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A TREATISE ON THE TRANSFORMATION OF
THE INTESTINAL FLORA
WITH SPECIAL REFERENCE TO THE
IMPLANTATION OF BACILLUS ACIDOPHILUS

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DISSERTATION.

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THE present volume is based on investigations conducted at the Sheffield Scientific School of Yale University and presented in lectures delivered during the autumn of 1920 by the senior author, on the Silliman Foundation.

PREFACE

FIFTY years have passed since Pasteur and Koch, the founders of modern bacteriology, made their first contributions to this new field of scientific study. No phase of the subject has been given more attention by investigators than the bacteriology of the digestive tract. However, success in these endeavors has been limited, with few exceptions, to the discovery of microorganisms which are manifestly disease-producing and which exert their baneful influence in comparatively short periods of time, as for example the microbial agents causing typhoid fever and epidemic dysentery. The more subtle bacterial processes which take place in the intestine, and the laws which govern the preponderance of one type of bacteria over another, have as yet been but little understood.

The authors have aimed to present here in as direct a manner as possible the results of an extended series of observations on: (1) the relation of diet to the character of the intestinal bacterial flora, and (2) the possibility of implanting bacteria of known physiological properties in place of those which ordinarily hold sway after early infancy.

The demonstration that lactose, dextrin and milk, when taken in sufficient amount, encourage a non-putrefactive flora, and that a non-putrefactive organism like *Bacterium acidophilus* may be established in the intestine by oral administration, should be of fundamentally scientific interest, irrespective of any important bearing that it may have on the many and complex problems related to intestinal disease and minor alimentary disturbances.

The descriptions of methods employed in the examination of fecal specimens are given in some detail for the use of other investigators who are engaged in studies similar to those recorded in this treatise. Particular attention is given also to the preparation and use of *Bacterium acidophilus* milk, since the experience of the last six months has shown that the administration of this product is the most effective and practical method of implanting the submissive aciduric organism in the digestive tract, and one which is not only well-borne by the subject but is as a rule welcomed by him because of its unique qualities as a beverage and a real food.

THE AUTHORS.

New Haven, Conn., October, 1920.

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TABLE OF CONTENTS

	PAGES
I. Historical review	1-10
II. Feeding and implantation experiments with white rats	11-64
Methods	11-13
Preliminary feeding experiments	13-16
Carbohydrate feeding	17-21
Administration of living suspensions of <i>Bacillus acidophilus</i>	21-34
<i>Bacillus acidophilus</i> and carbohydrate feeding	34-49
<i>Bacillus acidophilus</i> versus <i>Bacillus bulgaricus</i> feeding	49-52
Slow carbohydrate absorption in the intestine and its relation to <i>Bacillus acidophilus</i> implantation	52-61
Relation of hydrogen ion concentration to the character of the intestinal flora	61-63
Conclusions	63-64
III. Feeding and implantation experiments with human subjects	65-110
The basal daily diet	66-69
Carbohydrate feeding	70
Transforming influence of lactose	70
Transforming influence of dextrin	70-77
Implantation experiments with living cultures of <i>Bacillus acidophilus</i>	78-79
Simultaneous use of the special carbohydrates and <i>Bacillus acidophilus</i>	79-84
Transforming influence of milk cultures of <i>Bacillus acidophilus</i> on the intestinal flora	85-94
The use of <i>Bacillus acidophilus</i> milk reinforced with lactose or dextrin	94-99
Attempts to implant <i>Bacillus bulgaricus</i> in man	99-106
Incomplete absorption of lactose from the intestine	106-107
Relation of hydrogen ion concentration to the character of the intestinal flora	108-110
IV. A full account of the preparation of <i>Bacillus acidophilus</i> milk for human consumption, and of its known properties	111-113
V. Methods employed in the routine examination of feces	114-117
The use of whey agar plates	114-115
Veillon tubes	115-116
Direct microscopic examination of fecal suspensions	117
VI. General discussion and summary	118-124
Explanation of plates	125-126
Bibliography	127-135

I. HISTORICAL REVIEW

THE present studies mark a resumption of work begun in this laboratory eight years ago. The new data recorded here are the results of investigation into phases of intestinal bacteriology which are as yet comparatively new and the importance of which is attested by the almost universal interest which is manifested in them by physicians and laymen alike.

While it is a well-established fact that diet exercises a most profound influence in determining the predominance of one or another type of bacteria in the alimentary canal, the question of transforming and simplifying the ordinary mixed intestinal flora through diet in conjunction with the oral administration of bacteria, or by the latter process alone, is still in its incipency.

The first systematic study of intestinal bacteria appears to have been carried out by Escherich (1886) who made an extensive investigation of the microorganisms in infants' dejecta, both in health and disease. He observed the great predominance of Gram-positive rods in the stools of healthy nurselings, but failed to isolate two organisms to which considerable interest is attached at the present time, namely *B. bifidus* and *B. acidophilus*. Through the classical researches of Tissier (1900) and Moro (1900a) these two organisms were added to the list of known intestinal bacteria. The controversy as to whether Tissier's *B. bifidus* or Moro's *B. acidophilus* is the predominating type in the normal dejecta of breast-fed babies was ended by the admission by Moro of Tissier's claim (1900b and 1905a), which was substantiated by Rodella (1901), Cahn (1901), Cippolina (1902), Passini (1903), Weiss (1904), and finally by Jacobson (1908).

By means of a dextrose broth medium containing 0.5 per cent glacial acetic acid, as first suggested by Heymann (1900), Finkelstein (1900), working independently, isolated an organism identical with that of Moro, which he called "Säureliebender Bacillus."

The meconium of the new-born infant is sterile. Billroth (1874) is given the credit by Mannaberg (1898) of having first observed this fact. Senator (1880) found the intestine to be free from bacteria, and his statement was confirmed by Escherich (1885), Popoff (1892), Schild (1895), Szegö (1897), Tissier (1900) and Moro (1900a). While Breslau (1866) has shown that the early infection of the meconium may be entirely independent of feeding, the mouth and the anus

2 TRANSFORMATION OF THE INTESTINAL FLORA

serving as the chief portals of entry for bacteria, the establishment of the characteristic flora of the infant does not take place prior to the administration of food, as was pointed out by Escherich (1885), Tissier (1900) and Moro (1900a).

According to the substantiated claims of Tissier, his *B. bifidus* is the predominant organism in the stools of breast-fed infants. In the change from breast- to bottle-feeding *B. bifidus* is in a large measure replaced by *B. acidophilus*, as has been claimed by Logan (1914) and others. While cow's milk brings about a less homogeneous flora than mother's milk, there is a large proportionate increase of *B. acidophilus*, not infrequently to the exclusion of all other organisms. As the child grows older and the artificial feeding becomes more varied, there appears a more and more complex flora which in the course of time resembles that of an adult. Fischer (1903) was the first to call attention to this change. Sittler (1910) and others confirmed the observation.

The comparatively recent experiments of Cohendy (1912) and Küster (1913) have for the time at least answered the question first propounded by Pasteur, namely whether intestinal bacteria are necessary for the well-being of the host. On the other hand, the enormous numbers of bacteria in the intestine of man and animal have been the subject of numerous researches into methods of quantitative determination. Eberle (1896), Klein (1900) and Hehewerth (1900) employed the direct microscopic count; Winterberg (1898) for the first time made use of the Thoma-Zeiss blood-counting chamber in this connection, while Strasburger (1902) resorted to the gravimetric method, which proved very efficient. Others have by means of the plate culture and other methods estimated the total number of bacteria in feces. Thus, MacNeal, Latzer and Kerr (1909) have estimated the average number of fecal bacteria excreted daily by a normal adult subsisting on an ordinary diet as thirty-three times ten to the twelfth power (33×10^{12}), and that this number of bacteria is equivalent to 5.34 grams of dried bacteria or 0.585 gram of bacterial nitrogen. Matill and Hawk (1911) place the daily output at 8.27 grams of dried bacteria, and Sato (1910) at 8.54 grams. Berger and Tsuchiya's (1910) estimate was much lower, namely 3.023. Strasburger calculated that a healthy adult daily excretes about 8 grams of (dried) bacteria, or 128 trillions.

The influence of sterile food on the intestinal population was studied by several investigators. Sucksdorff (1886) and Brotzu (1895) claimed that sterilized food reduces the number of bacteria in the alimentary tract. Similar results were obtained by Gilbert and Dominici (1894). However, the researches of Stern (1892), Adrian (1895), Eberle (1896) and Ballner (1904) failed to confirm this assertion. Escherich had shown that the sterilization of the food had little or no influence on the number of intestinal organisms. Hammerl (1897),

Albu (1897), Wang (1899) and Belonowsky (1907a) also were unable to establish any direct relationship.

Intestinal antiseptics has received considerable attention. Bouchard (1887) may be regarded as the pioneer in this field. He administered charcoal, naphthalene and iodoform internally, and observed a reduction of toxicity of the stools and urine. Wassilieff (1882) claimed that calomel produced a diminution in the putrefactive products of the feces of dogs. Baumann (1886) also noticed that calomel was effective in reducing the ethereal sulphates in the urine. Sucksdorff (1886) concluded that quinine and naphthalene reduced the number of intestinal bacteria. Müller (1887) and Bartoschewitch (1893) noticed a disappearance of ethereal sulphates after the administration of calomel, and Fürbringer (1887) found that this agent materially reduced the number of intestinal microorganisms. Kumawaga (1888) reported a reduction of bacteria in the proportion of 37 to 1 through the use of acetanilid in the dog. Salkowski (1889) obtained a considerable drop in the number of bacteria in the intestine of a dog which had received chloroform water. Sehrwald (1889) also obtained positive results with naphthalene, and Griffith (1895) and Williams (1895) used chlorine in the treatment of typhoid patients with apparently excellent results. Rovighi (1892) employed turpentine, camphor, menthol and boric acid with moderate success.

Morax (1886) could not confirm Baumann's conclusions, and maintained that the apparent diminution of ethereal sulphates was due to rapid removal of the putrefactive products through active peristalsis. Stieff (1889) and Biernacki (1892) arrived at the same conclusions as Morax. Schuetz (1901) could demonstrate no favorable influence through the use of antiseptics. On the contrary, he observed an increase of intestinal bacteria after the administration of calomel. Von Mieczkowski (1902) pronounced bismuth, silver nitrate, tannopin and beta-naphthol as valueless, but did secure a reduction after the use of menthol. Strasburger (1903) obtained negative results with naphthalene, thymol, silver nitrate and beta-naphthol. Salicylic acid, however, caused some reduction. Schönenborn (1903) found that naphthalene, itrol and thymol increased the bacteria, while salicylic acid mixed with the food diminished the number. Müller (1898), after several years of observation, is of the opinion that disinfection of the alimentary canal with drugs is hardly possible, and that there are no useful intestinal antiseptics.

Hoffman (1906) showed that iodoform when given by mouth is an effective disinfectant and decreases the number of bacteria in the feces. Hertter (1907) noted that in certain instances salicylates, aspirin and salol exerted some action in decreasing the output of indican, but ultimately reached the conclusion that most of the so-called intestinal antiseptics do very little good in effecting diminution of the putrefactive organisms of the intestine. Feigen (1908) used calomel, magnesium

4 TRANSFORMATION OF THE INTESTINAL FLORA

dioxide and iodo-anisol and secured no reduction; and Harris (1912) failed to obtain any reduction in the bacterial count by employing salol, beta-naphthol and guaiacol carbonate as antiseptic agents. Oliver (1907), on the other hand, used beta-naphthol with excellent results; Ellis (1912) reports success with magnesium sulphate in chronic forms of diarrhea, and Hand (1912) speaks of bismuth salicylate as an antiseptic in acute diarrhea. On the whole, however, the use of intestinal antiseptics has proven more or less disappointing.

As early as 1868 Senator declared that the decomposition of protein within the alimentary canal under ordinary conditions results in the formation of substances toxic to the host. Nearly twenty years later Bouchard (1884) elaborated the theory of intestinal intoxication. He claimed that the amount of putrefactive products eliminated in the urine was a measure of the degree of intestinal putrefaction and called his measurements "Urotoxic Coefficients." Jaffe (1877), Salkowski (1878), Brieger (1878) and others introduced new methods which played an important part in extending our knowledge of the processes of intestinal putrefaction under different conditions.

Ortweiler (1886) and Müller (1886) demonstrated that the administration of carbohydrates tends to lessen putrefaction in the digestive tube. Krauss (1894) obtained similar results with dogs. Biernacki (1892), Eisenstadt (1897) and Bachmann (1902), however, noted only a weak inhibiting action of carbohydrates. Hirschler (1886) appears to be the first to conclude that particular carbohydrates, sucrose, lactose, dextrin and starch, as well as the alcohol glycerol, exercise some inhibiting influence on intestinal putrefaction. Poehl (1887), Biernacki (1892), Winternitz (1892) and others studied the influence of milk and found that a milk diet tended to decrease the undesirable products of protein decomposition by bacteria. These observations were further confirmed by Herter (1897) and Leva (1908). Herter and Kendall (1909) fed milk and dextrose to cats and monkeys and observed a marked decrease of the ethereal sulphates in the urine. Barker (1914) and Torrey (1915) reported favorable results from the feeding of lactose to typhoid patients.

Solukha (1896) and Kopetski (1900) sought to establish a definite relationship between specific constituents of milk and the diminution of intestinal putrefaction, and since previous workers, Hirschler (1886), Winternitz (1892) and Schmitz (1893) had demonstrated that lactose could inhibit putrefaction, their efforts were naturally directed to this sugar. They concluded that lactose will inhibit intestinal putrefaction when it is fed by mouth. Wereschtschagen (1895) claimed that glucose may exercise the same action.

