THE REGULATION OF THE INTESTINAL FLORA OF DOGS THROUGH DIET.*

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It is now well known that diet exercises a profound influence on the determination of the types of bacteria developing in the intestinal tract. In fact, under conditions of normal physiological functioning within the digestive tube, it is the fundamental factor. The work of Herter and Kendall[1] was the first to establish clearly this fact, although it was suggested by the earlier publications of Escherich,[2] Hirschler,[3] Macfayden, Nencki and Sieber,[4] and Lembke.[5] Herter and Kendall were the first, however, to definitely correlate specific types of intestinal flora with the chemical composition of the ingested food. These facts were established, for the most part, through feeding experiments with monkeys and cats. As a high carbohydrate diet, they fed cow's milk fortified with lactose, and as a result the feces within three or four days changed in appearance and the bacterial types came to resemble very closely those of the normal human nursling in which *B. bifidus* and *B. acidophilus* are typically dominant. On the other hand, following the prolonged feeding of a protein diet, containing no carbohydrate (in the form of eggs to monkeys, and meat to cats) large numbers of proteolytic bacteria were present in the feces and entirely supplanted and suppressed the purely fermentative types. Putrefactive products, such as indol, phenols, hydrogen sulphide and ammonia were also strongly in evidence in the fecal discharges.

Hull and Rettger[6] next demonstrated that not all carbohydrates exercise an equal transforming effect, but, according to their findings in feeding experiments with white rats,

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lactose, whether as present in milk or as added to a meat diet, alone of the sugars caused the development of a purely fermentative flora dominated by *B. acidophilus* and *B. bifidus*. In a brief paper by Distaso and Schiller,[7] published almost simultaneously, some feeding experiments with white rats were reported which indicated that, of various carbohydrates tested, lactose and dextrin alone exercised a marked transforming power on the intestinal flora, bringing about a predominance of *B. bifidus*. The addition of sucrose, dextrose or levulose to the bread diet had no such effect. Apparently the effect of the carbohydrates on the development of the *B. acidophilus* was not studied. In an earlier publication Sittler[8] called attention to the association in infants of certain intestinal fermentative bacteria with different dietaries. He attributed the predominance of *B. bifidus* in the fecal flora of breast-fed infants to the lactose in human milk, basing this conclusion on the observation that the addition of lactose to cow's milk, fed to infants, would cause the establishment of a *B. bifidus* flora, whereas the substitution of sucrose for lactose effected no such transformation. He explained the difference on the ground that in the hydrolysis of lactose dextrose is formed but no levulose, whereas the levulose produced in the inversion of sucrose is unfavorable for *B. bifidus*. He also noted large numbers of *B. acidophilus* in the stools of infants fed with malt soup. The writer,[9] in 1915, reported the results of an investigation, carried out through the courtesy of Dr. Warren Coleman, of the effect of various high-calory diets on the fecal flora of typhoid fever patients. It was shown that with some cases, if lactose was added in amounts of two hundred and fifty to three hundred grams a day to the other elements of the Coleman-Shaffer diet, there resulted a transformation of the fecal flora from the ordinary type to one strongly dominated by *B. acidophilus*. *B. bifidus* was occasionally encountered, but it is not likely to occur in large numbers in the stools of human adults except under conditions of intestinal abnormality.

The study reported here, carried on at intervals during the past four years, is an attempt to demonstrate experimentally the transforming influence of various food materials on the intestinal flora. The work has been essentially qualitative, viz., an effort to determine what sort of carbohydrate, protein, or fat exercises the transforming influence. Certain cultural methods, not employed heretofore in studies of this character, have been utilized, and it is believed that a much more comprehensive insight into bacterial conditions in the intestinal tract under various dietary conditions has been attained.

Dogs have been used in this investigation almost exclusively as experimental subjects. These animals present certain advantages for a study of this kind but have been used but seldom by other workers for this purpose. The intestinal flora of dogs more closely resembles that of human adults than does that of white rats or monkeys. The principal difference between the fecal flora of the normal human adult and that of the dog is that in the latter anaërobic spore-bearing bacteria of the *B. welchii* group are more prominent. Dogs, of course, are naturally carnivorous, but through domestication have become quite as omnivorous as is man. In fact, they may be induced to eat a great variety of diets or a monotonous diet of one or two food elements for a long period of time. Generally they eat the food cleanly and quickly without scattering it about. The stools are ample in amount, generally moist, and may easily be obtained fresh when wanted. In these respects they offer advantages as experimental subjects for diet experiments of this type over other common laboratory animals. Cats and monkeys are fastidious in their tastes, hard to manage, and, especially as regards monkeys, the fecal flora does not resemble that of human beings so closely as does that of dogs. Herter and Kendall[1] have noted in the fecal flora of monkeys an absence of *B. welchii* types and only small numbers of streptococci, whereas subtiloid types are present in abundance. Rat feces are apt to be dry, and are very compact. In the intestinal tracts of these animals, also, peculiar types of bacteria develop which never appear prominently in human fecal matter.

It has been my experience that the intestinal flora of dogs reacts very promptly and with great uniformity to changes in diet. This conclusion is diametrically opposed to that reached by Sisson[10] in similar experiments with puppies. He reported that a milk diet containing ten to fifteen per cent of lactose or of sucrose did not produce an intestinal flora essentially different from that following a diet of whole pasteurized milk; nor did a high protein milk diet produce any marked change. In fact, he failed to find any acidophilic organisms at any levels when using a very high carbohydrate diet. He admits, however, that the methods used may not have been suitable to bring to development bacteria of this type. It seems to the writer that this was probably the case, as the three per cent acid broth used was much too acid to encourage the growth of these bacilli, especially if it contained no utilizable carbohydrate. In fact, the dog flora seems more readily modified through diet than does the human intestinal flora. This may be due to the fact that the large intestine in dogs is relatively very short. Dietary conditions influence first bacterial types in the small intestine, and later, and in less degree, the flora of the large intestine. With a short large intestine, as in dogs, through which there occurs a rapid passage of the intestinal contents, one would expect to observe a more clearly marked bacterial response to food stimuli, as revealed in an examination of the stools, than would be the case with a comparatively long large intestine, as in human beings, favoring more or less stasis of the intestinal content.

Only about a dozen animals were used in these experiments, but between four hundred and five hundred fecal specimens were examined. Early in the work it was observed that changes in the intestinal flora were more readily effected in some individuals than in others, as was also noted by Hull and Rettger in their rat experiments. On the same balanced diet, some dogs would show a tendency to putrefactive conditions, whereas with others the fermentative bacteria would remain dominant. Accordingly, in order that the results obtained with different food elements might have a comparative value, it was found advisable to carry out a series of experiments with single animals, rather than to use a large number only once or twice.

The more immediate purpose of these experiments has been the determination of the comparative transforming influence of various sugars and starches, ordinarily present in foods, on the bacteria within the intestinal tract; the same data as regards animal and vegetable proteins; and the influence of fat in the diet on the number and types of bacteria.

Methods.

Feeding of dogs and collection of specimens. — The animals were given definite weighed amounts of single articles of diet or mixtures. The amount fed was generally sufficient to keep them in weight, and was so prepared that it was generally eaten up quickly and without waste. Any food unconsumed was weighed and subtracted from the total amount fed. The animals were given one meal a day, generally in the afternoon. They were kept in single cages with metal bottoms which could easily be cleaned. No sawdust or similar substance was spread on the bottom of the cages. The stools were, for the most part, natural movements and were collected the first thing in the morning. If at all dry, fresh ones were obtained through the medium of glycerine suppositories. The majority of the animals were quite young. It was not found, however, that age was an important factor in the readiness with which the fecal flora could be transformed by diet. Kendall,[11] on the other hand, reported better results when young animals were used.

