 McIntosh, Warwick James, and Lazarus Barlow described an organism belonging to the *acidophilus* group under the name of *B. acidophilus odontolyticus* which they considered played a very important part in the aetiology of dental caries. They describe two types: (1) a long thin bacillus which may occur in chains, and (2) a shorter bacillus usually occurring in chains. They found this organism in most mouths quite apart from dental caries. These authors isolated *B. acidophilus* from various sources in broth of a reaction of pH 3.5. Fifty strains were tested as regards sugar reactions, and it was found that 98.1 per cent. fermented glucose, 81.5 per cent. lactose, 74 per cent. saccharose, but only 4.4 per cent. dulcitol. Nineteen strains which had been grown in the carbohydrate media were tested with anti-sera prepared from types 1 and 2 *odontolyticus*. As a result of their experiments they consider that members of the *acidophilus* group isolated from various sources have a moderately close relationship. They obtained the assistance of Dr Dodds for certain chemical observations concerned and he showed that the principal acid formed by these bacteria is malic and next in order lactic. The experiments of McIntosh, Warwick James, and Lazarus Barlow are, in my opinion, the most important yet made on the *B. acidophilus*. If dental caries is due to *B. acidophilus odontolyticus*, then as the *acidophilus* group are so closely related it would appear to be an unwise procedure to employ large quantities daily of *acidophilus* milk for the treatment of chronic constipation. This may be regarded as an extreme view, but, in my opinion, it is the wise course to adopt until the relationship of the various members of this group of organisms to dental caries is finally settled.

SUMMARY AND CONCLUSIONS.

(1) Each sample of faeces for these investigations was dried on unglazed porous tiles by the method introduced by Dudgeon. The dried faecal powder was added to the various solid and liquid media used for the aerobic and anaerobic examinations. By this method a large quantity of the faeces was examined, blood agar and boiled blood agar media were used as a routine

procedure, and it was unnecessary to employ media which hindered the growth of one organism at the expense of another.

(2) It is essential to control the intake of food during the bacteriological investigations of the faecal flora.

(3) Among healthy individuals, dietetic errors, excess of alcohol, and active purgation may temporarily alter the bacterial content of the faeces and increase the numbers of bacteria present, even to the same extent as among patients suffering from lesions of the intestinal tract.

(4) Septic infections of the mouth, throat, respiratory, and urinary tracts may give abnormal bacteriological findings in the faecal flora.

(5) Film preparations of the faeces should be made in every instance, but care must be exercised that the correct technique is employed.

(6) Experience has shown that several media are essential for the aerobic and anaerobic investigations of the faecal flora in each case.

(7) Spirochaetes were found in the faeces in 7 per cent. of the cases and usually in large numbers. There was no evidence to show that they had produced a pathological process in the bowel wall. Short coarse and long delicate spirochaetes occurred. Experience has shown that it is necessary to examine the mouth and respiratory tract in such cases for spirochaetal infection.

(8) **Streptococci.** One hundred and fifteen cultures of streptococci isolated from the faeces in 50 cases of various kinds were investigated. Eighty-two out of the 115 cultures proved to be enterococci. The morphology of this organism is its most striking characteristic. Twenty-one out of the 82 strains were haemolytic, of which 14 were thermostable and 13 acidified mannite. Many strains believed to be haemolytic streptococci were found on more detailed examination to be enterococci.

Nineteen strains of long-chained streptococci were isolated, out of which 6 were haemolytic, all were thermolabile, but only 1 acidified mannite. Fourteen strains of medium and short-chained streptococci were isolated. Five of these strains were haemolytic, 2 were thermolabile and 8 acidified mannite.

Haemolytic streptococci have been cultivated from the tonsils or sputum with the same cultural reactions and heat resistance as among those found in the patient's faeces, sometimes in large numbers.

Streptococci with similar cultural and haemolytic reactions and heat resistance have been cultivated from the faeces of normal individuals and from patients suffering from various infective processes.

(9) **Haemolytic colon bacilli.** These occurred in the faeces under varying conditions in 6 per cent. of the cases, and in most instances were found in abundance. In urinary infections the same haemolytic strains may occur in the faeces and urinary pus. Some cases of toxæmia due to the presence of haemolytic colon bacilli in the faeces in abundance, have improved with specific vaccine treatment to a degree beyond that met with in any other form of intestinal infection.

(10) The mucus capsulatus group occurred in 5.5 per cent. of the cases, usually in patients suffering from an abnormal condition of the intestinal tract.

(11) Slow lactose fermenters were cultivated in 2 per cent. of the cases. They have been found occasionally in the faeces of patients suffering from acute urinary fever due to the same bacillus.

(12) **Staphylococci.** The *Staphylococcus aureus* and *albus* were present in the faeces in this series in about 35 per cent. in adults. They occurred in large numbers in some cases of diarrhoea, but especially in patients suffering from pulmonary infections, and their presence in the faeces in typhoid fever may be of considerable importance. When staphylococci, especially *S. aureus*, occur in the faeces in large numbers, or persist as shown by multiple examinations, then it is advisable to examine the patient for an inflammatory focus.

(13) Diphtheria-like bacilli were isolated from the faeces in 6 per cent. of the cases. They were larger and coarser than true diphtheria bacilli, but gave a well-marked reaction with one of the modifications of Neisser's stain. They were non-pathogenic to guinea-pigs. Their presence in the faeces appears to be harmless, but they are of importance in children suffering from enlarged tonsils owing to errors of diagnosis which may arise in connection with true diphtheria bacilli.

(14) **Anaerobes.** *B. welchii* was cultivated from the faeces in 35 per cent. of the total cases. Robertson's heart muscle medium was the most effective for primary culture. Occasionally gangrenous infections due to *B. welchii* occurred. There was no positive evidence to show, however, that *B. welchii* produced acute gastro-enteritis, or acute colitis, but its relationship to certain infections and toxæmias is discussed.

(15) Three types of intestinal flora are referred to: (1) fermentative or saccharolytic, (2) putrefactive or proteolytic, (3) facultative or normal. The importance of diet in all bacteriological investigations of the faeces is established, while an abnormal faecal flora may be rendered normal by a simple diet. The value of lactose, dextrin, and milk for the treatment of intestinal disorders is fully discussed.

(16) Intestinal antiseptics. My observations with so-called intestinal antiseptics has led me to believe that on the whole they are devoid of bactericidal action on the intestinal flora of man. Dimol and colloidal kaolin have been proved to be exceptions in some instances.

(17) The pathology of intestinal toxæmia based on the work of the American investigators by means of closed intestinal loops is considered.

(18) The characteristics of the *acidophilus* group of organisms is referred to, including their value in controlling the intestinal flora of man, and for the treatment of intestinal disorders by means of *acidophilus* milk.

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