Nencki and Sieber (1882) and Stadelmann (1883) administered lactic acid to their patients with apparently good results. Grundsach (1893), Schmitz (1894) and Singer (1901) also claimed that lactic acid lessens intestinal putrefaction, as measured by the decrease of

etheral sulphates in the urine; encouraging results were obtained also by Winfield (1912). On the other hand, Rovighi (1892) showed that lactic acid exerted only a slight inhibiting action, and Winternitz (1892) asserted that it has no inhibitory influence, whatever.

Poehl (1887) noted that sour milk when ingested decreased intestinal putrefaction. This observation was confirmed by Rovighi (1892), Embden (1894), Brudski (1900) and Fischer (1903). Tissier and Martelly (1902) stated that the chief agent in effecting inhibition of putrefying bacteria is probably the lactic acid produced by the lactic acid bacilli. Tissier and Gasching (1903) found that acid-producing bacilli are able in a sugar-containing medium to arrest the growth of putrefactive organisms, thus confirming the conclusions of Bienstock (1899).

The feeding of buttermilk to infants has long been a custom among the peasants of Holland. Within a short time after DeJager (1897) and Teixeira de Mattos (1902) brought this method of infant feeding before the medical profession, the administration of buttermilk to infants, especially those afflicted with gastro-intestinal disturbances, became quite popular, and most favorable results were reported by various investigators.

From the earliest historic times the use of both sweet and sour milk has been practiced by many Nomadic and Semi-Nomadic tribes. The consumption of soured milk undoubtedly became popular on account of its enhanced palatability and better keeping qualities. As the result of long-continued practice in the preparation of the sour milk by methods handed down from generation to generation the different countries or tribes had their own established product, which differed as a rule from those of other peoples. Among the best-known of these are the kefir of the Caucasus, leben raib of Egypt, koumiss of Asiatic Russia, matzoon of the Armenians, and yoghurt of the Balkan Peninsula.

Numerous instances are on record where persons lived and retained much of their early vigor to a very old age, particularly in Bulgaria, and where from all appearances they owed their long life to sour milk which was their staple, and in many cases practically the only diet. Metchnikoff was a close observer of these conditions which were being brought to his attention by his students and others, and as a result of these observations and further extensive studies he propounded his lactic acid bacillus and longevity theory and founded his sour milk therapy.

Cohendy (1906a) performed experiments upon himself and thirty patients, by feeding pure milk cultures of *B. bulgaricus*. He observed a decrease in intestinal putrefaction which was noticeable for seven weeks after the taking of the culture. In a second paper the same writer (1906b) pointed out that *B. bulgaricus* becomes so thoroughly acclimated in the human intestine as to be found there several weeks after

6 TRANSFORMATION OF THE INTESTINAL FLORA

ingestion, and was led to conclude that the lactic acid bacilli themselves were responsible for their implantation, without due regard to the milk as a factor.

Belonowski (1907b) concluded from the results of his own experiments with mice that *B. bulgaricus* becomes established in the intestine about the tenth day after ingestion and persists for some time; second, that the putrefactive types of the flora are greatly reduced; and, finally, that *B. bulgaricus* owes its anti-putrescent power, not to the lactic acid alone, but also to certain other inhibitory products formed by the bacilli. Leva (1908) investigated the effect of *B. bulgaricus* when fed in the form of "lactobacilline" on the excretion of putrefactive products, and reported a decrease in the amount of phenol, volatile fatty acids and aromatic oxyacids excreted. He believed that *B. bulgaricus* becomes acclimated in the intestine. Pochon (1907) drank milk soured by lactic acid bacilli and after several weeks observed a marked reduction in the amounts of indol and phenol in the urine.

Harrington (1912) and Bogert (1918) reported favorable results obtained from the administration of living cultures of *B. bulgaricus*. Moro (1906) found it necessary to use large quantities of bouillon cultures of lactic acid bacilli when fed per os, and showed that better results could be obtained when the bacteria are introduced per rectum. Dunn (1907) used buttermilk containing large numbers of living lactic acid organisms in the treatment of various intestinal disturbances in infants, with apparently excellent results. Piffard (1908) directed many of his patients to employ sour milk for both dietetic and directly remedial purposes. He admits, however, that such use of sour milk has been largely empirical. North (1909) reported encouraging results in the treatment of disorders of the nose and throat with *B. bulgaricus*. Brady (1910) used sour milk with apparently good success. La Fetra (1909), while disappointed in the use of acidified milk, felt that there was a distinct field for the employment of buttermilk, which had given him very encouraging results in cases of intestinal disturbance. Fansler (1917) claims to have obtained good results from the treatment of wounds with living cultures of the bulgarian bacillus. Liefmann (1909) suggested sour milk as a means of eliminating typhoid bacilli from carriers. Mayer (1910) held that the continued use of sweet milk by typhoid carriers reduces the number of typhoid organisms.

Wegele (1908) reported favorably on the use of milk soured with *B. bulgaricus*. He believed that the production of lactic acid in "*statu nascendi*" in the digestive tube is of greater importance than the mere presence of the acid already formed in the milk. Wejnert (1908) demonstrated that the administration of milk soured with *B. bulgaricus* reduces the number of bacteria in the feces. Litchfield (1914) fed fermented milk reinforced with lactose to his typhoid patients, with excellent results. Tollens (1908) reported favorably on the employment of kefir in intestinal disturbances, while Klotz (1908) used yoghurt to

the same advantage with his patients. Oehler (1911) conducted feeding experiments with yoghurt on mice and monkeys and stated that *B. bulgaricus* could be demonstrated with ease in the feces during the entire feeding period.

The claims of Metchnikoff and his followers could not be substantiated, however, by the following investigators. Luerssen and Kühn (1908) failed to implant *B. bulgaricus* in the intestine of man by the continued use of yoghurt. Herter and Kendall (1908) found that on feeding a monkey for two weeks exclusively on milk fermented with *B. bulgaricus* of Massol the organism in question did not establish itself below the level of the ileo-caecal valve. Spiegel (1911) stated that the therapeutic value of bulgarian milk or tablets is at least doubtful and that there is insufficient evidence of beneficial action. Heinemann (1912) claimed that *B. bulgaricus* is not a panacea for intestinal putrefaction. Blodgett (1913) concluded that the administration of this organism in cases of glycosuria is of no benefit. Distaso and Schiller (1914) fed the organism to white rats and were forced to the conclusion that it is impossible to bring about the acclimatization of *B. bulgaricus* in the intestine.

Macfayden, Nencki and Sieber (1891), Lembke (1896) and Hammerl (1897) failed to establish any definite relation between ingested food and types of intestinal bacteria. Lembke (1897) continued his investigation and was ultimately able to demonstrate that by changing the diet, namely by the substitution of bread for meat, a marked difference could be brought about in the character of the intestinal flora. The investigator did not, however, attribute any prominence to the aciduric organisms.

Tissier's extensive study of the bacteriology of infants' stools marks a big step in advance. He observed three distinct phases in bacterial infection of the intestinal tract of infants, namely, the period of sterility, the period of mixed or promiscuous infection, and the period of transition resulting in the establishment of the characteristic nursing's flora. At birth the alimentary canal and its contents are sterile, as had been shown by Senator, Escherich and others. The first indications of bacterial contamination of the meconium are discernible several hours after birth. The early invaders are adventitious microbes in every respect resembling those which are commonly met with in the baby's environment, and probably gain entrance to the intestinal canal through the mouth and anus.

As soon as the digestive tract becomes the recipient of mother's milk and its digestion products a marked change is recognizable in the intestinal flora. The heterogeneous aggregation of microbes gives way to a simplified flora dominated by *B. bifidus*, which persists as long as the child's diet is confined to breast milk alone. When breast feeding gives way, however, to artificial feeding and the diet becomes more varied the simplified and essentially *B. bifidus* flora gradually shifts to

one of a more complex character, which eventually resembles the mixed flora of an ordinary adult.

Moro (1900a) claimed that his *Bacillus acidophilus* was the predominating organism in nurselings' stools, but later admitted Tissier's claims. Passini (1903), Moro (1905), Tissier (1905), Rodella (1905), Sittler (1908) and Bahrtdt and Beifeld (1910) concluded that diet plays an important rôle in determining the types of intestinal micro-organisms. They corroborated Tissier's earlier observation, namely that *Bacillus bifidus* is the predominant organism in the stools of breast-fed infants and that there is a more varied and less constant flora in the feces of bottle-fed babies. Spiegelberg (1899) reported a scarcity or absence of liquefying bacteria in the stools of normal nurselings.

Rettger and Horton (1914) noted a marked simplification of the intestinal flora of white rats soon after they were shifted from the usual mixed diet of grains and vegetables to one containing starch, lard, protein-free milk and purified proteins, the Gram-positive organisms of the acidophilus and bifidus types constituting eighty-five to one hundred per cent of the flora. Hull and Rettger (1914) observed that mixed grain feed tends to transform the flora of the rat, but that no profound change takes place until milk or lactose is administered in addition to the regular diet. The preponderance of *B. acidophilus* over other organisms was brought about within three days after a diet of vegetables and bread was followed by one of mixed grain and milk. When lactose was fed in appreciable amounts the transformation period was very short, and the change in type of bacteria was practically 100 per cent. It was noted, however, that the *B. acidophilus* phase was often more or less temporary, this organism giving way to *B. bifidus*. In the milk-feeding experiments the acidophilus phase was, as a rule, the more permanent, *B. bifidus* seldom gaining the ascendancy. Of particular interest in this investigation was the failure of the authors to implant *B. bulgaricus* by the ingestion of milk cultures and water suspensions of this organism.

Rettger, Kirkpatrick, Jones and Card (1914 and 1915) employed several thousand chicks in their studies on the influence of milk feeding on growth and mortality, and found that the unique properties of this food exist in the milk *per se*, and not in any milk acids or milk-souring bacteria. The chicks received unpasteurized, sterilized and naturally soured milk, as well as milk soured with pure cultures of *B. bulgaricus*, and no differences could be observed in the beneficial effects of the different preparations. These observations impressed upon the writers the fundamental fallacies of Metchnikoff's interpretation of the sour-milk therapy. Hull and Rettger (1917) confirmed the results of their earlier work. They found, further, that two to three grams of lactose are sufficient to establish an aciduric flora within three days. It required a longer time to transform the flora by milk feeding than by the use of lactose, and the change was not so nearly complete. Meat or

high protein diet caused an increase in the relative numbers of putrefactive bacteria, which could be reduced again by the addition of lactose to the meat diet. Starch appeared to foster the amylolytic types. They concluded that milk owes its beneficial action to the lactose, which is absorbed slowly from the intestine. On several occasions it was found in the feces of rats to which it had been fed. It was claimed that the transforming influence of lactose could not be due to lactic acid produced from the sugar, because it was impossible to demonstrate increased acidity of the intestinal contents of the white rats.

Throughout the above series of investigations Rettger and his associates observed that *B. acidophilus* was an almost constant inhabitant of the intestines of chickens of all ages and of white rats, and that the administration of milk and lactose, and to a limited extent raw grains, stimulated proliferation of this organism, and that unless such favorable dietary agents were fed the number of acidophilus bacilli was held at a low level. It was quite apparent then why the ingestion of milk soured with *B. bulgaricus* has such a marked transforming influence on the intestinal flora, and that what at first sight appears to be *B. bulgaricus* is nothing more than an invigorated *B. acidophilus* (1915, page 27).

Weiss (1904) had demonstrated the presence of large numbers of *B. acidophilus* in the intestine of man after the administration of milk. Sittler (1908) called attention to the relation of intestinal organisms to the diet. He attributed the predominance of *B. bifidus* in the feces of breast-fed infants to the lactose in the mother's milk, basing his conclusions largely on the observation that cow's milk reinforced with lactose causes the establishment of a similar flora. He noted also that malt soup exerts the same influence, while sucrose effected no transformation. De Gasperi (1911) claimed that bread or mixed grain caused the establishment of *B. bifidus*, whereas the substitution of meat for bread or grain brought about a preponderance of *B. coli* and *Proteus vulgaris*.

That a mixed diet of milk and other staple foods causes a more marked change in the bacterial flora than an ordinary diet without the milk was shown by Fischer (1903) and confirmed by Sittler (1910). Steele (1908) came to the conclusion that the regulation of the amount and character of food is the most efficient means of checking excessive bacterial activities in the intestine, while Friedenwald and Leitz (1909) held that regulation of diet and evacuation of the bowels constitute the most effective method of reducing the excessively high bacterial content of the bowel. Harris (1912) fully agreed with the conclusions of Friedenwald and Leitz.

Herter and Kendal (1909) noted a definite correlation between specific types of bacteria and the chemical composition of the ingested food. They observed a gradual but rapid substitution of an acidophilic, non-proteolytic type of flora for one that is strongly proteolytic, when the diet was changed from meat and eggs to milk and

10 TRANSFORMATION OF THE INTESTINAL FLORA

dextrose. Distaso and Schiller (1914) reported that, of the various carbohydrates used by them, lactose and dextrin alone exercised a profound influence on the intestinal flora, bringing about a predominance of *B. bifidus*. No mention is made of *B. acidophilus*. Wollstein (1912) studied the effect upon infants of high carbohydrate and protein diets, and found that the former encourage the development of cocci, *B. coli*, *B. acidophilus* and *B. bifidus*, whereas the high protein diets favored *B. mesentericus*, and brought about a very great reduction in the numbers of *B. acidophilus* and *B. bifidus*.

Torrey (1915) found that 250 to 300 grams of lactose were required to transform the fecal flora of typhoid patients from the usual mixed type to one dominated by *B. acidophilus*. *B. bifidus* was occasionally seen, but it was never present in sufficient numbers to be of very much significance. In his very recent investigation on dogs Torrey (1919) demonstrated that fifty grams of lactose or dextrin, when added to a meat and rice diet, completely transformed the ordinary flora to one strongly dominated by *B. acidophilus*. He showed further that the feeding of a bread and milk diet favored the development of a flora consisting almost entirely of *B. acidophilus*. Saccharose, maltose and glucose exercised only a comparatively moderate, if indeed any, transforming influence. Starchy foods in the form of white bread, potatoes and beans tended to bring *B. acidophilus* into prominence, while rice was less effective. Torrey claimed that while animal proteins encouraged a strongly proteolytic flora, vegetable proteins were without this effect.