Five hundred milligrams of the fecal matter were weighed and emulsified in fifty cubic centimeters of normal saline solution. Accordingly, each cubic centimeter held the bacteria present in ten milligrams of the fecal matter. This was labeled " Dilution 1," and from this a series of dilutions were prepared in multiples of ten up to 1–10,000, numbered respectively 2, 3, 4 and 5. As the stool specimens varied so much in their water content, this weighing of definite amounts of the fecal matter is of doubtful value. The important point to be determined is the relative numbers of the various bacterial types, and in the tables the count for each type of bacteria is not given, but merely its percentage of the total count. This total count included *B. coli*, streptococci, *B. acidophilus* and spore-bearing bacteria.

The selection of the best cultural methods has been a matter of difficulty, but one of great importance. The aim has been to employ procedures and media which would best give a general insight into the numbers and biological activities of the important groups of bacteria capable of establishing themselves and pullulating in some part of the intestinal tract. In that way it is possible to correlate particular types of fecal flora with specific dietary conditions. The methods used by the writer in the study of the fecal flora in typhoid fever were in part discarded and replaced by others, which enables one to gain a clearer view of the relative predominance and activity of both the fermentative and putrefactive bacteria, including aërobic and anaërobic forms.

The following media were used, and in all instances incubation was carried out at 37° C.

A. Plate cultures:

1. Endo plates (with either lactose or sucrose). — These plates give an insight into the relative numbers of aërobic bacteria which develop rather characteristically on this medium, such as those of the colon and intermediate group, *B. proteus*, *B. mesentericus*, *B. pyocyaneus* and streptococci.

2. Sugar-free nutrient agar, neutral to phenolphthalein. — Poured plates were made with this medium after seeding with 1 cubic centimeter of a suitable dilution of the fecal matter. This medium was chosen because *B. coli*, *B. proteus* and streptococci develop freely and quite characteristically in it, but *B. acidophilus* is almost entirely suppressed.

3. Liver glucose agar, +4. to phenolphthalein. — This solid plate medium is emphatically the one of choice as a differential one for the aciduric bacteria. The advantages in the use of this medium for quantitative determination of *B. acidophilus* in fecal specimens have been described by the writer in a recent article.[12] In brief, it greatly favors the development of these bacilli, and the colonies are characteristic. On the other hand, streptococci are entirely suppressed and *B. coli* largely so, except acid-tolerant strains which become, at times, surprisingly abundant. The colonies, however, are generally easily distinguished from those of *B. acidophilus*.

4. Liver glucose blood agar, +1. to phenolphthalein. — In the article by the writer, referred to above, the preparation and use of this medium for the isolation of *B. bifidus* was described. The superiority of this medium lies in the fact that, under suitable anaërobic conditions, *B. bifidus* not only develops luxuriantly, but the colonies are distinctive in appearance after forty-eight hours' incubation at 37° C. Several other types of bacteria, which find Endo medium unfavorable, are brought to development following surface seeding on this medium.

5. Anaërobic plates for spore-bearing bacteria. — Poured anaërobic plates were prepared with liver glucose agar +1. medium to determine the numbers of *B. welchii* and related bacilli present in the spore stage in the fecal specimen. A suitable dilution of the fecal emulsion was heated at 80° C. for ten minutes and poured plates in deep Petri dishes were prepared with this medium. After the seeded liver agar had hardened, it was covered with a layer of sterile plain agar and further anaërobic conditions were obtained through the method of Zinnser.[13] *B. welchii* types, which are frequently so prevalent in canine feces, especially when on a meat diet, develop very satisfactorily and speedily under these cultural conditions.

It was not considered worth while to determine the numbers of aërobic spores, as bacteria of that type occur merely fortuitously in the intestinal tract, and are not of any significance from a biological standpoint.

B. In addition to the above plate cultures, the following fluid media were seeded with appropriate dilutions of the fecal suspension.

6. Peptone lead acetate broth.[14] — This lead acetate broth of Pake has been found useful for an estimation of the H_2S-producing properties of mixtures of fecal bacteria. The larger the amount of H_2S produced by bacteria growing in this medium the blacker the precipitate becomes. As applied to this work, a series of tubes of this medium were seeded with graded dilutions of the fecal specimen. If the specimen contained an unusual number of bacteria capable of forming H_2S from peptone the tube seeded with dilution 5 (1–100,000) would show blackening after three days' incubation. These especially active hydrogen sulphide-producing bacteria were members of *B. proteus* group, almost exclusively. Other less active formers of hydrogen sulphide were certain strains of the colon group, either communis or communior, and more rarely *B. lactis aërogenes.*

7. Fermentation tubes. — Two of these were employed in each examination, one containing glucose broth +1. to phenolphthalein and the other neutral sugar-free broth. A previous article[9] contained a discussion of the information to be gained through the use of sugar broths in fermentation tubes in studies of this character. Recent experience has also shown that implantations in neutral sugar-free broth in fermentation tubes will yield much information and bring to development types of bacteria, especially putrefactive anaërobic forms, which do not multiply in any of the other media employed. An examination of the sediments in these tubes, after three or four days' incubation, enables one to form an estimate of the putrefactive propensities of the fecal flora. With a putrefactive flora the sediment will generally reveal an abundance of free spores and spore-bearing bacteria, generally of the *B. welchii* type, but also occasionally those of the *B. putrificus* type. Also there may be seen many short and long slender gram-negative bacilli and curved threads, together with gram-positive and gram-negative single cocci and diplococci, often very minute and collected in bunches. Few typical *B. coli*-like rods will be observed. Streptococci, in chain or diplococcus form, may or may not be numerous. If, on the other hand, the fecal flora is purely fermentative in type, one finds many typical *B. coli*-like organisms in the sediment, also many *B. acidophilus*-like and many gram-positive diplococci, but few or no spore-bearing bacteria. These distinctions have also been found to be valid, in the main, for human stool specimens.

The dextrose broth fermentation tube was found of chief value in bringing *B. bifidus* to development, and especially the bifid form. The sediment, also, in a way, indicates the relative dominance of different types of fermentative bacteria. The amount of gas formation in itself, however, has little significance. The same abnormality in the amount of gas production may follow seeding the glucose fermentation tube with a markedly fermentative combination of fecal bacteria as that resulting from the growth of a mixture of certain bacteria with frankly putrefactive tendencies.

8. Acetic acid glucose broth. — The use of glucose broth containing three strengths of acetic acid, viz., N/20, N/10, N/5, yields information in regard to the aciduric properties of the strains of *B. acidophilus* which develop in response to the stimulus of various carbohydrate diets.

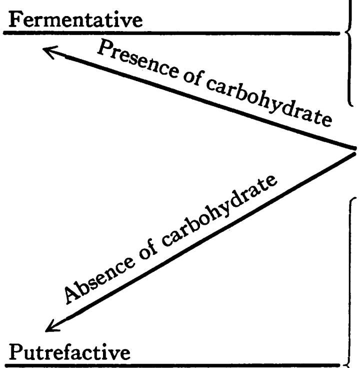
Microscopic examination of a gram-stained film made directly from the fecal emulsion will give much information to an observer who is familiar with the morphology of fecal bacteria which are essentially fermentative, on the one hand, and putrefactive on the other. Such an examination is of special value in studies of the intestinal bacteria of man. In these experiments, however, the cultural results were so well defined and definite that little additional information was gained by examinations of the fecal films, except as regarded the relative predominance of *B. bifidus*.

Factors determining the development of fecal floras of various types. — The factors of weight in determining the development and continued predominance of bacterial types within the intestinal tract are,— (1) the chemical character of the diet, particularly the specific availability of the decomposition products of various food materials for the metabolism of different types of intestinal bacteria, (2) the completeness of the digestion and absorption of food within the small intestine, (3) the rapidity and degree to which the food residue is eliminated, (4) symbiosis and antagonism between bacterial groups. With man, factors (2) and (3) are of fundamental importance, and unfavorable bacterial conditions within the intestinal tract resulting fron abnormalities in these factors cannot always be satisfactorily corrected by dietary changes. With animals, such as dogs, however, digestion and elimination is so vigorous that almost any type of fecal flora may be established by merely changing the diet.

Fecal bacteria in general. — The types of bacteria which find conditions favorable for development at some level of the intestinal tract may be divided conveniently into those which are obligatively or characteristically fermentative and those in which the tendency of the bacterial metabolism is strongly putrefactive, or which, although functionally fermentative, are found generally in greatest numbers in association with a putrefactive flora, as is the case with *B. welchii*. Intermediate between these two groups, we have the very important *B. coli* group which may participate either in fermentative or putrefactive activities, depending upon the nature of the food residue. A grouping on this basis is illustrated in Table I.

TABLE I.

Grouping of intestinal bacteria according to their metabolic tendencies.



Fermentative:
- *B. acidophilus*
- *B. bifidus*
- Streptococci (Enterococci)
- *B. welchii*

B. coli group

Putrefactive:
- *B. sporogenes* (Klein)
- *B. maligni œdmatis* (Koch)
- *B. putrificus*
- *B. mesentericus*
- *B. proteus*
- *B. pyocyaneus*
- *B. fluorescens*
- Staphylococci

A purely fermentative flora, developed as the result of feeding a diet in which certain carbohydrates predominate, is simple in type and embraces only a few varieties of bacteria. In adults it is dominated by the *B. acidophilus*, and in breast-fed infants by *B. bifidus*. In a fermentative flora of a far more common type among adults, *B. coli* is predominant. On the other hand, a markedly putrefactive flora is complex and contains many varieties of bacteria. In the following sections the tendency of various carbohydrates, on the one hand, to bring into existence a simplified fermentative flora, and, on the other hand, the tendency of diets containing a considerable percentage of various proteins to encourage the development of a complex putrefactive flora is demonstrated.

Balanced diets. — In order that the deviation from what may be termed the normal association of intestinal bacterial types in dogs may be appreciated, the results following the feeding of a fairly well balanced ratio of carbohydrate, fat and protein, are reported first. The figures given for the various elements in the diets in all of the following tables were not obtained through analyses of each lot of food, but were taken from standard tables,[15] and are sufficiently accurate for the purpose. The figures given for fat are probably the least accurate, but, as will be shown presently, the amount of this food element in ordinary amount plays a very slight part in the determination of the types and numbers of bacteria which develop. This table is also presented first so that the individuality factor among dogs may be appreciated. Whether because of varying rate or degree of digestion, absorption and elimination of the food ingested, individual dogs exhibit certain idiosyncrasies in the nature of the response of their fecal flora to various diet stimuli. The general nature of the reaction is the same in each animal if the stimulus is strong enough, but the pullulation of certain intestinal bacterial types is more readily effected through diet in the case of some dogs than in that of others. These individual peculiarities have also been noted by Hull and Rettger for white rats and by the writer for man. Accordingly, in making a comparative study of, for instance, the transforming influence of certain sugars on the bacterial flora, it is desirable to carry out a series of experiments with single individuals. When the individual peculiarities have been gauged, the response of the intestinal bacteria to various food stimuli may be anticipated with precision.

This table brings out the fact that, although in the diet of rice and meat there was a larger amount of carbohydrate than in the one consisting of bread and meat, yet the former tended to establish a preponderance of *B. coli*, whereas the latter encouraged an overgrowth of *B. acidophilus* and, in some measure, of *B. bifidus*. As will presently be shown, the *B. acidophilus*-stimulating properties of the diet containing bread is due to its dextrin content. Of these two diets the one consisting of rice and meat is much the better suited as a basic one for testing the transforming influence of various sugars, as it permits the development of a moderate degree of putrefaction, a high percentage of *B. coli*, and a low one of *B. acidophilus*. The bacterial distribution, in fact, is very

TABLE II.

Relative numbers and activities of various types of bacteria appearing in fecal specimens from dogs on a "normal, balanced" diet.

Dog	1.	7.	9.	11.	1.	3.
Period of feeding (in days)	14	14	14	14	11	11
Diet (in grams)	Rice (boiled), 500. Beef heart, 100.	Same.	Same.	Same.	Bread, 200. Beef heart, 100.	Same.
Grams: C-H Fat Protein	122.0 20.5 30.0	Same.	Same.	Same.	103.0 34.8 39.2	Same.
B. coli	91	51	36	79	3	8
B. acidophilus	2	15	14	12	88	38
Streptococcus	4	33	48	4	10	38
Anaërobic spores (*B. welchii* mostly)	3	1	2	14	7	16
B. bifidus	−	−	−	−	+	+
H_2S-producing bacteria	+++	+++	+++	+	++	++
Degree of putrefaction (sugar-free broth sediment)	++	−	++	++	+	−
Gas production (glucose broth)	55	18	20	45	35	35
Acetic acid, N/20 Glucose broth, N/10	++ −	+++ −	+ −	+ −	++ −	++ −
Gram-stained film from fecal suspension.	Gram-negative, fermentative.	Gram-negative, fermentative.	Moderately putrefactive.	Gram-negative, moderately putrefactive.	Gram-positive, fermentative.	Gram-positive, fermentative.

similar to what one frequently encounters in an examination of fecal specimens from human adults on an ordinary fairly well balanced, meat-containing diet. This combination of rice and meat is, in fact, so unfavorable for the multiplication of *B. acidophilus* that, if it is continued for some time, these aciduric bacteria tend to die out entirely. This is shown in the following table (III.), in which are summarized the bacterial findings at various levels of the intestinal tract of a dog fed with a rice and meat diet for seven weeks.

In obtaining these specimens, the animal, after a fast of eighteen to twenty-four hours, was chloroformed and, when at the point of death, various sections of the intestinal tract were tied off, beginning with the pylorus, then the end of the duodenum, about the middle of the small intestine, eight inches above the ileo-cæcal valve, the cæcum, and the end of the rectum. Emulsions of the intestinal content from these various sites were prepared in normal saline solution, making them all, as far as possible, to the same density, in order that an estimate might be made through cultural methods of the relative numbers and types of bacteria at the various levels.

Above the upper limit of the ileum very few bacteria were found either through cultural procedures or by direct microscopic examination of films. The *B. welchii* and a few gram-positive diplococci were the only organisms encountered. The *B. welchii* dominated the situation, as was shown by its overgrowth in the glucose broth fermentation tubes with the production of one hundred per cent of gas. The *B. coli* became in evidence first in the ileum, and here also were enormous numbers of *B. welchii*. In the large intestine a greater number of types of bacteria were encountered, with *B. coli* dominant and a partial suppression of *B. welchii*. The *B. acidophilus* was detected alone in the colon-rectum and only in small numbers. It is of interest and importance to note that putrefaction, as judged by the types and biological activities of the bacteria, was much more marked in the ileum than in the large intestine. It seems not improbable that this may at times be the case with human sufferers from auto-intoxication of intestinal origin, and may explain the fact that colonic irrigations do not always afford relief.

TABLE III.