II. FEEDING AND IMPLANTATION EXPERIMENTS WITH WHITE RATS

THE first investigation reported here is an attempt to implant *B. acidophilus* within the alimentary canal of the white rat. The experiments consisted essentially in the feeding, in connection with a basic diet made up of bread and beef, of such special carbohydrates as lactose and dextrin, and of uniform suspensions of *B. acidophilus* in saline solution.

In studying the transforming influence of these agents an effort was made to determine the minimum amounts required to bring about an appreciable change in the intestinal flora. Bacteriological examinations of the feces were made on alternate days. White rats were used exclusively. They offer many advantages, at least in this particular instance. They were easily obtained in large numbers, and with little cost to the laboratory; they occupy little room; they may be induced to live on a monotonous and simple diet, and the feces may be obtained fresh at the time they are needed. During the investigation 66 rats were employed, and between 1200 and 1300 fecal specimens were examined. The total number of complete rat experiments was 118, some of the rats being used more than once.

METHODS

The rats were kept separately in wire cages having floors made of half inch wire mesh and placed over paper-covered trays for collecting the droppings. The basic diet consisted of bread and beef, ten and three grams respectively daily. This was fed for at least five days prior to the administration of the test substances. The bread and meat diet was found to be sufficient to keep the rats in good physical condition, and was as a rule consumed without waste. There was but one feeding a day, usually in the morning.

The feces were collected easily according to the method of Hull and Rettger (1917). The rats were held by the tails and gently rubbed on the back. The samples may be collected directly into test tubes with very little or no contamination. The specimens thus obtained were moist and soft and were readily reduced to a more or less uniform suspension by vigorous shaking with the aid of broken glass in test tubes containing ten cubic centimeters of physiological saline solution.

12 TRANSFORMATION OF THE INTESTINAL FLORA

These suspensions were diluted to match tube eight of the McFarland (1907) nephelometer scale. The methods of bacteriological examination of fecal specimens are given at some length in the special chapter on methods (pages 114-117) which goes into the preparation and use of whey agar plates, Veillon tubes and Gram-stained microscopic slides.

The amount of gas formation and the relative numbers of *B. acidophilus* colonies in the Veillon tubes are recorded by the following system:

Gas production		<i>B. acidophilus</i> colonies	
—	none	—	none
+—	very slight	+—	very few
+	slight	+	few
++	moderate	++	moderate
+++	marked	+++	marked
++++	very marked	++++	very marked

Special tests for *B. welchii* were at times made by the milk culture and heat test, but these were regarded unnecessary as a routine procedure, since the Veillon tubes provide the right conditions for development of this organism.

In selecting methods for the bacteriological examination of intestinal contents and fecal specimens the aim has been to employ simple and yet sufficiently reliable procedures to obtain a general insight into the numbers and biological activities of important groups of bacteria which are capable of establishing themselves in the intestinal tract, and to make it possible to correlate particular types of organisms with specific dietary conditions. The methods chosen enable one to gain a clear view of the relative predominance and activities of the fermentative and so-called putrefactive bacteria, both aerobic and anaerobic.

During the investigation pure stock cultures have been grown in either whey broth, whey agar, or in milk. The hydrogen ion concentration of the broth and agar, unless otherwise noted, was adjusted to pH 6.8. Whey agar has been found to be of particular value for preserving the different strains of *B. acidophilus* and *B. bulgaricus*, transplants being made onto the entire slope of the agar with a liberal amount of the inoculum.

Samples of intestinal contents were procured in the following manner. After a fast of twenty-four hours the rats were killed with chloroform, the abdominal cavity opened and the entire intestinal tract spread out on a sheet of paper. The various sections of the intestine, namely the duodenum, jejunum, ileum, caecum and the colon and rectum, were then removed with shears. The contents of each section were forced out by a process of stripping with forceps into test tubes containing sterile physiological saline solution and broken glass. Standard emulsions were prepared and subjected to precisely the same bacteriological examinations as the samples of feces.

In order to conserve space, quite a number of charts, curves and photographic plates originally prepared for publication have been left out of this book. Those which do appear have been selected because of their representative character. The curves were plotted to show average figures.

Throughout the present treatise the generic term "Bacillus" has been applied to the acidophilus and bulgaricus organisms. "Bacterium" has the greater claim to recognition here, from the standpoint of both motility and spore production. Furthermore, such usage would be in harmony with the general recommendation of the Committee on Classification of the Society of American Bacteriologists which, however, recommends the use of the generic term "*Lactobacillus*" for the group of milk-souring bacteria to which *B. acidophilus* and *B. bulgaricus* belong. The term "Bacillus" has been retained because of its universal use in connection with these two organisms by both bacteriologists and men of the medical profession, and because of the lack of definite action on the point at issue taken by organized societies.

PRELIMINARY FEEDING EXPERIMENTS

While chief emphasis was placed on the implantation of *B. acidophilus* in the intestine of the white rat, considerable attention was given also to the influence of various carbohydrates on the intestinal flora, both with and without the administration of suspensions of *B. acidophilus*, and to the possibility of establishing *B. bulgaricus* in the digestive tract. In every experiment the animals were first given a basal diet which was found to encourage the development of the usual complex flora without completely eliminating *B. acidophilus*, a normal inhabitant of the alimentary tract of man and animals. This diet, consisting of bread (ten grams) and chopped lean beef (three grams), encourages to a certain extent the "putrefactive" and gas-producing types of intestinal bacteria. It was consumed freely by the rats, and appeared in every way to serve, for the relatively short periods in which it was fed, as a complete and wholesome food.

Tables 1 and 2 give the results of examinations of the intestinal flora of rats receiving the basal or "normal balanced" diet alone. In the first of these tables will be seen the relative numbers of *B. acidophilus*, gas production in the Veillon tubes, and the description of Gram-stained films of the fecal suspensions, while the second table shows the distribution of *B. acidophilus* and other types in the alimentary canal, namely the duodenum, jejunum, ileum, caecum and the colon and rectum. Few bacteria were found above the ileum. In the ileum, and to a still greater extent in the large intestine, there were large numbers of bacteria, with *B. coli* as the dominating organism. *B. acidophilus* was found in the large intestine only, and even here it was comparatively rare. (See Tables 1 and 2, and Charts 1 and 2.)

TABLE 1

RESULTS WITH RATS FED ON THE BASIC DIET

Days after Initial Administra- tion of Diet	B. Acidoph. Per Cent of	Veillon Tubes						Gram-Stained Films from Fecal Suspensions
		—1—		—2—		—3—		
		Gas	Acidoph.	Gas	Acidoph.	Gas	Acidoph.	
Rat 15								
2	1	+++	—	++	—	+	—	Streptococci predom. Many Gram neg. rods. Few Gram pos. rods.
4	0	+++	—	+++	—	++	—	Gram neg. rods and Strep. about equal. Very few Gram pos. rods.
6	0	+++	—	+++	—	++	—	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
8	3	+++	—	+++	—	+	—	Gram neg. rods predom. Many Streptococci. Very few Gram pos. rods.
10	2	+++	—	+++	—	++	—	Gram neg. rods predom. Many Streptococci. Very few Gram pos. rods.
12	0	+++	—	+++	—	++	—	Gram neg. rods strongly predom. Many Streptococci. Few Gram pos. rods.
14	1	+++	—	+++	—	+	—	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
Rat 16								
2	0	+++	—	++	—	+	—	Gram neg. rods and Streptococci about equal. Very few Gram pos. rods.
4	3	+++	—	+++	—	+	—	Gram neg. rods predom. Many Streptococci. Very few Gram pos. rods.
6	1	+++	—	+++	—	+	—	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
8	0	+++	—	+++	—	+	—	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
10	3	++	—	+++	—	+	—	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
12	1	+++	—	+++	—	++	—	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
14	2	+++	—	++	—	+	—	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
Rat 17								
2	3	++	—	++	—	++	—	Diet in Grams—Bread 10, Beef 3
4	1	+++	—	+++	—	++	—	Many Streptococci. Few Gram pos. rods.
6	0	+++	—	+++	—	+	—	Many Streptococci. Few Gram pos. rods.
8	1	+++	—	+++	—	++	—	Many Streptococci. Few Gram pos. rods.
10	3	+++	—	+++	—	++	—	Many Streptococci. Few Gram pos. rods.
12	1	+++	—	+++	—	++	—	Many Streptococci. Few Gram pos. rods.
14	0	+++	—	+++	—	+	—	Many Streptococci. Few Gram pos. rods.

TABLE 2

DISTRIBUTION OF *B. ACIDOPHILUS* IN THE ALIMENTARY CANAL OF RATS
PERIOD OF FEEDING, 15 DAYS

Section of Alimentary Canal	B. Acidoph. Per Cent of	Veillon Tubes		Gram-Stained Films from Intestinal Contents		
		1	2			
		Gas	B. Acidoph.			Gas
Rat 15						
Duodenum	0	++	—	+	Gram neg. rods predom. Few Strep. Only occas. Gram pos. rods. Bact. not numerous.	Diet in Grams—Bread 10, Beef 3
Jejunum	0	++	—	++	Gram neg. rods predom. Few Strep. Occas. Gram pos. rods. Bact. not num.	
Ileum	0	++	—	++	Gram neg. rods predom. Few Strep. Very few Gram pos. rods. Bact. quite numerous.	
Caecum	1	+++	—	++	Gram neg. rods strongly predom. Many Strep. Few Gram pos. rods. Bact. very numerous.	
Colon Rectum	3	+++	—	++	Gram neg. rods strongly predom. Many Strep. Few Gram pos. rods. Bact. very numerous.	
Rat 16						
Duodenum	0	++	—	+	Gram neg. rods predom. Few Strep. Occas. Gram pos. rods. Bact. not num.	Diet in Grams—Bread 10, Beef 3
Jejunum	0	++	—	+	Gram neg. rods predom. Few Strep. Very few Gram pos. rods. Bact. not numerous.	
Ileum	0	++	—	++	Gram neg. rods predom. Few Strep. Very few Gram pos. rods. Bact. not numerous.	
Caecum	3	++	—	++	Gram neg. rods strongly predom. Many Strep. Few Gram pos. rods. Bact. very numerous	
Colon Rectum	2	+++	—	++	Gram neg. rods strongly predom. Many Strep. Few Gram pos. rods. Bact. very numerous.	
Rat 17						
Duodenum	0	++	—	+	Gram neg. rods predom. Few Strep. Occas. Gram pos. rods. Bact. not num.	Diet in Grams—Bread 10, Beef 3
Jejunum	0	++	—	+	Gram neg. rods predom. Few Strep. and Gram pos. rods. Bact. quite num.	
Ileum	2	+++	—	++	Gram neg. rods predom. Few Strep. and Gram pos. rods. Bact. very num.	
Caecum	2	+++	—	+++	Gram neg. rods predom. Many Strep. Few Gram pos. rods. Bact. very num.	
Colon Rectum	8	+++	—	+++	Gram neg. rods predom. Many Strep. Few Gram pos. rods. Bact. very num.	

CHART 1

Curve indicating average percentages of *B. acidophilus* appearing in fecal specimens from rats fed on a "Normal, balanced" diet

CHART 2

Graph indicating average percentages of *B. acidophilus* in the various parts of the alimentary canal of rats fed on a "Normal, balanced" diet

CARBOHYDRATE FEEDING

In all of the experiments with rats the test carbohydrate was added in dry form directly to the regular diet, namely ground bread and meat. The meals were completely consumed. By comparison of the results of daily examinations with each other and with those obtained during the preliminary period the progressive changes in the intestinal flora could be discerned easily. Each of the carbohydrate feeding experiments was carried on for two weeks.

Lactose.—This sugar was fed to sixteen rats. The ingestion of two grams daily effected a radical transformation of the fecal flora from the ordinary mixed type to one almost entirely dominated by *B. acidophilus*. (See Tables 3 and 4.) A progressive change was clearly evident two days after the first administration of lactose, but did not reach its maximum until at least the fourth day. In some instances six to eight days were required for this maximum. The acidophilus phase persisted with very little or no fluctuation as long as the diet remained unchanged.

With the actual increase in the numbers of *B. acidophilus* in the feces there was practically complete suppression or elimination of the types constituting the ordinary mixed flora. Large numbers of the characteristic fluffy colonies of the Moro bacillus appeared in the Veillon tubes and agar plates within a few days after the first administration of the lactose, and there was gradual diminution in the amount of gas production in the Veillon tubes until finally it was entirely absent. *B. bifidus* could be observed only occasionally in these tubes by its more or less typical smooth, disc-shaped colonies occurring in the deeper layers of the whey agar. The direct microscopic examination of Gram-stained films offered additional confirmation of the transition to the acidophilus type of flora. After the first six or eight days the films contained practically nothing but Gram-positive rods. (See Table 3 and Chart 3.)

Table 4 and Chart 4 show the relative numbers of *B. acidophilus* in the different parts of the intestine. The prominence of the aciduric type, particularly in the lower intestine, is again noteworthy.