Distribution of various types of bacteria in the intestinal tract of a dog on a diet consisting of boiled rice, 500 grams, and beef heart, 100 grams. Period of feeding, seven weeks.

	Duodenum.	Jejunum.	Ileum.	Cæcum.	Colon, Rectum.
B. coli	O	O	Numerous.	V. numerous.	V. numerous.
B. acidophilus	O	O	O	O	Few.
Streptococcus	Few.	Few.	Few.	Few.	Numerous.
B. welchii	Few.	Few.	V. numerous.	Numerous.	Few.
B. bifidus	O	O	O	O	O
H_2S-producing bacteria	O	+	++	+++	+++
Degree of putrefaction (sugar-free broth sediment)	–	–	++	–	–
Gas production (glucose broth)	100 *B. welchii.*	100 *B. welchii.*	70	35	40
Acetic acid Glucose broth } N/10	–	–	–	–	+
Gram-stained film from fecal suspension.	A few *B. welchii*-like.	Like duodenum.	*B. welchii*-like. Outnumber other types.	G– rods predominate. G+ diplococci.	Bacteria very numerous. G– types strongly predominate.

Transforming effect of various sugars. — In the writer's experiments with dogs, the various sugars tested were added at first to a diet of meat and bread. As bread, however, contains more or less dextrinized starch, the basal diet was changed to one of rice and meat so balanced as to favor neither the overgrowth of fermentative nor putrefactive bacteria. In each instance the sugar tested was dissolved in a small amount of water and thoroughly mixed with the food. Hull and Rettger,[16] in a similar test of sugars, with rats as subjects, added them directly to meat, but such a procedure is not practicable with dogs. As a preliminary measure, however, in order to create as uniform experimental conditions as possible, each animal was first fed meat alone for eight to fourteen days, then boiled rice and meat for eight days, and finally one of the sugars was added to the rice and meat diet in the amount indicated, generally fifty grams of sugar to each one hundred grams of meat. Fecal specimens were examined on the day the sugar was added to the diet. These control results are given for several dogs in Table II., and in estimating the change effected by the various sugars comparison should be made with the bacterial conditions established through the rice and meat or the bread and meat diet alone.

As is clearly indicated in Table IV., my results are more nearly in accord with those reported by Distaso and Schiller than with those of Hull and Rettger, in that both lactose and dextrin were found to effect a radical transformation in the fecal flora, and not lactose alone. This change consisted not alone of the marked increase in the numbers of *B. bifidus* as reported by Distaso and Schiller but more especially of a striking overgrowth of *B. acidophilus*. The change in the predominance of bacterial types was clearly evident after two days of feeding, but reached its maximum on the fourth to the eighth day and persisted to a marked degree to at least the twentieth day. This transformation, in the main, was characterized by the enormous relative and actual increase in the numbers of the *B. acidophilus* appearing in the stools, the complete suppression of *B. welchii* and other bacterial

TABLE IV.

Results with dogs fed diets containing various sugars.

Dog	9.	9.	9.	1.	1.	7.	11.	11.	3.	3.	3.	1.
Period of feeding (in days)	21	8	9	21	12	8	4	21	8	4	3	8
Diet (in grams)	Rice (boiled), 500. Beef heart, 100. Dextrose, 50.	Rice (boiled), 500. Beef heart, 100. Saccharose, 50.	Rice (boiled), Beef heart, 1 Dextrin (white	Rice (boiled), 500. Beef heart, 100. Dextrin (yellow), 50.	Bread, 200. Beef heart, 100. Maltose, 30.	Rice (boiled), 500. Beef heart, 100. Saccharose, 50.	Rice (boiled), 500. Beef heart, 100. Lactose, 50.	Same.	Bread, 200. Meat, 100. Lactose, 30.	Bread, 200. Meat, 100. Lactose, 50.	Bread, 200. Meat, 100. Lactose, 100.	Bread, 200. Meat, 100. Saccharose, 50.
Grams: C–H	172.0	172.0	172.0	172.0	133.0	172.0	172.0	Same.	133.0	153.0	203.0	153.0
Grams: Fat	20.5	20.5	20.5	20.5	34.8	20.5	20.5		34.8	34.8	34.8	34.8
Grams: Protein	30.0	30.0	30.0	30.0	39.2	30.0	30.0		39.2	39.2	39.2	39.2
B. coli	70	39	.3	3	9	1.3	.01	1.6	52	14	11	53
B. acidophilus	19	9	96.0	97	30	98.5	99.98	97.5	48	74	88	19
Streptococcus	0	13	3.7	0	60	0.2	.01	.8	0	12	1	20
Anaërobic spores (*B. welchii*)	11	39	0.0	0	1	0.0	.00	.1	0	0	0	8
B. bifidus	+++	++++	–	+	++	+	+	++++	++	++	?	+
H_2S-producing bacteria	++	+++	+	+	+++	+++	–	++	++	++	+++	+++
Degree of putrefaction (sugar-free broth sediment)	+	++	–	–	–	+	–	–	–	–	–	–
Gas production (glucose broth)	80	70	5	47	42	15	0	45	38	18	12	50
Acetic acid, N/20	++	++	++	+++	+++	++	++	++	++	++	++	++
Glucose broth, N/10	–	++	++	–	++	++	+	+	1	+	++	+
Gram-stained film from fecal suspension.	G– bacteria predominant. *B. coli*-like very numerous.		G+ bacteria dominant. Ba not numerous.	lmost entirely *B. cidophilus*-like.	G+ bacteria predominant. *B. bifidus* numerous.	G+ and G– about equal.	G+ strongly predominant. Bacteria not numerous.	*B. bifidus* outnumber *B. acidophilus*-like.	G+ strongly predominant. Bacteria not numerous.	As last.		G+ rather predominant. Bacteria numerous.

types associated with putrefaction, the almost complete elimination of *B. coli* and other gas-producers, of streptococci, of hydrogen suphide-forming bacteria; in brief, the establishment of a purely fermentative non-gas-producing intestinal flora. The addition of either of these two carbohydrates to the diet had an especially disastrous effect upon *B. welchii*, which is clearly apparent within two days by their gram-negative staining, vacuolated appearance, and inability to form spores.

Kendall[17] has suggested that the feeding of liberal amounts of lactose to infants may cause an abnormal development of *B. welchii* within the intestinal tract due to the occasional presence of spores in this sugar. It has been my experience, that, although the *B. welchii* finds very congenial conditions within the intestines of dogs, the feeding of several different samples of lactose has never caused an increase in their number, but always an almost complete elimination. Dextrin as a transforming agent has been found certainly as effective as lactose, and perhaps rather more so. The final results with either lactose or dextrin was, in general, the same, viz., the dominance of the fecal flora by *B. acidophilus*. With dextrin in every dog tested, this dominance was complete and other types of fecal bacteria were strongly suppressed, whereas with lactose in the case of some dogs (1 and 3) the supremacy of *B. acidophilus* was not so complete as when dextrin was fed. Several samples of commercial dextrin were tested, and the same result was attained whether a yellow or a white variety was used. As regards the minimum amount of lactose or dextrin necessary to effect this change, no very definite data were obtained. It appeared, however, that twenty-five or thirty grams of lactose or dextrin a day caused a marked increase of *B. acidophilus*, but the maximum change followed feeding fifty grams each day. Hull and Rettger[16] have also observed in their experiments with white rats that "the amounts of lactose required to bring about a complete transformation of the flora varied with the different animals, but in general one to two grams of lactose per day were sufficient to bring about a simplification, although complete transformation required amounts somewhat in excess of two grams."

In this connection it may be noted that Hull and Rettger reported that the feeding of dextrin did not cause any noticeable multiplication and overgrowth of the *B. acidophilus* and associated types. As regards this point their results are in complete disagreement with mine, although it should be borne in mind that different species of animals were employed in the feeding experiments.

The following tabulation (V.) shows the bacterial types found to be present at various levels of the intestinal tract following the ingestion of the lactose-rice-meat diet for six days. It will be observed that the *B. acidophilus* was multiplying throughout, even as high as the duodenum. In fact they were the only bacteria found in the small intestine above the ileum with the exception of a few streptococci and *B. coli*. In the jejunum there were a number of gram-negative capsulated bacilli, probably belonging in the *B. lactis* aërogenes group. Films from material in the ileum showed few bacteria, certainly no more than in the jejunum. The *B. coli* and streptococci were found here and in larger numbers than the *B. acidophilus*. The *B. welchii* was not encountered at any level of the small intestine, nor was the *B. bifidus* with the exception of a few in the duodenum. Beyond the ileo-cæcal valve from the cæcum to the anus, *B. acidophilus* was strongly predominant. In fact, the only other organism encountered in the rectum was the *B. bifidus* which was also present in considerable numbers throughout the large intestine. Larger numbers of viable *B. coli* were found in the colon than at any other level. Bacteria producing hydrogen sulphide were represented throughout the intestinal tract, but were rather more active in the ileum than elsewhere. In another animal, fed for thirty days on this meat-rice-lactose diet, the only locality in which there was some evidence of putrefaction was the ileum. Here, not only the bacteria cultured gave evidence of putrefactive activities, but the contents was distinctly alkaline to litmus, whereas at other points it was practically neutral to litmus or slightly acid as in the large intestine. These findings emphasize the more or less appreciated fact that generalizations in regard to the bacterial conditions in

TABLE V.

Distribution of bacteria in the intestinal tract of a dog on a lactose diet (boiled rice, 500; beef heart, 100; lactose 50 grams).

	Duodenum.	Jejunum.	Ileum.	Cæcum.	Colon.	Rectum.
B. coli	O	Few.	Numerous.	Numerous.	Numerous.	Few.
B. acidophilus	Numerous.	V. numerous.	Numerous.	V. numerous.	V. numerous.	V. numerous.
Streptococcus	Few.	Few.	Numerous.	O	Few.	Few.
B. welchii	O	O	O	O	O	O
B. bifidus	Few.	O	O	Numerous.	Numerous.	Numerous.
H_2S-producing bacteria	++	+++	+++	++	++	+
Degree of putrefaction (sugar-free broth sediment)	—	—	—	—	—	—
Gas production (glucose broth)	40	17	40	28	50	O
Acetic acid / Glucose broth } N/20	+++	+++	+	+	+	+
Gram-stained film from intestinal material suspension.	Only gram-positive rods.	G+ and G− bacteria. Capsulated G− rods. Single G+ cocci.	Like jejunum.	Strongly G+. *B. acidophilus* and *B. bifidus*.	Like cæcum, also yeasts.	Like colon, also G+ single cocci.

the upper levels of the intestinal tract, based on examinations of fecal specimens, should be made with caution. Sisson,[10] however, in the light of similar experiments with puppies, came to the conclusion that the flora was essentially the same on any intestinal level without regard to the character of the diet employed. This may be largely true as far as the results with a high carbohydrate diet are concerned, but with an ordinary mixed diet or one containing much meat protein the writer's experiments show that the flora at various levels differ markedly.

Of other sugars tested, including saccharose, maltose and dextrose, none were entirely without transforming effect upon the intestinal flora, but the only one which approached lactose and dextrin at times in effectiveness as a simplifying agent was saccharose. With most dogs, especially those exhibiting a tendency to putrefactive conditions, the addition of fifty grams of saccharose to the meat and rice diet had no appreciable effect on the intestinal flora (compare Dog 9, TablesII. and IV.) except to cause a marked increase in the numbers of *B. bifidus*, whereas in the case of one animal with a tendency to the development of a fermentative flora (Dog 7, Tables II. and IV.) the saccharose brought about as complete an overgrowth of *B. acidophilus* as did lactose and dextrin, and one which persisted for at least thirty days. Another dog of this type was given a diet containing fifty grams of saccharose for a month (Table VI.), and then cultures were made from the various levels of the intestine. A comparison of these findings with those obtained from the lactose-fed animal (Table V.) shows marked differences. On the lactose diet marked simplification of the intestinal flora was effected throughout the small and large intestine, with a strong predominance of the *B. acidophilus* and a total suppression of obligate putrefactive bacteria. This was especially evident in the specimen from the rectum where even *B. coli* was suppressed through the complete overgrowth of aciduric bacteria. With the saccharose diet, however, the development of *B. acidophilus* was almost entirely confined to the large intestine. Although in this particular animal (Table VI.)

TABLE VI.

Distribution of bacteria in the intestinal tract of a dog on a saccharose diet (boiled rice, 500; beef heart, 100; saccharose, 50 grams).

	Duodenum.	Jejunum.	Ileum.	Colon.	Rectum.
B. coli	O	O (?)	Numerous.	V. numerous.	V. numerous.
B. acidophilus	O	Few.	Few.	V. numerous.	V. numerous.
Streptococcus	O	Numerous.	Numerous.	V. numerous.	V. numerous.
B. welchii	Few.	Few.	Few.	V. numerous.	Numerous.
B. bifidus	O	O	O	Few.	Few.
H_2S- producing bacteria	+ *B. proteus.*	++ *B. proteus.*	+++ *B. proteus.*	+++ *B. proteus.*	+++ *B. proteus.*
Degree of putrefaction (sugar-free broth sediment)	–	–	–	+	++
Gas production (glucose broth)	30	65	35	90	70
Acetic acid, N/20	–	+++	+	+++	+++
Glucose broth, N/10	–	–	–	–	+++
Gram-stained film from intestinal material suspension.	Very few bacteria. No G– bacilli. No *B. welchii*-like.	Similar to duodenum.	Very few bacteria. Some G– rods and some streptococci in short chains.	Bacteria very numerous. *B. welchii*-like and *B. coli*-like in equal numbers. G+ diplococci numerous.	More types of bacteria than in colon. Many spores. Some *B. welchii*-like, others *B. putrificus*-like.

large numbers of *B. acidophilus* were encountered in the content of the large gut, there was evident no suppression of other types of bacteria, especially those of putrefactive propensities, which was so strikingly associated with the lactose diet. It would seem, then, that saccharose or its hydrolytic products do not consistently offer that specific stimulus for the overgrowth of the aciduric bacteria which is inherent in lactose feeding.

A single experiment was carried out with maltose, and only thirty grams were used in each feeding on account of the high cost of the pure product. This experiment, however, indicated that this sugar has certainly little more transforming influence than has saccharose. Dextrose in the diet offered the least stimulus of any of the sugars tested to the development of *B. acidophilus*. In only one experiment did the dextrose fed cause a slight increase in the percentage of *B. acidophilus*, and this did not persist for more than four days. In most instances it was entirely devoid of transforming effect. These results, then, indicate that the sugars tested may be ranked in about the following order as regards their tendency to establish in dogs a simplified intestinal flora dominated by the *B. acidophilus:* dextrin, lactose, maltose, saccharose and dextrose.

Dextrose, maltose and saccharose, in the amount employed, all exercised a certain amount of anti-putrefactive action, although not nearly so marked as did lactose and dextrin. This action was evidenced in the decrease in numbers of putrefactive types of bacteria, both as observed in the gram-stained films made directly from the fecal matter and from the sediments in the sugar-free broth fermentation tube and also by the drop in the numbers of H_2S-producing bacteria and of *B. welchii* as revealed through cultural procedures.