Dextrin.—The sixteen rats employed in the lactose-feeding experiments were again placed on the basal diet until the simplified flora had completely given way to the usual mixed type. The rats were then given two grams daily of ordinary commercial dextrin, and the feces examined as before. A change in the character of the intestinal flora became apparent within two to four days. The dextrin stimulated the proliferation of *B. acidophilus* in the course of the first four to six days to such an extent as to overshadow all other bacterial types. The typical colonies of *B. acidophilus* became very abundant on the whey agar plates and in the Veillon tubes, often to the exclusion of practically all other forms, and gas was absent from the Veillon tubes. The Gram-

TABLE 3
RESULTS WITH RATS FED SPECIAL DIET CONTAINING LACTOSE

Days after Initial Administra- tion of Diet	B. Acidoph. Per Cent of	Veillon Tubes						<i>Gram-Stained Films from Fecal Suspensions</i>
		—1—	—2—	—3—	Gas	B. Acidoph.	Gas	
Rat 12								
2	39	++	+	+	+	+	+	Diet in Grams—Bread 10, Beef 3, Lactose 2
4	60	+	+	++	+	+	+	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
6	82	—	—	+++	—	++	—	Gram pos. rods predom. Many Gram neg. rods. Few Streptococci.
8	90	—	—	+++	—	+++	—	Gram pos. rods strongly predom. Few Streptococci. and Gram neg. rods.
10	95	—	—	+++	—	+++	—	Gram pos. rods strongly predom. Few Strep. Very few Gram neg. rods.
12	99	—	—	+++	—	+++	—	Practically all Gram positive rods (Acidoph.-like).
14	98	—	—	+++	—	+++	—	Practically all Gram positive rods (Acidoph.-like).
Rat 13								
2	42	++	+	+	+	+	+	Diet in Grams—Bread 10, Beef 3, Lactose 2
4	64	+	+	++	—	+	+	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
6	80	+	—	+++	—	++	—	Gram pos. rods predom. Many Gram neg. rods. Few Strep.
8	94	—	—	+++	—	+++	—	Gram pos. rods strongly predom. Few Streptococci and Gram neg. rods.
10	99	—	—	+++	—	+++	—	Gram pos. rods strongly predom. Very few Streptococci and Gram neg. rods.
12	99	—	—	+++	—	+++	—	Gram pos. rods strongly predom. Very few Streptococci and Gram neg. rods.
14	99	—	—	+++	—	+++	—	Practically all Gram positive rods (Acidoph.-like).
Rat 14								
2	34	++	+	+	+	+	+	Diet in Grams—Bread 10, Beef 3, Lactose 2
4	56	++	+	++	+	+	+	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
6	78	+	—	+++	—	++	—	Gram pos. rods predom. Many Streptococci. and Gram neg. rods.
8	89	—	—	+++	—	+++	—	Gram pos. rods strongly predom. Many Streptococci. Few Gram neg. rods.
10	92	—	—	+++	—	+++	—	Gram pos. rods strongly predom. Few Streptococci and Gram neg. rods.
12	94	—	—	+++	—	+++	—	Gram pos. rods strongly predom. Few Strep. Very few Gram neg. rods.
14	97	—	—	+++	—	+++	—	Practically all Gram positive rods (Acidoph.-like).

TABLE 4

DISTRIBUTION OF B. ACIDOPHILUS IN THE ALIMENTARY CANAL OF RATS
PERIOD OF FEEDING, 15 DAYS

Section of Alimentary Canal	B. Acidoph. Per Cent of	Veillon Tubes				Gram-Stained Films from Intestinal Contents
		—1—		—2—		
		Gas	B. Acidoph.	Gas	B. Acidoph.	
Rat 12						
Duodenum	89	—	++	—	++	Gram pos. rods predom. Few Strep. and Gram neg. rods. Bact. not num.
Jejunum	93	+	+++	—	+++	Gram pos. rods predom. Few Strep. and Gram neg. rods. Bact. not num.
Ileum	91	—	++	—	++	Gram pos. rods strongly predom. Very few Strep. and Gram neg. rods. Bact. quite numerous.
Caecum	99	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like). Bact. very numerous.
Colon Rectum	99	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like). Bact. very numerous.
Rat 13						
Duodenum	83	—	+	—	+	Gram pos. rods predom. Few Strep. and Gram neg. rods. Bact. not num.
Jejunum	88	—	++	—	++	Gram pos. rods predom. Few Strep. and Gram neg. rods. Bact. quite num.
Ileum	96	—	+++	—	+++	Practically all Gram pos. rods (Acidoph.-like). Bact. quite numerous.
Caecum	99	—	+++	—	+++	Practically all Gram pos. rods (Acidoph.-like). Bact. very numerous.
Colon Rectum	99	—	+++	—	+++	Practically all Gram pos. rods (Acidoph.-like). Bact. very numerous.
Rat 14						
Duodenum	87	—	+	—	+	Gram pos. rods predom. Few Strep. and Gram neg. rods. Bact. not num.
Jejunum	92	—	++	—	++	Gram pos. rods predom. Few Strep. and Gram neg. rods. Bact. not num.
Ileum	91	—	+++	—	+++	Gram pos. rods strongly predom. Very few Strep. and Gram neg. rods. Bact. quite numerous.
Caecum	99	—	+++	—	+++	Practically all Gram pos. rods (Acidoph.-like). Bact. very numerous.
Colon Rectum	99	—	+++	—	+++	Practically all Gram pos. rods (Acidoph.-like). Bact. very numerous.

CHART 3

Curve indicating average percentages of *B. acidophilus* appearing in fecal specimens from rats fed on a lactose diet

CHART 4

Graph indicating average percentages of *B. acidophilus* in the various parts of the alimentary canal of rats fed on a lactose diet

stained films frequently appeared to be almost purely Gram-positive rods of the *B. acidophilus* type. (See Table 5 and Chart 5.)

The rise in the acidophilus curve is slightly more marked even when two grams of dextrin are fed than during the administration of the same amounts of lactose, the maximum in the former instance being reached on the eighth day. In other words, dextrin has shown itself to be at least as effective in simplifying the intestinal flora as lactose.

Several of the dextrin-fed rats were killed for the purpose of making bacteriological examinations of different sections of the intestine. *B. acidophilus* was present throughout the intestine, even as high as the duodenum. With the exception of a few Gram-negative rods and streptococci, it was practically the only organism found above the ileo-caecal valve; whereas below this level it was strongly predominant. (See Table 6 and Chart 6.) Gas-forming bacteria appeared to be almost entirely eliminated.

The results obtained thus far demonstrate conclusively that both lactose and dextrin, when fed in sufficient quantities to albino rats, have a most pronounced influence on the character of the intestinal flora, transforming it from the ordinary complex type to one in which the dominant organism is *B. acidophilus*.

When smaller amounts of lactose or dextrin than two grams were fed daily the transformations were less profound. Thus, one gram encouraged the development of *B. acidophilus* to only about 50 per cent of the total fecal flora, as will be seen in subsequent pages (35-40).

Glucose, maltose and sucrose.—None of these sugars, when fed in the same manner and in the same amounts as the lactose and dextrin, exerted any transforming influence upon the intestinal flora. This was clearly brought out in the agar plates, the Veillon tubes and the Gram-stained slides. Throughout these feeding experiments the flora presented the same character as when the rats were kept on the "normal balanced" diet of bread and meat. A total of six rats was employed, two for each sugar. (See Table 7 and Charts 7, 9 and 11.)

Bacteriological examinations of the different levels of the intestine gave negative results also. *B. acidophilus* was absent from the small intestine, and was found below the ileo-caecal valve in numbers only that are normally met with in rats subsisting on the basal diet. (See Table 8 and Charts 8, 10 and 12.)

The above results are in harmony with those of Hull and Rettger (1914b, 1917).

ADMINISTRATION OF LIVING SUSPENSIONS OF

B. ACIDOPHILUS

All of the strains of *B. acidophilus* that were employed in these experiments were isolated from the feces of albino rats during periods of lactose or dextrin feeding. Typical colonies of this organism were

TABLE 5
RESULTS WITH RATS FED SPECIAL DIET CONTAINING DEXTRIN

Days after Initial Administra- tion of Diet	B. Acidoph. Per Cent of	Veillon Tubes						<i>Gram-Stained Films from Fecal Suspensions</i>
		—1—		—2—		—3—		
		Gas	B. Acidoph.	Gas	B. Acidoph.	Gas	B. Acidoph.	
Rat 19								
2	28	++	—	+	—	+	—	Diet in Grams—Bread 10, Beef 3, Dextrin 2
4	52	+	++	+	+	—	++	Gram neg. rods predom. Many Streptococci and Gram pos. rods.
6	88	—	+++	—	+++	—	++	Gram pos. rods predom. Many Streptococci and Gram neg. rods.
8	94	—	+++	—	+++	—	+++	Gram pos. rods predom. Many Streptococci and Gram neg. rods.
10	92	—	+++	—	+++	—	+++	Gram pos. rods strongly predom. Few Streptococci and Gram neg. rods.
12	97	—	+++	—	+++	—	+++	Gram pos. rods strongly predom. Very few Strep. Occas. Gram neg. rods.
14	99	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
Rat 20								
2	32	++	+	+	+	+	+	Diet in Grams—Bread 10, Beef 3, Dextrin 2
4	66	+	++	+	++	—	+	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
6	92	—	+++	—	+++	—	++	Gram pos. rods predom. Many Streptococci and Gram neg. rods.
8	90	—	+++	—	+++	—	+++	Gram pos. rods strongly predom. Few Streptococci and Gram neg. rods.
10	99	—	+++	—	+++	—	+++	Gram pos. rods strongly predom. Few Strep. Occas. Gram neg. rods.
12	96	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
14	98	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
Rat 21								
2	24	++	—	+	—	+	—	Diet in Grams—Bread 10, Beef 3, Dextrin 2
4	68	+	+	+	+	+	+	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
6	91	—	+++	—	+++	—	++	Gram pos. rods strongly predom. Many Gram neg. rods. Few Streptococci.
8	98	—	+++	—	+++	—	+++	Gram pos. rods strongly predom. Few Streptococci and Gram neg. rods.
10	99	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
12	99	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
14	99	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).

picked off whey agar plates, introduced into whey broth and incubated at 37° C. for forty-eight to seventy-two hours. By this simple technique eighteen strains were procured from the rats, and three from human subjects who had been taking 300 grams of lactose daily in connection with their regular diets.

The bacterial suspensions were prepared by washing off with sterile saline solution the surface growths of large whey agar tubes which had been incubated for forty-eight hours at 37° C. These washings were reduced by dilution to the degree of turbidity corresponding to tube five of the McFarland nephelometer. This bacterial concentration was adhered to in all of the feeding experiments in which living bacilli were employed. The final suspensions were added to the basal diet, being thoroughly incorporated in the ground bread. No fewer than three, and as many as eighteen, mixed strains were administered in individual experiments.

The bacterial suspensions were given in amounts varying from one to five cubic centimeters, and the usual bacteriological examinations of the feces made. After several preliminary trials, beginning with five cubic centimeters, two cubic centimeters of the standardized suspension were finally chosen, as less than this amount was found to be inadequate for bringing about a profound change in the intestinal flora.

CHART 5

Curve indicating average percentages of B. acidophilus appearing in fecal specimens from rats fed on a dextrin diet

TABLE 6
DISTRIBUTION OF B. ACIDOPHILUS IN THE ALIMENTARY CANAL OF RATS
PERIOD OF FEEDING, 15 DAYS

Section of Alimentary Canal	B. Acidoph. Per Cent of	Veillon Tubes		Gram-Stained Films from Intestinal Contents	
		Gas	Acidoph. B.	Gas	Acidoph. B.
		1	2		
Rat 19					
Diet in Grams—Bread 10, Beef 3, Dextrin 2					
Duodenum	85	—	++	—	++
Jejunum	90	—	++	—	++
Ileum	95	—	+++	—	+++
Caecum	99	—	+++	—	+++
Colon Rectum	99	—	+++	—	+++
Gram pos. rods predom. Few Gram neg. rods. Occas. Strep. Bact. not num.					
Gram pos. rods strongly pred. Few Strep. and Gram neg. rods. Bact. not num.					
Practically all Gram positive rods (Acidoph.-like). Bacteria very numerous.					
Practically all Gram positive rods (Acidoph.-like). Bacteria very numerous.					
Rat 20					
Diet in Grams—Bread 10, Beef 3, Dextrin 2					
Duodenum	83	—	+	—	+
Jejunum	92	—	++	—	++
Ileum	88	—	+	—	++
Gram pos. rods strongly predom. Few Strep. Occas. Gram neg. rods. Bact. quite numerous.					
Practically all Gram positive rods (Acidoph.-like). Bacteria very numerous.					
Practically all Gram positive rods (Acidoph.-like). Bacteria very numerous.					
Rat 21					
Diet in Grams—Bread 10, Beef 3, Dextrin 2					
Duodenum	78	—	+	—	+
Jejunum	87	+	+	—	+
Ileum	85	—	++	—	++
Caecum	99	—	+++	—	+++
Gram pos. rods strongly predom. Very few Strep. Occas. Gram neg. rods. Bact. very numerous.					
Gram pos. rods strongly predom. Very few Strep. Occas. Gram neg. rods. Bacteria very numerous.					

CHART 6

Graph indicating average percentages of *B. acidophilus* appearing in the various parts of the intestine of rats fed on a dextrin diet

Of the many experiments conducted on the implantation of *B. acidophilus* in the absence of any added carbohydrate the following are presented here as typical. Three rats were placed on the basal diet for ten days; after developing the usual mixed flora, two cubic centimeters of the suspension of living organisms were added daily to the bread and meat. Two days after the first feeding of the suspension *B. acidophilus* was observed in appreciable numbers in the plates and Veillon tubes. The maximum transformation was reached within three to eight days and persisted throughout the experiment. *B. acidophilus* was found to predominate to the same extent as in the experiments in which lactose and dextrin were fed. (See Table 9 and Chart 13.)

Post-mortem examinations of the different sections of the digestive tract revealed the presence of the above organism throughout the intestine. (See Table 10 and Chart 14.) There was a higher proportional representation of *B. acidophilus* in the duodenum and jejunum than in the lactose- and dextrin-feeding experiments; in all other respects the bacteriological findings were essentially the same.