The amount of gas produced in glucose broth by the fecal flora of an animal on the lactose or dextrin diet was generally very scanty, due to the fact that the few gas-producing bacteria present were overgrown by *B. acidophilus*. The dextrose, maltose and saccharose diets also brought to development a fecal flora which produced less gas than that associated with meat alone, or meat and bread, or a meat and rice diet. These findings, then, do not substantiate Herter's suggestion that "a diet containing an abundance of carbohydrate probably would lead to a greater gas production than a diet in which carbohydrates are much restricted." As regards human conditions, also, it would seem that an excessive amount of meat in the diet may have much more to do with the establishment of a troublesome gas-producing flora than has the carbohydrate content, unless the ability to digest the latter is seriously impaired. As far as the *B. coli* group is concerned, the feeding of a certain sugar, such as saccharose or lactose, did not lead to a quantitative increase of the types splitting such sugars, but rather, as it happened, to the reverse. As a polysaccharid, such as saccharose, is hydrolyzed to monosaccharids to a very large extent, if not entirely, before the food residue reaches the large intestine, it is obvious that the sort of sugar fed would make little difference in the development of specific fermenting types among bacteria frequenting that locality.

Apparently *B. bifidus* does not find as congenial conditions for development in the intestinal tract of dogs as in that of white rats. Rettger and Hull have stated that with white rats on continued lactose feeding *B. acidophilus* is often supplanted by *B. bifidus*. A similar statement was made by Distaso and Schiller as applied to both lactose and dextrin. It has been my experience with dogs that the *B. bifidus* rarely develops to such an extent that it dominates the fecal flora. Of the sugars, lactose was most likely to cause an increase in their numbers, but never to the extent that they entirely supplanted the *B. acidophilus*. Dextrose and saccharose also in some instances caused a marked development of *B. bifidus* (Table IV., Dog 9). In fact, the increases and decreases of this organism occurred very irregularly and could not be correlated with any exact dietary conditions. There was one carbohydrate, however, which seemed to actually discourage their development, and that was dextrin. Although dextrin regularly and quickly caused an enormous development of *B. acidophilus*, in the course of no one of four experiments, in which this substance was added to the diet, was *B. bifidus* observed. It may be, however, that their presence in small numbers was masked by the enormous multiplication of *B. acidophilus*.

B. bifidus has apparently only slight anti-putrefactive action. In a few instances in which *B. bifidus* was present in great numbers in the fecal flora with few *B. acidophilus* (Table IV., Dog 9) putrefactive bacteria had also developed quite freely. This was never observed in fecal specimens in which *B. acidophilus* was predominant. An examination by the writer of a large number of stool specimens from human subjects with putrefactive fecal floras and exhibiting the symptoms of intestinal auto-intoxication has also revealed the interesting fact that almost invariably they contain large numbers of *B. bifidus*, but few or no *B. acidophilus*.

The only certain method for the demonstration of *B. bifidus* in fecal specimens lies in cultural tests. A useful plating procedure through which *B. bifidus* forms colonies distinctive in appearance has been described by the writer.[12] Hitherto investigators have depended upon the peculiar morphology of these bacilli, as observed in gram-stained films from the fecal specimens, or on what was considered distinctive colony formation in anaërobic glucose agar plate cultures. The objection to the first method of identification is that *B. bifidus* quite frequently does not assume the captate or bifid form and cannot be distinguished from *B. acidophilus*; and to the second, that the lenticular deep colonies of *B. bifidus* cannot certainly be differentiated from those of *B. coli*.

Beside, in connection with these diets containing various sugars, *B. bifidus* would appear sporadically in considerable numbers in the stools of dogs on other diets, except meat alone. A bread diet, whether white or whole wheat, seemed especially apt to encourage their multiplication. Other favorable combinations were bread and milk, bread and meat, bread, meat and milk, but not milk alone or meat and rice. Just what the nutrient conditions were which stimulated the most active multiplication of *B. bifidus* could not be determined with certainty. In this respect this bacillus stood in marked contrast to the *B. acidophilus*.

A high percentage of carbohydrate in the diet tends to stimulate the development of streptococcal types, especially those generally known as *M. ovalis*. This is true in particular as regards dextrose, maltose and saccharose; all of which tend to cause a marked relative increase in the numbers of streptococci during the first week of feeding. Lactose and dextrin bring about such an intense multiplication of *B. acidophilus* that the streptococci are crowded out almost immediately. Starchy diets favor the growth of intestinal types of streptococci, as does also a milk diet and one containing much casein.

Milk diets. — Two puppies of the same litter, about six weeks old, were fed on a diet consisting solely of pasteurized milk (Grade C) and then for eight days on the same grade of milk boiled for five minutes. With the unboiled milk *B. coli* was slightly predominant, with streptococci next in numbers and *B. acidophilus* last; whereas on the boiled milk diet *B. acidophilus* or streptococci outnumbered *B. coli* in the fecal specimens. *B. acidophilus*, however, never was found to predominate to anything like the extent as was the case in association with the use of the lactose or dextrin diets. Streptococci were always very numerous, but few *B. welchii* developed. *B. bifidus* occurred, but were not numerous. The putrefactive bacteria were not in evidence, and gas production in glucose broth was about normal in amount. The number of viable bacteria generally was high, running into the millions per milligram of fecal matter. These counts for viable bacteria were not reduced at all following a change from pasteurized milk diet to one of sterilized (boiled) milk. Escherich and others have also reported that whether the food is sterilized or not makes little difference in the number of the bacteria present in the intestinal tract of children.

If bread is now added to the milk in about equal weight, the *B. acidophilus* begins to multiply rapidly until it frequently constitutes ninety to ninety-five per cent of the total viable bacteria, crowding out practically all of the other fecal bacteria except streptococci. The much higher percentage of

B. acidophilus on the bread and milk diet over that developing on a diet of milk alone was due without doubt to the dextrin content of the bread.

Starches. — According to the experience of Hull and Rettger,[16] the feeding of cornstarch to white rats did not materially alter the relative numbers and distribution of the common intestinal bacteria, although it did tend to cause an increase in numbers of the strongly amolytic types. In these experiments with dogs it has been observed that some starchy foods exercised a marked transforming effect on the intestinal bacteria, whereas others were less effective. These diets included starch in the form of white bread, boiled rice, boiled potatoes and a mixture of beans and rice. White bread was made more appetizing by moistening it with a weak infusion of meat extract. This bread diet generally brought about a marked development of *B. acidophilus* and an almost complete suppression of putrefactive bacteria, but the relative and actual numbers of *B. acidophilus* seldom reached as high a figure as when lactose or dextrin were fed, nor did they persist as long. In the case of one dog (Table VII., column 3) which did not develop a markedly acidophilic flora on white bread alone, the addition of a small amount of meat to the bread stimulated the development of *B. acidophilus* quite markedly. In this connection it may be noted that Hull and Rettger in their experiments with white rats found that a white bread diet did not foster an aciduric flora. Rice and white potatoes, in a comparative test, exercised very much the same degree of stimulating action on the development of *B. acidophilus*, which was, in fact, about equal to that of white bread. Rice, however, seemed to permit a greater development of putrefactive bacteria than the other starch foods. Beans, although carrying a high protein content, caused a very marked development of *B. acidophilus* and a complete suppression of putrefactive types. As will be pointed out presently, the vegetable proteins exhibit a marked contrast to the animal proteins in that their presence in foods, even in large amounts, does not lead to the development of a putrefactive intestinal flora. All of these starchy foods, if combined with a small amount of

TABLE VII.

Results with dogs on various starchy diets.

Dog	1.	6.	6.	8.	8.	7.
Period of feeding (in days)	8	8	8	9	9	8
Diet (in grams)	Bread (white), 300.	As last.	Bread, 250. Beef heart, 50.	Potato (boiled), 262. Beef heart, 33.	Rice (boiled), 500. Beef heart, 50.	Beans (dry), 80. Rice (dry), 35.
Grams C–H	153.3	As last.	127.7	54.3	122.0	74.2
Grams Fat	4.2		13.5	7.2	10.5	1.5
Grams Protein	28.8		32.0	11.5	22.0	20.0
B. coli	0.1	61.0	8.0	25.0	7.0	12.0
B. acidophilus	83.0	39.0	55.0	53.0	53.0	85.0
Streptococci	6.9	0.0	35.0	20.0	36.0	3.0
Anaërobic spores (*B. welchii* mostly)	0.0	0.0	1.5	2.0	4.0	0.0
B. bifidus	+	–	–	+	–	+
H_2S-producing bacteria	–	+++	+++	++	+++	–
Degree of putrefaction (sugar-free broth sediment)	–	–	–	–	++	–
Gas production (glucose broth)	15	45	20	40	5	15
Acetic acid, N/20	+++	+	++	+++	+++	++
Glucose broth, N/10	+++	+	++	++	+++	+
Gram-stained film from fecal suspension.	G+ bacteria strongly predominate. Bacteria few in number.	G+ and G– about equal. Bacteria fairly numerous.	As last.	G+ and G– about equal. Bacteria very numerous.	G– bacteria predominate. G+ diplococci numerous.	G+ bacteria strongly predominate. *B. acidophilus* very numerous.

meat, stimulate the multiplication of streptococci, but apparently not otherwise.

Protein. — It is well known that a diet consisting entirely or largely of meat encourages the development of an extreme type of putrefactive intestinal flora, but the effect of such proteins as casein, fish and those of vegetable origin on intestinal bacteria has not been determined very definitely. A considerable number of dogs were kept for varying lengths of time on a diet of boiled beef hearts. There followed quickly and invariably the establishment of a putrefactive flora of pronounced and quite uniform type. Under these conditions gram-positive, spore-bearing, anaërobic bacilli, many of them of the *B. welchii* type, multiplied to such an extent as to dominate the flora to much the same extent as did *B. acidophilus* following a diet containing lactose or dextrin. These *B. welchii*-like organisms are found throughout the length of the intestine, and often in almost pure culture in the small gut. As partially digested muscle forms a most favorable culture medium for *B. welchii*, its luxuriant growth is not surprising. On this meat diet the *B. coli* were markedly suppressed and the *B. acidophilus* and *B. bifidus* practically eliminated. Streptococci and single gram-positive cocci might, however, be present in large numbers. The bacteria producing hydrogen sulphide from peptone were markedly numerous, especially *B. proteus*. Sediments from the sugar-free broth fermentation tubes frequently showed large numbers of spores of the *B. welchii* and *B. putrificus* types, very slender gram-negative rods and threads, and minute gram-negative cocci and diplococci, gram-negative, spindle-shaped bacilli. Most or all of these organisms are obligate anaërobes commonly associated with putrefactive conditions.

In Table VIII. is shown also the effect of a fish diet on the intestinal flora. The results stand in marked contrast to those obtained in connection with a purely meat diet. For one thing, fish protein does not encourage the growth of spore-bearing bacteria of either aërobic or anaërobic type. With a fish diet, too, instead of a predominance of *B. welchii* we find that the great majority of viable bacteria belong in the

TABLE VIII.

Results with dogs on high-protein diets of various types.

Dog	1.	3.	9.	9.	1.	1.	14.	1.
Period of feeding (in days)	8	8	14	8	4	8	8	8
Diet (in grams)	Beef heart, 300.	Same.	Rice (boiled), 500. Casein, 50.	Rice (boiled), 500. Casein, 164.	Protosac, 300.	Same.	Fish, 300. (Boiled Cod)	Fish, 200. Rice (boiled), 500.
Grams: C-H	0.0	Same.	122.0	122.0	83.0	Same.	0.0	122.0
Grams: Fat	60.0		.5	1.6	4.2		1.5	1.3
Grams: Protein	48.0		64.0	164.0	89.5		56.1	47.0
B. coli	2.0	16.0	7.0	38.0	1.0	0.4	97.5	49.0
B. acidophilus	0.0	0.0	91.0	19.0	99.0	99.4	0.5	2.0
Streptococcus	44.5	6.0	0.0	34.0	0.0	0.2	0.0	30.0
Anaërobic spores (*B. welchii* mostly)	53.4	78.0	2.0	9.0	0.0	0.0	2.5	21.0
B. bifidus	−	−	−	+	+++	+	−	−
H_2S-producing bacteria	+++	+++	++	++	++	+	+++++ (*B. proteus.*)	++++ (*B. proteus.*)
Degree of putrefaction (sugar-free broth)	+++	++++	−	+++	−	−	+	+
Gas production (glucose broth)	80	45	20	30	35	48	50	20
Acetic acid, N/20	+	−	+++	++	+++	+++	−	+
Glucose broth, N/10	−	−	+	++	+++	+++	−	−
Gram-stained film from fecal suspension.	G − strongly predominate. Most G − rods probably dead.	As last.	G + and G − about equal. Bacteria numerous.	G + predominate. Bacteria numerous.	G + strongly predominate. *B. bifidus* numerous.	G + strongly predominate. Few bacteria.	G − slightly predominate. No *B. welchii*-like. Few free spores.	

B. coli group. *B. welchii*, *B. acidophilus* and streptococci are either few in number or altogether absent. The fish diet, however, seemed to bring to development bacteria capable of forming hydrogen sulphide from peptone to a greater degree than any other food element. These bacteria apparently belong for the most part in the *B. proteus* group. Other than this it cannot be said that fish encourages the growth of obligate putrefactive types of intestinal bacteria; certainly in far less degree than does meat. When rice was fed with the fish there occurred a development of a greater diversity of bacterial types than with fish alone. Streptococci and spore-bearing bacteria were, also, much more numerous, and there occurred a slight development of *B. acidophilus*. The findings with the same animal (Dog 1) on a meat and rice diet are shown in Table II. The results are quite similar, although it should be noted that a larger amount of protein was given in the fish and rice than in the meat and rice diet.

Although milk casein is an animal protein it does not tend to create putrefactive conditions in the intestinal tract to nearly the same degree as does meat. It requires two to five times (Table VIII., Dog 9) as much casein as meat protein by weight to start and maintain the development of putrefactive bacteria within the intestinal tract; and even with a very large proportion of casein in the diet, such that the amount of protein greatly exceeds that of the carbohydrate, the fecal flora does not become markedly putrefactive, nor does the percentage of spore-bearing bacteria of the *B. welchii* type tend to increase as would be the case if meat were substituted. Large amounts of casein in the diet tended to stimulate the development of streptococci. This same result was associated with a milk diet; apparently not because of its lactose, but on account of its casein content.