TABLE 7
RESULTS WITH RATS FED SPECIAL DIETS CONTAINING VARIOUS SUGARS

Days after Initial Administra- tion of Diet	B. Acidoph. Per Cent of	Veillon Tubes						<i>Gram-Stained Films from Fecal Suspensions</i>
		—1—		—2—		—3—		
		Gas	B. Acidoph.	Gas	B. Acidoph.	Gas	B. Acidoph.	
Rat 59								
2	2	++	—	++	—	+	—	Gram neg. rods predom. Many Streptococci. Very few Gram pos. rods.
4	6	+++	—	+++	—	+	—	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
6	4	+++	—	+++	—	+	—	Gram neg. rods strongly predom. Many Streptococci. Few Gram pos. rods.
8	4	+++	—	+++	—	+	—	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
10	2	+++	—	+++	—	+	—	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
12	0	+++	—	+++	—	+	—	Gram neg. rods strongly predom. Many Streptococci. Few Gram pos. rods.
14	1	+++	—	++	—	+	—	Gram neg. rods strongly predom. Many Streptococci. Few Gram pos. rods.
Rat 63								
Diet in Grams—Bread 10, Beef 3, Sucrose 2								
2	2	+++	—	++	—	+	—	Streptococci and Gram neg. rods about equal. Few Gram pos. rods.
4	1	+++	—	+++	—	+	—	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
6	5	+++	—	+++	—	+	—	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
8	1	+++	—	+++	—	+	—	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
10	0	+++	—	+++	—	+	—	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
12	3	+++	—	+++	—	—	—	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
14	1	+++	—	+++	—	+	—	Gram neg. rods strongly predom. Few Streptococci. Few Gram pos. rods.
Rat 65								
Diet in Grams—Bread 10, Beef 3, Glucose 2								
2	1	+++	—	++	—	+	—	Gram neg. rods strongly predom. Many Streptococci. Few Gram pos. rods.
4	1	+++	—	++	—	+	—	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
6	3	+++	—	++	—	+	—	Gram neg. rods and Streptococci about equal. Few Gram pos. rods.
8	0	+++	—	+++	—	+	—	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
10	1	+++	—	+++	—	+	—	Gram neg. rods predom. Many Streptococci. Very few Gram pos. rods.
12	2	+++	—	+++	—	+	—	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
14	1	+++	—	++	—	+	—	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.

TABLE 8

DISTRIBUTION OF B. ACIDOPHILUS IN THE ALIMENTARY CANAL OF RATS
PERIOD OF FEEDING, 15 DAYS

Section of Alimentary Canal	B. Acidoph. Per Cent of	Veillon Tubes						Gram-Stained Films from Fecal Suspensions	
		—1—	—2—	—3—	Gas	B. Acidoph.	Gas		
Rat 59									
Duodenum	0	++	—	—	+	—	—	Gram neg. rods predom. Few Strep. Occas. Gram pos. rods. Bact. not num.	
Jejunum	0	++	—	—	++	—	—	Gram neg. rods predom. Few Strep. Occas. Gram pos. rods. Bact. not num.	
Ileum	0	++	—	—	++	—	—	Gram neg. rods predom. Few Strep. and Gram pos. rods. Bact. quite num.	
Caecum	4	+++	—	—	++	—	+	Gram neg. rods predom. Many Strep. Few Gram pos. rods. Bact. quite num.	
Colon Rectum	2	+++	—	—	++	—	+	Gram neg. rods strongly predom. Many Strep. Few Gram pos. rods. Bacteria very numerous.	
Rat 63									
Duodenum	0	++	—	—	+	—	—	Diet in Grains—Bread 10, Beef 3, Sucrose 2	
Jejunum	0	++	—	—	+	—	—	Gram neg. rods predom. Few Strep. Occas. Gram pos. rods. Bact. not num.	
Ileum	0	++	—	—	++	—	+	Gram neg. rods predom. Few Strep. and Gram pos. rods. Bact. quite num.	
Caecum	5	+++	—	—	++	—	+	Gram neg. rods predom. Many Strep. Few Gram pos. rods. Bact. very num.	
Colon Rectum	2	+++	—	—	++	—	+	Gram neg. rods predom. Many Strep. Few Gram pos. rods. Bact. very num.	
Rat 65									
Duodenum	0	++	—	—	+	—	—	Diet in Grains—Bread 10, Beef 3, Glucose 2	
Jejunum	0	++	—	—	+	—	—	Gram neg. rods predom. Few Strep. Very few Gram pos. rods. Bact. not num.	
Ileum	2	++	—	—	+	—	—	Gram neg. rods predom. Few Strep. Very few Gram pos. rods. Bact. not num.	
Caecum	4	+++	—	—	++	—	+	Gram neg. rods predom. Many Strep. and Gram pos. rods. Bact. quite num.	
Colon Rectum	5	+++	—	—	++	—	+	Gram neg. rods predom. Many Strep. Few Gram pos. rods. Bact. very num.	

CHART 7

Curve indicating average percentages of *B. acidophilus* appearing in fecal specimens from rats fed on a diet containing glucose

CHART 8

Graph indicating average percentages of *B. acidophilus* in the various parts of the alimentary canal of rats fed on a diet containing glucose

CHART 9

Curve indicating average percentages of *B. acidophilus* appearing in fecal specimens from rats fed on a diet containing maltose

CHART 10

Graph indicating average percentages of *B. acidophilus* in the various parts of the alimentary canal of rats fed on a diet containing maltose

TABLE 9
RESULTS WITH RATS FED SPECIAL DIET CONTAINING B. ACIDOPHILUS

Days after Initial Administra- tion of Diet	B. Acidoph. Per Cent of	Veillon Tubes						<i>Gram-Stained Films from Fecal Suspensions</i>
		—1—		—2—		—3—		
		Gas	B. Acidoph.	Gas	B. Acidoph.	Gas	B. Acidoph.	
Rat 34								
2	32	++	+	+	+	—	+	Diet—Bread 10, Beef 3 grams, B. Acidophilus (suspension in saline), 2 cc. Gram neg. rods predom. Many Gram pos. rods. Few Streptococci.
4	88	+	++	—	++	—	++	Gram pos. rods predom. Few Streptococci and Gram neg. rods.
6	94	—	+++	—	+++	—	+++	Gram pos. rods predom. Few Streptococci and Gram neg. rods.
8	99	—	+++	—	+++	—	+++	Gram pos. rods strongly predom. Very few Strep. Occas. Gram neg. rods.
10	98	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
12	99	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
14	98	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
Rat 35								
2	36	++	+	+	+	+	+	Diet—Bread 10, Beef 3 grams, B. Acidophilus (suspension in saline), 2 cc. Gram neg. rods predom. Many Streptococci and Gram pos. rods.
4	90	+	++	—	++	—	++	Gram pos. rods predom. Few Streptococci and Gram pos. rods.
6	98	+	+++	—	+++	—	+++	Gram pos. rods strongly predom. Few Streptococci. Occas. Gram neg. rods.
8	94	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
10	95	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
12	99	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
14	99	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
Rat 36								
2	28	++	+	+	+	+	+	Diet—Bread 10, Beef 3 grams, B. Acidophilus (suspension in saline), 2 cc. Gram neg. rods predom. Many Streptococci and Gram pos. rods.
4	84	+	++	+	++	—	++	Gram pos. rods predom. Many Gram neg. rods. Few Streptococci.
6	93	—	+++	—	+++	—	+++	Gram pos. rods strongly predom. Very few Strep. Occas. Gram neg. rods.
8	90	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
10	98	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
12	99	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
14	99	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).

CHART 11

Curve indicating average percentages of *B. acidophilus* appearing in fecal specimens from rats fed on a diet containing sucrose

CHART 12

Graph indicating average percentages of *B. acidophilus* in the various parts of the alimentary canal of rats fed on diet containing sucrose

TABLE 10
DISTRIBUTION OF B. ACIDOPHILUS IN THE ALIMENTARY CANAL OF RATS
PERIOD OF FEEDING, 15 DAYS

Section of Alimentary Canal	B. Acidoph. Per Cent of	Veillon Tubes				Gram Stained Films from Intestinal Contents
		Gas	1	2	Gas	
Rat 34						
Duodenum	82	—	++	—	++	Diet—Bread 10, Beef 3 grams, B. Acidophilus (suspension in saline), 2 cc. Practically all Gram positive rods (Acidoph.-like). Bacteria quite numerous.
Jejunum	88	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like). Bacteria very numerous.
Ileum	92	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like). Bacteria very numerous.
Caecum	99	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like). Bacteria very numerous.
Colon Rectum	98	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like). Bacteria very numerous.
Rat 35						
Duodenum	86	—	++	—	++	Diet—Bread 10, Beef 3 grams, B. Acidophilus (suspension in saline), 2 cc. Gram pos. rods pred. Very few Strep. Occas. Gram neg. rods. Bact. not num.
Jejunum	90	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like). Bacteria quite numerous.
Ileum	96	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like). Bacteria quite numerous.
Caecum	99	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like). Bacteria very numerous.
Colon Rectum	94	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like). Bacteria very numerous.
Rat 36						
Duodenum	88	—	++	—	++	Diet—Bread 10, Beef 3 grams, B. Acidophilus (suspension in saline), 2 cc. Practically all Gram positive rods (Acidoph.-like). Bacteria not numerous.
Jejunum	92	—	++	—	++	Practically all Gram positive rods (Acidoph.-like). Bacteria quite numerous.
Ileum	98	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like). Bacteria quite numerous.
Caecum	99	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like). Bacteria very numerous.
Colon Rectum	99	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like). Bacteria very numerous.

Gram-Stained Films from Intestinal Contents

CHART 13

Curve indicating average percentages of *B. acidophilus* appearing in fecal specimens from rats fed on a diet containing *B. acidophilus*

CHART 14

Graph indicating average percentages of *B. acidophilus* appearing in the various parts of the intestine of rats fed on a diet containing *B. acidophilus*

These experiments indicate that *B. acidophilus* is able to establish itself in the intestine of the white rat when administered per os without any accompanying carbohydrates. In every instance where two cubic centimeters of the suspension were given it became the predominating form throughout the enteric tract, almost completely eliminating or suppressing all of the other bacterial types. The results warrant the assumption that this organism is capable of adapting itself to intestinal conditions and that positive implantation can be brought about.

As will be seen in the following pages, the administration of only one cubic centimeter of the standard *B. acidophilus* suspension did not suffice to maintain for more than six to eight days the characteristic acidophilus flora established through combined *B. acidophilus* and lactose feeding. From the experiments thus far conducted it appears as if amounts of the suspension much smaller than two cubic centimeters do not possess any marked transforming influence.

B. ACIDOPHILUS AND CARBOHYDRATE FEEDING

The purpose of this series of experiments was to determine the combined influence of relatively small amounts of either lactose or dextrin and suspensions of *B. acidophilus* which by themselves had very little or no transforming influence on the intestinal flora. Accordingly, a number of white rats were put on the basal diet until the flora was found to be of the usual mixed type, when they received the different test substances according to a plan involving eighteen individual rat experiments.

Three of the rats were given two grams of lactose daily along with the bread and meat; three received two grams of dextrin each; three, one gram of lactose; three, one gram of dextrin; three, one gram of lactose and one cubic centimeter of *B. acidophilus* suspension, and three one gram of dextrin and one cubic centimeter of the bacterial suspension.

In all of the rats which were fed two grams of the lactose or dextrin there was a marked simplification of the intestinal flora.* This was demonstrated by the examinations of the fecal suspensions and of materials from the different levels of the enteric tract. In striking contrast to these findings, however, were the results obtained in the feeding of one gram of each of these sugars. The small amount of lactose or dextrin stimulated only a partial transformation, and *B. acidophilus* at the best attained a maximum of only about 50 per cent of the total bacterial population. There was no radical suppression of other types of ordinary intestinal microorganisms, and gas formation in the Veillon tubes, while considerably reduced, persisted throughout the experiment. (See Tables 11 to 14, and Charts 15 to 18.)

*The tables are omitted to conserve space. The results were essentially the same as in Tables 3 to 6.

TABLE 11

RESULTS WITH RATS FED SPECIAL DIETS CONTAINING LACTOSE

Days after Initial Administra- tion of Diet	B. Acidoph. Per Cent of	Veillon Tubes						Gram-Stained Films from Fecal Suspensions
		—1—		—2—		—3—		
		Gas	B. Acidoph.	Gas	B. Acidoph.	Gas	B. Acidoph.	
Rat 49								
2	22	++	—	++	—	+	—	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
4	30	+++	—	++	—	—	—	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
6	62	++	+	++	+	—	+	Gram neg. rods predom. Many Streptococci and Gram pos. rods.
8	58	++	+	++	+	+	+	Gram neg. rods predom. Many Streptococci and Gram pos. rods.
10	54	++	+	++	+	—	—	Gram neg. rods predom. Many Streptococci and Gram pos. rods.
12	56	++	+	++	+	—	—	Gram neg. and Gram pos. rods about equal. Few Streptococci.
14	60	++	+	++	+	—	+	Gram neg. rods predom. Many Gram pos. rods. Few Streptococci.
Rat 50								
2	18	++	—	++	—	—	—	Diet in Grams—Bread 10, Beef 3, Lactose 1
4	32	+++	—	++	—	—	—	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
6	42	+++	+	++	—	—	+	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
8	44	++	+	++	+	—	+	Gram neg. rods predom. Many Streptococci and Gram pos. rods.
10	48	++	+	++	+	—	—	Gram neg. rods predom. Many Streptococci and Gram pos. rods.
12	50	++	+	++	+	—	+	Gram pos. and Gram neg. rods about equal. Few Streptococci.
14	46	++	+	++	+	+	—	Gram neg. rods predom. Many Gram pos. rods. Few Streptococci.
Rat 51								
2	24	+++	—	++	—	+	—	Diet in Grams—Bread 10, Beef 3, Lactose 1
4	36	+++	—	++	—	—	—	Streptococci predom. Many Gram neg. rods. Few Gram pos. rods.
6	40	++	+	++	+	—	+	Streptococci predom. Many Gram pos. rods and Gram neg. rods.
8	48	++	+	++	+	—	—	Streptococci and Gram pos. rods about equal. Many Gram neg. rods.
10	42	++	+	++	+	+	—	Streptococci predom. Many Gram pos. rods. Few Gram neg. rods.
12	51	++	+	++	+	—	+	Gram pos. rods predom. Many Streptococci. Few Gram neg. rods.
14	46	++	+	++	+	—	—	Streptococci predom. Many Gram pos. rods. Few Gram neg. rods.

TABLE 12
DISTRIBUTION OF B. ACIDOPHILUS IN THE ALIMENTARY CANAL OF RATS
PERIOD OF FEEDING, 15 DAYS

Section of Alimentary Canal	B. Acidoph. Per Cent of	Veillon Tubes				Gram-Stained Films from Intestinal Contents
		Gas	B. Acidoph.	Gas	B. Acidoph.	
Rat 49						
Duodenum	36	+	—	—	—	Diet in Grams—Bread 10, Beef 3, Lactose 1
						Gram neg. rods predom. Very few Strep. Occas. Gram pos. rods. Bact. not numerous.
Jejunum	48	+	+	—	+	Gram neg. rods predom. Few Strep. and Gram pos. rods. Bact. not num.
Ileum	51	+	+	—	+	Gram neg. rods predom. Few Strep. and Gram pos. rods. Bact. quite num.
Caecum	55	+	+	+	+	Gram neg. rods predom. Many Gram pos. rods. Few Strep. Bact. very num.
Colon Rectum	56	++	+	+	+	Gram neg. rods predom. Many Gram pos. rods. Few Strep. Bact. very num.
Rat 50						
Duodenum	30	+	—	—	—	Diet in Grams—Bread 10, Beef 3, Lactose 1
Jejunum	38	+	+	—	+	Gram neg. rods predom. Few Gram pos. rods. Occas. Strep. Bact. not num.
Ileum	40	+	+	—	+	Gram neg. rods predom. Few Strep. and Gram pos. rods. Bact. not num.
Caecum	48	++	+	+	+	Gram neg. rods predom. Many Gram pos. rods. Few Strep. Bact. quite num.
Colon Rectum	47	++	+	+	+	Gram neg. rods predom. Many Gram pos. rods. Few Strep. Bact. very num.
Rat 51						
Duodenum	38	—	+	—	—	Diet in Grams—Bread 10, Beef 3, Lactose 1
Jejunum	44	—	+	—	+	Streptococci predom. Few Gram neg. and Gram pos. rods. Bact. not num.
Ileum	46	+	+	—	+	Strep. and Gram neg. rods about equal. Few Gram pos. rods. Bact. quite num.
Caecum	48	+	+	—	+	Strep. predom. Many Gram pos. rods. Few Gram neg. rods. Bact. very num.
Colon Rectum	58	++	+	+	+	Strep. predom. Many Gram pos. rods. Few Gram neg. rods. Bact. very num.

CHART 15

Curve indicating average percentages of *B. acidophilus* appearing in fecal specimens from rats fed on a diet containing lactose

CHART 16

Graph indicating average percentages of *B. acidophilus* appearing in the different parts of the intestine of rats fed on a diet containing lactose

TABLE 13

RESULTS WITH RATS FED SPECIAL DIETS CONTAINING DEXTRIN

Days after Initial Administra- tion of Diet	B. Acidoph. Per Cent of	Veillon Tubes				Gram-Stained Films from Fecal Suspensions			
		—1—	—2—	—3—	—4—	Gas	B. Acidoph.	Gas	B. Acidoph.
Rat 52									
2	16	++	—	—	—	—	—	—	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
4	18	+++	—	—	—	—	—	—	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
6	46	++	+	+	+	+	+	+	Gram neg. rods predom. Many Streptococci and Gram pos. rods.
8	44	++	+	+	+	+	—	—	Gram neg. rods predom. Many Streptococci and Gram pos. rods.
10	48	++	+	+	+	+	—	—	Gram neg. rods predom. Many Streptococci and Gram pos. rods.
12	45	++	+	+	+	+	—	—	Gram neg. rods predom. Many Streptococci and Gram pos. rods.
14	52	++	+	+	+	+	—	—	Gram neg. rods predom. Many Streptococci and Gram pos. rods.
Rat 53									
2	22	+++	—	—	—	—	—	—	Streptococci predom. Many Gram neg. rods. Few Gram pos. rods.
4	34	+++	+	+	+	+	—	—	Gram neg. rods and Streptococci about equal. Few Gram pos. rods.
6	40	++	+	+	+	+	—	—	Gram neg. rods predom. Many Streptococci and Gram pos. rods.
8	48	++	+	+	+	+	—	—	Streptococci predom. Many Gram neg. and Gram pos. rods.
10	42	+	+	+	+	+	—	—	Streptococci predom. Many Gram neg. and Gram pos. rods.
12	46	++	+	+	+	+	—	—	Gram neg. rods predom. Many Streptococci and Gram pos. rods.
14	48	++	+	+	+	+	—	—	Gram neg. rods predom. Many Streptococci and Gram pos. rods.
Rat 54									
2	24	+++	—	—	—	—	—	—	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
4	31	+++	—	—	—	—	—	—	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
6	52	++	+	+	+	+	—	—	Gram neg. rods predom. Many Streptococci and Gram pos. rods.
8	50	++	+	+	+	+	—	—	Gram pos. rods and Streptococci about equal. Many Gram neg. rods.
10	52	+	+	+	+	+	—	—	Gram pos. rods predom. Many Streptococci and Gram neg. rods.
12	48	++	+	+	+	+	—	—	Gram neg. rods predom. Many Gram pos. rods. Few Streptococci.
14	51	++	+	+	+	+	—	—	Gram neg. rods predom. Many Gram pos. rods. Few Streptococci.

Diet in Grams—Bread 10, Beef 3, Dextrin 1

Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
 Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
 Gram neg. rods predom. Many Streptococci and Gram pos. rods.
 Gram neg. rods predom. Many Streptococci and Gram pos. rods.
 Gram neg. rods predom. Many Streptococci and Gram pos. rods.
 Gram neg. rods predom. Many Streptococci and Gram pos. rods.
 Gram neg. rods predom. Many Streptococci and Gram pos. rods.

Diet in Grams—Bread 10, Beef 3, Dextrin 1

Streptococci predom. Many Gram neg. rods. Few Gram pos. rods.
 Gram neg. rods and Streptococci about equal. Few Gram pos. rods.
 Gram neg. rods predom. Many Streptococci and Gram pos. rods.
 Streptococci predom. Many Gram neg. and Gram pos. rods.
 Streptococci predom. Many Gram neg. and Gram pos. rods.
 Gram neg. rods predom. Many Streptococci and Gram pos. rods.
 Gram neg. rods predom. Many Streptococci and Gram pos. rods.

Diet in Grams—Bread 10, Beef 3, Dextrin 1

Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
 Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
 Gram neg. rods and Streptococci about equal. Many Gram neg. rods.
 Gram pos. rods predom. Many Streptococci and Gram neg. rods.
 Gram neg. rods predom. Many Gram pos. rods. Few Streptococci.
 Gram neg. rods predom. Many Gram pos. rods. Few Streptococci.

TABLE 14

DISTRIBUTION OF B. ACIDOPHILUS IN THE ALIMENTARY CANAL OF RATS
PERIOD OF FEEDING, 15 DAYS

Section of Alimentary Canal	B. Acidoph. Per Cent of	Veillon Tubes				<i>Gram-Stained Films from Intestinal Contents</i>
		—1—		—2—		
		Gas	B. Acidoph.	Gas	B. Acidoph.	
Rat 52						
Duodenum	48	—	+	—	+	Diet in Grams—Bread 10, Beef 3, Dextrin 1 Strep. predom. Few Gram neg. and Gram pos. rods. Bact. not numerous.
Jejunum	50	—	+	—	+	Strep. and Gram pos. rods about equal. Few Gram neg. rods. Bact. not num.
Ileum	56	+	+	—	+	Gram neg. rods predom. Many Strep. and Gram pos. rods. Bact. quite num.
Caecum	65	+	+	+	+	Gram neg. rods predom. Many Strep. and Gram pos. rods. Bact. very num.
Colon Rectum	68	++	+	+	+	Gram neg. rods predom. Many Strep. and Gram pos. rods. Bact. very num.
Rat 53						
Duodenum	46	+	+	—	+	Diet in Grams—Bread 10, Beef 3, Dextrin 1 Gram neg. rods predom. Few Strep. and Gram pos. rods. Bact. not num.
Jejunum	58	+	+	—	+	Gram neg. rods predom. Few Strep. and Gram pos. rods. Bact. quite num.
Ileum	60	+	+	+	+	Gram neg. rods predom. Many Gram pos. rods. Few Strep. Bact. very num.
Caecum	62	++	+	+	+	Gram neg. rods predom. Many Strep. and Gram pos. rods. Bact. very num.
Colon Rectum	61	++	+	+	+	Gram neg. rods predom. Many Strep. and Gram pos. rods. Bact. very num.
Rat 54						
Duodenum	32	+	—	—	—	Diet in Grams—Bread 10, Beef 3, Dextrin 1 Strep. predom. Few Gram pos. rods. Very few Gram neg. rods. Bact. not numerous.
Jejunum	44	+	+	—	+	Gram pos. rods predom. Few Strep. and Gram neg. rods. Bact. not num.
Ileum	52	+	+	—	+	Gram neg. rods predom. Many Gram pos. rods. Few Strep. Bact. quite num.
Caecum	55	++	+	+	+	Gram neg. rods predom. Many Gram pos. rods. Few Strep. Bact. very num.
Colon Rectum	54	++	+	+	+	Gram neg. rods predom. Many Gram pos. rods. Few Strep. Bact. very num.

CHART 17

Curve indicating average percentages of *B. acidophilus* appearing in fecal specimens from rats fed on a dextrin diet

CHART 18

Graph indicating average percentages of *B. acidophilus* appearing in the different parts of the intestine of rats fed on a diet containing dextrin

When, however, the diets furnishing only one gram of lactose or dextrin were reinforced with one cubic centimeter of the standard suspension of *B. acidophilus*, a very marked transformation of the intestinal flora from the mixed type to one completely dominated by *B. acidophilus* was effected. Within six to eight days after the administration of these diets this organism constituted 90 to 99 per cent of the total flora. The Veillon tubes contained numerous colonies of *B. acidophilus*, and no gas, while the stained films revealed a picture of practically all Gram-positive acidophilus-like bacilli. (See Tables 15 to 18, and Charts 19 to 22.)

The following experiments which extended over a period of thirty days were undertaken to determine whether the ingestion of one cubic centimeter of *B. acidophilus* suspension alone would maintain the simplified character of a flora already established by the feeding of two grams of lactose or dextrin, or the above-mentioned combination of sugar and *B. acidophilus* suspension. Nine rats which had been on the basic diet for five days and had developed the typical mixed flora were divided into three lots of three each. The first lot was given two grams of lactose daily; the second, one gram of lactose and one cubic centimeter of the bacterial suspension; and the third, two cubic centimeters

CHART 19

Curve indicating average percentages of *B. acidophilus* appearing in fecal specimens from rats fed on a diet containing lactose and *B. acidophilus*

TABLE 15

RESULTS WITH RATS FED SPECIAL DIET CONTAINING LACTOSE AND B. ACIDOPHILUS

Days after Initial Administra- tion of Diet	B. Acidoph. Per Cent of	Veillon Tubes						<i>Gram-Stained Films from Fecal Suspensions</i>
		1—	2—	3—	4—	5—	6—	
		Gas	B. Acidoph.	Gas	B. Acidoph.	Gas	B. Acidoph.	
Rat 37								
								Diet—Bread 10, Beef 3, Lactose 1 gram, B. Acidophilus (suspension in saline), 1 cc.
2	38	+	++	—	++	—	++	Gram neg. rods predom. Many Streptococci and Gram pos. rods.
4	92	—	+++	—	+++	—	+++	Gram pos. rods predom. Few Streptococci and Gram neg. rods.
6	99	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
8	96	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
10	97	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
12	99	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
14	95	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
Rat 38								
								Diet—Bread 10, Beef 3, Lactose 1 gram, B. Acidophilus (suspension in saline), 1 cc.
2	30	++	+	—	+	—	+	Gram neg. rods and Streptococci about equal. Few Gram pos. rods.
4	86	+	++	—	++	—	++	Gram pos. rods predom. Few Streptococci and Gram neg. rods.
6	96	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
8	99	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
10	94	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
12	98	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
14	94	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
Rat 39								
								Diet—Bread 10, Beef 3, Lactose 1 gram, B. Acidophilus (suspension in saline), 1 cc.
2	37	+	++	+	++	—	++	Gram neg. rods predom. Many Streptococci and Gram pos. rods.
4	90	—	++	—	++	—	++	Gram pos. rods predom. Few Streptococci and Gram neg. rods.
6	98	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
8	96	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
10	99	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
12	99	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).
14	99	—	+++	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).