A vegetable protein food material, uncombined with considerable amounts of carbohydrate, is not available. Although beans contain a relatively high percentage of protein for a vegetable food yet it is closely combined with nearly three times as much carbohydrate. A food of vegetable origin in which the protein element exceeded the carbohydrate was found in a bread called "Protosac," produced for the use of diabetic patients. Although this bread, according to trustworthy analyses, contains three times as much protein and only one half as much carbohydrate as ordinary white bread, it brought about the development of an even more purely fermentative flora in which *B. acidophilus* constituted over ninety-nine per cent. of the viable bacteria and the complete suppression of putrefactive types. This high-protein vegetable food, in fact, exhibited as strong an anti-putrefactive action as the diets containing large amounts of lactose and dextrin combined with a minimum of meat protein. This marked difference between the animal and vegetable proteins as regards their tendencies to create putrefactive conditions within the intestinal tract lies probably in the fact that the decomposition products of vegetable proteins furnishes a pabulum entirely unsuited to the needs of the putrefactive types, whereas it actually stimulates the growth of the purely fermentative *B. acidophilus* types.

Fats. — Very few bacteria are able to split fats, and probably none of those normally vegetating in the intestinal tract exercise this property. The addition of fat in the form of butter to the diet apparently brought to development no new types of intestinal bacteria, but merely tended to suppress some of those normally present. The bacterial group especially affected seemed to be the streptococci. They were much reduced in numbers on a diet containing much fat. The numbers of *B. coli* were frequently reduced, but aciduric bacteria of the *B. acidophilus* type were least affected and might even increase in relative numbers. The total counts of viable bacteria in the stools were somewhat diminished on a fat-rich diet, as was also found by the writer to be the case in a similar study with typhoid patients. In association with the addition of butter in large amounts to the dietary, putrefactive bacteria showed little tendency to increase in numbers with the exception of *B. proteus* types; but, on a diet containing much lard, putrefactive bacteria became much more in evidence, especially spore-bearing types with the morphology of *B. putrificus*. Otherwise the results with lard were essentially the same as those with butter.

TABLE IX.

Results with dogs on various fat diets.

Dog	3.	3.	3.	3.	3.	1.	1.
Period of feeding (in days)	10	6	5	11	12	11	8
Diet (in grams)	Bread, 206. Skim milk, 175 (boiled). Beef heart, 57.	Bread, 141. Skim milk, 132 (boiled). Beef heart, 36. Butter, 20.	Bread, 150. Skim milk, 150 (boiled). Beef heart, 50. Butter, 50.	Bread, 200. Beef heart, 100.	Bread, 200. Beef heart, 100. Butter, 50.	Bread, 200. Beef heart, 100.	Bread, 200. Beef heart, 100. Lard, 50.
Grams C-H	114.1	78.7	83.0	102.2	102.2	102.2	102.2
Grams Fat	14.8	26.5	56.3	22.8	65.3	22.8	72.8
Grams Protein	34.7	24.2	18.0	25.2	25.7	25.2	25.2
B. coli	0.3	10.0	53.0	8.0	4.0	3.0	7.0
B. acidophilus	97.0	89.5	41.0	40.0	88.3	79.0	80.0
Streptococcus	1.3	0.0	0.0	40.0	0.7	10.0	0.0
Anaërobic spores (*B. welchii* mostly)	1.4	2.5	3.0	12.0	7.0	8.0	13.0
B. bifidus	−	++	+	+	−	+	−
H_2S-producing bacteria	++	+++	++	++	+++	+++	+
Degree of putrefaction (sugar-free broth sediment)	+	++	++	−	++	−	++++
Gas production (glucose broth)	8	20	30	35	35	35	22
Acetic acid, N/20	+++	+++	+++	++	++	++	++
Glucose broth, N/10	+++	+++	+++	−	+	+	−
Grain-stained film from fecal suspension.	G+ bacteria strongly predominant. Bacteria few.	As last.	G+ and G− bacteria about equal in number. Bacteria fairly numerous.	G− bacteria predominant. Bacteria very numerous.	G+ bacteria strongly predominant. Bacteria few.	G− bacteria slightly predominant. Bacteria numerous.	G− bacteria predominant, many not developing in cultures. Bacteria very numerous.

SUMMARY.

Under normal physiological conditions, the fundamental factor controlling the types of bacteria vegetating in the intestinal tract is the chemical character of the food ingested. Secondary controlling factors of almost equal weight are the rate and degree of the digestion and absorption of the food and the character of the end products of the digestive process. It has been demonstrated experimentally in this investigation with dogs that, on the one hand, not all carbohydrates have an equal tendency to establish a purely fermentative intestinal flora, and, on the other hand, not all protein foods encourage putrefactive conditions in a like degree.

In feeding experiments with dogs it has been shown that two carbohydrates, lactose or dextrin, when added to a meat and rice diet, caused such a marked development of aciduric bacteria of the *B. acidophilus* type that they completely dominated the fecal flora and effected the almost complete suppression of proteolytic types commonly found in the canine intestinal tract, even including *B. coli*. This purely fermentative flora would, furthermore, persist as long as the diet was continued; there being no tendency to reversion to the so-called normal flora. *B. bifidus* sometimes increases greatly under these dietary conditions, but generally was soon overgrown and suppressed by *B. acidophilus*, and, in fact, very rarely became the dominant type. A diet of bread and milk, which naturally contains both lactose and dextrin, was also followed by the establishment of a fecal flora consisting almost entirely of *B. acidophilus*. With other sugars tested, viz., saccharose, maltose and glucose, this transformation did not occur or was far less striking. Glucose, even in large amounts, exercised no transforming influence on the types of bacteria present in the intestinal tract, and the same seemed to be true for maltose. Saccharose, however, fed in large amounts, did cause a moderate increase in the numbers of obligate fermentative bacteria and a partial suppression of proteolytic types, but in no way was the change commensurate with that effected through the use of lactose or dextrin.

With the grade of pasteurized milk used (Grade C), some difference was noted in the results obtained, depending upon whether it was fed in an unboiled or boiled condition. With the unboiled milk, *B. coli* and streptococci predominated, whereas with boiled milk streptococci and *B. acidophilus* outnumbered other types of viable bacteria. To obtain, however, a simplification of the intestinal flora with a strong predominance of aciduric bacteria it was found necessary to add bread to the milk.

Starchy foods all tended to effect a simplification of the intestinal flora and an elimination of obligate putrefactive bacteria. These foods with a large starch content differed in some degree in their efficiency as transforming agencies. White bread, potatoes and beans all tended to bring about a predominance of *B. acidophilus*, whereas rice proved rather less effective as an anti-putrefactive agent.

Various proteins were found to differ radically in their effect upon the intestinal flora, depending upon their source. Of the varieties tested, the proteins of mammalian tissues were the only ones which markedly encouraged the growth and activity of the obligate putrefactive bacteria within the intestinal tract. A diet of fish brought to development a flora which was entirely different from that appearing in association with the feeding of beef hearts. Spore-bearing bacteria did not appear in the fecal specimens in more than insignificant numbers. There was a notable absence of the *B. welchii* types which constitute so large a part of the flora in connection with a meat diet. On the other hand, bacteria of the *B. coli* and *B. proteus* types were strongly predominant. Milk casein, as an article of diet, exhibited far less tendency to give rise to intestinal putrefaction than did meat protein. Experiments in feeding casein seemed to indicate that the large numbers of streptococci present in the feces in connection with a milk diet were due to this element in milk rather than to the lactose. Vegetable proteins stand in strong contrast to animal proteins, especially meat, in that they do not offer the slightest encouragement to the growth of intestinal putrefactive types of bacteria. In fact, with a bread containing a very high protein content with a minimum of carbohydrate as marked an overgrowth of aciduric intestinal bacteria occurred as was observed in connection with diets to which considerable amounts of lactose or dextrin had been added.

Fat, as an element of diet, seemed to play no part in determining the development of bacterial types within the intestine. The only effect of large amounts of fat of animal origin was apparently a reduction in the relative numbers within certain bacterial groups.

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