TABLE 16
DISTRIBUTION OF B. ACIDOPHILUS IN THE ALIMENTARY CANAL OF RATS
PERIOD OF FEEDING, 15 DAYS

Section of Alimentary Canal	B. Acidoph. Per Cent of	Veillon Tubes				Gram-Stained Films from Intestinal Contents	
		—1—	Gas	B. Acidoph.	—2—		Gas
Rat 37							Diet—Bread 10, Beef 3, Lactose 1 gram, B. Acidophilus (suspension in saline), 1 cc.
Duodenum	97	—	++	—	++	Practically all Gram positive rods (Acidoph.-like).	Bacteria not numerous.
Jejunum	98	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).	Bacteria quite numerous.
Ileum	99	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).	Bacteria very numerous.
Caecum	99	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).	Bacteria very numerous.
Colon Rectum	99	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).	Bacteria very numerous.
Rat 38							Diet—Bread 10, Beef 3, Lactose 1 gram, B. Acidophilus (suspension in saline), 1 cc.
Duodenum	94	—	++	—	++	Practically all Gram positive rods (Acidoph.-like).	Bacteria not numerous.
Jejunum	96	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).	Bacteria quite numerous.
Ileum	98	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).	Bacteria quite numerous.
Caecum	99	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).	Bacteria very numerous.
Colon Rectum	99	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).	Bacteria very numerous.
Rat 39							Diet—Bread 10, Beef 3, Lactose 1 gram, B. Acidophilus (suspension in saline), 1 cc.
Duodenum	90	—	+	—	+	Practically all Gram positive rods (Acidoph.-like).	Bacteria not numerous.
Jejunum	93	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).	Bacteria quite numerous.
Ileum	99	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).	Bacteria very numerous.
Caecum	99	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).	Bacteria very numerous.
Colon Rectum	99	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like).	Bacteria very numerous.

CHART 20

Graph indicating average percentages of *B. acidophilus* appearing in the different parts of the intestine of rats fed on a diet containing dextrin and *B. acidophilus*

of the standard suspension for the first two weeks, followed by one cubic centimeter of the same suspension during the remainder of the experiment.

The results obtained with the different rats during the first fourteen days were very consistent and essentially the same as in previous experiments with the special flora-simplifying diets, *B. acidophilus* becoming the dominant organism and remaining so to the end of the fourteen days' period. At the close of this period one cubic centimeter was substituted for the two cubic centimeters of *B. acidophilus* suspension administered to the third set of rats, and this plan followed for the remaining sixteen days of the experiment. The diet of the other rats remained unchanged.

The daily administration of one cubic centimeter of *B. acidophilus* tended to preserve the simple character of the intestinal population for six to eight days, after which a somewhat sudden reversion to the usual mixed flora took place. (See Chart 23.) On the other hand, the flora of the other six rats remained simplified and completely dominated by *B. acidophilus*. These results lead to the conclusion that in the white rat

TABLE 17

RESULTS WITH RATS FED SPECIAL DIET CONTAINING DEXTRIN AND B. ACIDOPHILUS

Days after Initial Administra- tion of Diet	B. Acidoph. Per Cent of	Veillon Tubes						<i>Gram-Stained Films from Fecal Suspensions</i>
		—1—	—2—	—3—	Gas	B. Acidoph.	Gas	
Rat 22								Diet—Bread 10, Beef 3, Dextrin 1 gram, B. Acidophilus (suspension in saline), 1 cc.
2	36	+	+	+	—	—	—	Gram neg. rods predom. Many Streptococci and Gram pos. rods.
4	72	—	++	++	—	—	—	Gram pos. rods predom. Many Streptococci. Few Gram neg. rods.
6	88	—	+++	+++	—	—	—	Gram pos. rods predom. Few Streptococci and Gram neg. rods.
8	85	—	+++	+++	—	—	—	Gram pos. rods predom. Few Streptococci. Very few Gram neg. rods.
10	94	—	+++	+++	—	—	—	Gram pos. rods strongly predom. Few Streptococci. Very few Gram neg. rods.
12	96	—	+++	+++	—	—	—	Gram pos. rods strongly predom. Very few Strept. Occas. Gram neg. rods.
14	99	—	+++	+++	—	—	—	Practically all Gram positive rods (Acidoph.-like).
Rat 23								Diet—Bread 10, Beef 3, Dextrin 1 gram, B. Acidophilus (suspension in saline), 1 cc.
2	44	++	+	+	+	+	+	Gram neg. rods and Streptococci about equal. Many Gram pos. rods.
4	69	+	++	++	—	—	—	Gram pos. rods predom. Many Streptococci and Gram neg. rods.
6	86	—	+++	+++	—	—	—	Gram pos. rods predom. Few Streptococci and Gram neg. rods.
8	93	—	+++	+++	—	—	—	Gram pos. rods strongly predom. Few Streptococci. Occas. Gram neg. rods.
10	99	—	+++	+++	—	—	—	Gram pos. rods strongly predom. Very few Strept. Occas. Gram neg. rods.
12	97	—	+++	+++	—	—	—	Practically all Gram positive rods (Acidoph.-like).
14	99	—	+++	+++	—	—	—	Practically all Gram positive rods (Acidoph.-like).
Rat 24								Diet—Bread 10, Beef 3, Dextrin 1 gram, B. Acidophilus (suspension in saline), 1 cc.
2	32	++	+	+	+	+	+	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
4	70	+	++	++	—	—	—	Gram pos. rods predom. Many Streptococci and Gram neg. rods.
6	92	—	+++	+++	—	—	—	Gram pos. rods strongly predom. Few Streptococci and Gram neg. rods.
8	99	—	+++	+++	—	—	—	Gram pos. rods strongly predom. Very few Strept. Occas. Gram neg. rods.
10	98	—	+++	+++	—	—	—	Practically all Gram positive rods (Acidoph.-like).
12	99	—	+++	+++	—	—	—	Practically all Gram positive rods (Acidoph.-like).
14	98	—	+++	+++	—	—	—	Practically all Gram positive rods (Acidoph.-like).

at least the ingestion of one cubic centimeter of *B. acidophilus* suspension is insufficient to stabilize and maintain a simplified flora of the purely aciduric type, although two cubic centimeters of the bacterial suspension when fed daily are able, not only to bring about a complete transformation, but also to preserve the acidophilus picture indefinitely, that is as long as the suspension is administered. However, one cubic centimeter of the *B. acidophilus* suspension, which in itself exerts little or no transforming influence, becomes very effective in the course of but a few days after it is reinforced with one gram of lactose or dextrin. The same results are obtained, of course, when one cubic centimeter of the acidophilus suspension is added daily to the diet containing one gram of lactose or dextrin which in itself brings about only partial transformation.

The conclusions arrived at thus far regarding the transforming influence of lactose, dextrin and *B. acidophilus*, when given in sufficient amounts, alone or in combination, were further corroborated by the following experiments. Nine rats harboring the usual mixed flora were divided into three groups of three each. The first group received two grams of dextrin; the second, two cubic centimeters of *B. acidophilus* suspension; and the third, one gram of dextrin and one cubic centimeter of the bacterial suspension. The results were quite uniform throughout

CHART 21

Curve indicating average percentages of *B. acidophilus* appearing in fecal specimens from rats fed on a diet containing dextrin and *B. acidophilus*

TABLE 18

DISTRIBUTION OF B. ACIDOPHILUS IN THE ALIMENTARY CANAL OF RATS
PERIOD OF FEEDING, 15 DAYS

Section of Alimentary Canal	B. Acidoph. Per Cent of	Veillon Tubes					Gram-Stained Films from Intestinal Contents
		—1—	Gas	B. Acidoph.	Gas		
Rat 22							
Duodenum	90	—	—	+++	—	++	Diet—Bread 10, Beef 3, Dextrin 1 gram, B. Acidophilus (suspension in saline), 1 cc. Gram pos. rods strongly predom. Few Gram neg. rods. Occas. Strep. Bact. not numerous.
Jejunum	89	—	—	+++	—	++	Practically all Gram positive rods (Acidoph.-like). Bacteria very numerous.
Ileum	99	—	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like). Bacteria very numerous.
Caecum	99	—	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like). Bacteria very numerous.
Colon Rectum	98	—	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like). Bacteria very numerous.
Rat 23							
Duodenum	88	—	—	++	—	++	Diet—Bread 10, Beef 3, Dextrin 1 gram, B. Acidophilus (suspension in saline), 1 cc. Gram pos. rods predom. Few Strep. and Gram neg. rods. Bact. not numerous.
Jejunum	94	—	—	+++	—	+++	Gram pos. rods strongly predom. Very few Gram neg. rods. Occas. Strep. Bact. quite numerous.
Ileum	92	—	—	++	—	++	Practically all Gram positive rods (Acidoph.-like). Bacteria very numerous.
Caecum	99	—	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like). Bacteria very numerous.
Colon Rectum	97	—	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like). Bacteria very numerous.
Rat 24							
Duodenum	86	+	—	++	—	+	Diet—Bread 10, Beef 3, Dextrin 1 gram, B. Acidophilus (suspension in saline), 1 cc. Gram pos. rods predom. Few Strep. and Gram neg. rods. Bact. not num.
Jejunum	92	—	—	++	—	++	Gram pos. rods strongly predom. Occas. Strep. and Gram neg. rods. Bact. not numerous.
Ileum	96	—	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like). Bacteria very numerous.
Caecum	96	—	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like). Bacteria very numerous.
Colon Rectum	99	—	—	+++	—	+++	Practically all Gram positive rods (Acidoph.-like). Bacteria very numerous.

CHART 22

Graph indicating average percentages of *B. acidophilus* appearing in the different parts of the intestine of rats fed on a diet containing lactose and *B. acidophilus*

CHART 23

Curve indicating average percentages of *B. acidophilus* appearing in fecal specimens from rats fed on a diet containing *B. acidophilus*

the experiment. The transformation of the flora was practically complete in each of the three groups of rats, and *B. acidophilus* was present in the fecal specimens to such an extent as to almost completely exclude all other types. The examination of the different sections of the intestine showed the presence of *B. acidophilus* throughout the length of the digestive tube, and in relatively large numbers.*

B. ACIDOPHILUS VERSUS *B. BULGARICUS* FEEDING

The methods of preparing and administering the suspensions of living organisms in these experiments were identical with those employed in the preceding work. The five strains of *B. bulgaricus* which were used were isolated from commercial preparations placed on the market by five different laboratories. The strains of *B. acidophilus* were the same as those employed in the previous experiments.

Since two cubic centimeters of the standard suspension of *B. acidophilus*, as well as one cubic centimeter of the same suspension reinforced with one gram of lactose, were found to effect profound transformation in the flora of the albino rat, parallel sets of experiments were set up in which the administration of *B. bulgaricus* and attempts to recover this organism from the feces constituted part of the plan.

As it is now a well-known fact that colony formation, morphology, staining properties, etc., are unsatisfactory bases for distinguishing between *B. acidophilus* and *B. bulgaricus*, and that even the milk acidity test is not always dependable, the fermentation test of Rahe (1914) was employed. The pure strains of these two organisms were inoculated into maltose broth adjusted to pH 6.8, and incubated at 37° C. for forty-eight to ninety-six hours. The maltose solution and the broth were sterilized separately. None of the five laboratory strains of *B. bulgaricus* were able to attack the maltose with acid production, while all of the many strains on hand of *B. acidophilus* fermented the sugar with sufficient production of acid to be readily demonstrable. This method of distinguishing between the two types of aciduric bacteria has been of very material assistance in the carrying on of this phase of the investigation.

In all of the experiments in which *B. bulgaricus* was administered its ingestion had absolutely no influence on the character of the intestinal flora. At no time during the entire investigation was *B. bulgaricus* recovered from the feces, regardless of whether the suspension was fed alone or in the presence of lactose as an energy-supplying pabulum. In all of the animals receiving the bulgaricus suspension alone the usual mixed flora prevailed to the end of the feeding experiment. Upon the

*For a complete record of results the reader is referred to the doctorate thesis of H. A. Cheplin in the Yale University Library.

ingestion of one gram of lactose in connection with one cubic centimeter of the bacterial suspension there was an increase in the number of aciduric bacteria in the same way and to the same extent as in the experiments in which one gram of lactose or dextrin alone was fed. The aciduric organisms were found to be *B. acidophilus*, and not *B. bulgaricus*. (See Charts 24 and 25.) The characteristic fluffy colonies were picked off the whey agar plates and inoculated into the maltose broth.

The stimulation of the proliferation of *B. acidophilus*, and not of *B. bulgaricus*, as the result of the ingestion of one gram of lactose and one cubic centimeter of the standard bulgaricus bacillus suspension, is a clear indication that *B. bulgaricus* is incapable of adapting itself to the intestinal environment of the white rat. The intestinal flora of the control rats receiving two cubic centimeters of *B. acidophilus* suspension or one cubic centimeter of the suspension plus one gram of lactose was completely transformed from the complex to the simple aciduric type.

In order to determine whether any of the ingested bulgaricus bacilli survive for any length of time in the intestine, the test animals were killed twenty-four hours after the last feeding of *B. bulgaricus* material, and bacteriological examinations of the different sections of the intestine made. It became quite apparent that *B. bulgaricus* did not survive in any portion of the enteric tract, and the results were the same as when one gram of lactose or dextrin alone was fed.

CHART 24

Curves indicating average percentages of *B. bulgaricus* and *B. acidophilus* appearing in fecal specimens from rats fed on a diet containing lactose and *B. bulgaricus*

CHART 25

Graphs indicating average percentages of *B. bulgaricus* and *B. acidophilus* appearing in the different portions of the intestine of rats fed on a diet containing lactose and *B. bulgaricus*

In the following experiments larger amounts of *B. bulgaricus* suspensions were employed than heretofore, namely two to five cubic centimeters. When these amounts were administered to rats daily without any accompanying carbohydrates no change whatever could be observed in the character of the intestinal flora, and *B. bulgaricus* failed to appear in the feces.

Nine rats which had been on the basal diet for seven days were divided into three groups of three each. One of these groups received two cubic centimeters of the standard suspension of *B. bulgaricus*; another five cubic centimeters of the same material, while the remaining three rats were given two cubic centimeters of the living suspension together with two grams of lactose. The results were similar to those obtained in the preceding experiments. The fecal flora of the first two groups of rats remained apparently unchanged. The ingestion of even five cubic centimeters of *B. bulgaricus* suspension exercised no transforming influence

whatever. (See Tables 19 and 20, and Charts 26 and 27.) In both the plates and the Veillon tubes essentially the same colony picture was obtained as in the preliminary tests when the rats were receiving the basal diet alone. Gas formation in the Veillon tubes was very pronounced at all times. There was some increase in the relative number of Gram-positive rods in the films prepared from the fecal suspensions directly, due in all probability to the passage through the alimentary canal of dead organisms of the *B. bulgaricus* type.

It was impossible, also, to recover *B. bulgaricus* from the feces of the rats that were fed two cubic centimeters of the living bulgaricus suspension together with two grams of the lactose. However, the intestinal flora of these animals was thoroughly simplified, the transformation effect being indistinguishable from that obtained with the feeding of two grams of lactose alone.

Post-mortem examinations, made twenty-four hours after the last feeding, failed to reveal the presence of *B. bulgaricus* in the different parts of the digestive tube. (See Table 20.) Not one of the nine rats receiving the suspensions of *B. bulgaricus* showed any evidence of this organism in any portion of the intestine. On the other hand, all of the animals which received two grams of lactose in connection with two cubic centimeters of the bulgaricus suspension harbored a flora in the feces and at the various levels of the intestinal canal which was wholly dominated by *B. acidophilus*. (See Tables 21 and 22, and Charts 28 and 29.)

The foregoing results corroborate the earlier findings of Hull and Rettger and in themselves most conclusively demonstrate that *B. bulgaricus* cannot be implanted in the intestine of the white rat even when administered in enormous numbers. The decisive results obtained with *B. acidophilus* proved beyond a doubt its adaptability to intestinal conditions and its capability of implantation within the intestinal tract of the albino rat.

SLOW CARBOHYDRATE ABSORPTION IN THE INTESTINE AND ITS RELATION TO *B. ACIDOPHILUS* IMPLANTATION

Of the various carbohydrates fed to white rats lactose and dextrin alone exerted a simplifying action on the intestinal flora, bringing *B. acidophilus* into great prominence. Since this organism is a normal inhabitant of the lower intestine, it was thought that perhaps both lactose and dextrin, in virtue of their slow absorption, reach the colon where they provide optimum cultural and environmental conditions by serving as readily utilizable sources of energy for *B. acidophilus* and the closely related *B. bifidus* of Tissier. According to this theory, the

TABLE 19

RESULTS WITH RATS FED SPECIAL DIET CONTAINING B. BULGARICUS

Days after Initial Administra- tion of Diet	B. Acidoph. Per Cent of	Veillon Tubes						<i>Gram-Stained Films from Fecal Suspensions</i>	
		—1—		—2—		—3—			
		Gas	B. Acidoph.	Gas	B. Acidoph.	Gas	B. Acidoph.		
Rat 43									
2	0	+++	—	++	—	++	—	Diet—Bread 10, Beef 3 grams, B. Bulgaricus (suspension in saline), 5 cc.	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
4	0	+++	—	+++	—	+++	—		Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
6	0	+++	—	+++	—	+++	+		Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
8	0	+++	—	+++	—	+++	+		Gram neg. rods predom. Many Streptococci and Gram pos. rods.
10	0	+++	—	+++	—	+++	++		Gram neg. rods predom. Many Streptococci and Gram pos. rods.
12	0	+++	—	+++	—	+++	++		Gram neg. rods predom. Many Streptococci and Gram pos. rods.
14	0	+++	—	++	—	++	++		Gram neg. rods predom. Many Streptococci and Gram pos. rods.
Rat 44									
2	0	+++	—	+	—	+	—	Diet—Bread 10, Beef 3 grams, B. Bulgaricus (suspension in saline), 5 cc.	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
4	0	+++	—	+++	—	+++	++		Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
6	0	+++	—	+++	—	+++	+		Gram neg. rods and Streptococci about equal. Few Gram pos. rods.
8	0	+++	—	+++	—	+++	++		Gram neg. rods predom. Many Streptococci and Gram pos. rods.
10	0	+++	—	+++	—	+++	++		Gram neg. rods predom. Many Streptococci and Gram pos. rods.
12	0	+++	—	+++	—	+++	+		Gram neg. rods predom. Many Streptococci and Gram pos. rods.
14	0	+++	—	++	—	++	++		Gram neg. rods predom. Many Streptococci and Gram pos. rods.
Rat 45									
2	0	+++	—	++	—	++	—	Diet—Bread 10, Beef 3 grams, B. Bulgaricus (suspension in saline), 5 cc.	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
4	0	+++	—	+++	—	+++	+		Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
6	0	+++	—	+++	—	+++	+		Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
8	0	+++	—	+	—	+	—		Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.
10	0	+++	—	+++	—	+++	++		Gram neg. rods predom. Many Streptococci and Gram pos. rods.
12	0	+++	—	+++	—	+++	++		Gram neg. rods predom. Many Streptococci and Gram pos. rods.
14	0	+++	—	++	—	++	+		Gram neg. rods predom. Many Streptococci and Gram pos. rods.

TABLE 20

DISTRIBUTION OF B. BULGARICUS IN THE ALIMENTARY CANAL OF RATS
PERIOD OF FEEDING, 15 DAYS

Section of Alimentary Canal	B. Bulgar. Per Cent of	Veillon Tubes				<i>Gram-Stained Films from Intestinal Contents</i>
		—1—	B. Bulgar.	Gas	—2—	
Rat 43						
Diet—Bread 10, Beef 3 grams, B. Bulgaricus (suspension in saline), 5 cc.						
Duodenum	0	+	—	+	—	Strep. predom. Few Gram neg. and Gram pos. rods. Bacteria not numerous.
Jejunum	0	++	—	+	—	Strep. predom. Few Gram neg. and Gram pos. rods. Bact. quite numerous.
Ileum	0	+++	—	+++	—	Gram neg. rods predom. Many Strep. and Gram pos. rods. Bact. very num.
Caecum	0	+++	—	+++	—	Gram neg. rods predom. Many Strep. and Gram pos. rods. Bact. very num.
Colon Rectum	0	+++	—	+++	—	Gram neg. rods predom. Many Strep. and Gram pos. rods. Bact. very num.
Rat 44						
Diet—Bread 10, Beef 3 grams, B. Bulgaricus (suspension in saline), 5 cc.						
Duodenum	0	++	—	+	—	Gram neg. rods predom. Few Strep. and Gram pos. rods. Bact. not num.
Jejunum	0	++	—	+	—	Gram neg. rods predom. Few Strep. and Gram pos. rods. Bact. quite num.
Ileum	0	+++	—	+++	—	Gram neg. rods predom. Many Strep. and Gram pos. rods. Bact. very num.
Caecum	0	+++	—	+++	—	Gram neg. rods predom. Many Strep. and Gram pos. rods. Bact. very num.
Colon Rectum	0	+++	—	+++	—	Gram neg. rods predom. Many Strep. and Gram pos. rods. Bact. very num.
Rat 45						
Diet—Bread 10, Beef 3 grams, B. Bulgaricus (suspension in saline), 5 cc.						
Duodenum	0	++	—	+	—	Gram neg. rods predom. Few Strep. and Gram pos. rods. Bact. not num.
Jejunum	0	++	—	+	—	Gram neg. rods predom. Few Strep. and Gram pos. rods. Bact. not num.
Ileum	0	+++	—	+++	—	Gram neg. rods predom. Many Strep. and Gram pos. rods. Bact. very num.
Caecum	0	+++	—	+++	—	Gram neg. rods predom. Many Strep. and Gram pos. rods. Bact. very num.
Colon Rectum	0	+++	—	+++	—	Gram neg. rods predom. Many Strep. and Gram pos. rods. Bact. very num.

CHART 26

Curve indicating average percentages of *B. bulgaricus* appearing in fecal specimens from rats fed on a diet containing *B. bulgaricus*

CHART 27

Graph indicating average percentages of *B. bulgaricus* appearing in the different parts of the intestine of rats fed on a diet containing *B. bulgaricus*

TABLE 21
RESULTS WITH RATS FED SPECIAL DIET CONTAINING LACTOSE AND B. BULGARICUS

Days after Initial Administra- tion of Diet	B. Acidoph. Per Cent of	Veillon Tubes						B. Acidoph. Per Cent of	Gram-Stained Films from Fecal Suspensions	
		1			2					
		Gas	B. Acidoph.	Gas	Gas	B. Acidoph.	Gas			
Rat 46										
2	38	++	+	+	+	+	+	0	Diet—Bread 10, Beef 3, Lactose 2 grams, B. Bulgaricus (suspension in saline), 2 cc.	
4	68	+	++	+	+	+	+	0	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.	
6	94	—	+++	—	+++	—	+++	0	Gram pos. rods predom. Few Streptococci and Gram neg. rods.	
8	93	—	+++	—	+++	—	+++	0	Practically all Gram pos. rods (Acidoph.-like).	
10	98	—	+++	—	+++	—	+++	0	Practically all Gram pos. rods (Acidoph.-like).	
12	99	—	+++	—	+++	—	+++	0	Practically all Gram pos. rods (Acidoph.-like).	
14	98	—	+++	—	+++	—	+++	0	Practically all Gram pos. rods (Acidoph.-like).	
Rat 47										
2	36	++	+	++	+	+	—	+	Diet—Bread 10, Beef 3, Lactose 2 grams, B. Bulgaricus (suspension in saline), 2 cc.	
4	66	++	++	++	++	++	—	+	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.	
6	92	++	++	—	++	++	—	++	Gram neg. and Gram pos. rods about equal. Few Streptococci.	
8	90	+	+++	—	++	++	—	++	Gram pos. rods predom. Few Streptococci and Gram neg. rods.	
10	98	—	+++	—	++	++	—	+++	Practically all Gram pos. rods (Acidoph.-like).	
12	98	—	+++	—	++	++	—	+++	Practically all Gram pos. rods (Acidoph.-like).	
14	98	—	+++	—	++	++	—	+++	Practically all Gram pos. rods (Acidoph.-like).	
Rat 48										
2	34	++	+	++	+	+	—	+	Diet—Bread 10, Beef 3, Lactose 2 grams, B. Bulgaricus (suspension in saline), 2 cc.	
4	62	++	+	+	+	+	—	+	Gram neg. rods predom. Many Streptococci. Few Gram pos. rods.	
6	88	+	++	—	++	++	—	++	Gram neg. rods predom. Many Gram pos. rods. Few Streptococci.	
8	90	—	+++	—	++	++	—	++	Gram pos. rods predom. Few Gram neg. rods and Streptococci.	
10	96	—	+++	—	++	++	—	++	Gram pos. rods predom. Few Streptococci. Very few Gram neg. rods.	
12	99	—	+++	—	++	++	—	+++	Practically all Gram pos. rods (Acidoph.-like).	
14	94	—	+++	—	++	++	—	+++	Practically all Gram pos. rods (Acidoph.-like).	

TABLE 22

DISTRIBUTION OF B. ACIDOPHILUS IN THE ALIMENTARY CANAL OF RATS
PERIOD OF FEEDING, 15 DAYS

Section of Alimentary Canal	B. Acidoph. Per Cent of	Veillon Tubes				Per Cent of B. Bulgar.	Gram-Stained Films from Intestinal Contents
		—1—		—2—			
		Gas	B. Acidoph.	Gas	B. Acidoph.		
Rat 46							
Duodenum	84	—	++	—	+	0	Diet—Bread 10, Beef 3, Lactose 2 grams, B. Bulgaricus (suspension in saline), 2 cc. Gram pos. rods predom. Very few Strep. Occas. Gram neg. rods. Bact. not numerous.
Jejunum	88	—	++	—	+	0	Gram pos. rods strongly predom. Very few Strep. Occas. Gram neg. rods. Bacteria quite numerous.
Ileum	90	—	+++	—	+++	0	Practically all Gram pos. rods (Acidoph.-like). Bacteria very numerous.
Caecum	99	+	+++	—	+++	0	Practically all Gram pos. rods (Acidoph.-like). Bacteria very numerous.
Colon Rectum	99	+	+++	—	+++	0	Practically all Gram pos. rods (Acidoph.-like). Bacteria very numerous.
Rat 47							
Duodenum	80	—	+	—	+	0	Diet—Bread 10, Beef 3, Lactose 2 grams, B. Bulgaricus (suspension in saline), 2 cc. Gram pos. rods predom. Few Strep. Occas. Gram neg. rods. Bact. not numerous.
Jejunum	82	—	++	—	++	0	Gram pos. rods predom. Very few Strep. Occas. Gram neg. rods. Bact. not numerous.
Ileum	91	—	+++	—	+++	0	Gram pos. rods strongly predom. Occas. Strep. and Gram neg. rods. Bact. very numerous.
Caecum	98	—	+++	—	+++	0	Practically all Gram pos. rods (Acidoph.-like). Bacteria very numerous.
Colon Rectum	96	—	+++	—	+++	0	Practically all Gram pos. rods (Acidoph.-like). Bacteria very numerous.
Rat 48							
Duodenum	87	—	++	—	++	0	Diet—Bread 10, Beef 3, Lactose 2 grams, B. Bulgaricus (suspension in saline), 2 cc. Gram pos. rods predom. Very few Strep. Occas. Gram neg. rods. Bact. not numerous.
Jejunum	90	—	+++	—	+++	0	Gram pos. rods strongly predom. Occas. Strep. and Gram neg. rods. Bact. not numerous.
Ileum	92	—	+++	—	+++	0	Practically all Gram pos. rods (Acidoph.-like). Bacteria quite numerous.
Caecum	95	—	+++	—	+++	0	Practically all Gram pos. rods (Acidoph.-like). Bacteria very numerous.
Colon Rectum	98	—	+++	—	+++	0	Practically all Gram pos. rods (Acidoph.-like). Bacteria very numerous.

other carbohydrates used would disappear from the enteric tract before the colon is reached and hence would not offer the favorable pabulum to the aciduric organisms.

To establish the validity of the above assumption, at least in so far as the availability of the carbohydrates in the lower intestine is concerned, the following experiments were conducted in addition to the examination of fecal specimens for reducing substances. A number of rats which had been receiving one or another of the carbohydrates, lactose, dextrin, maltose, sucrose and glucose, as well as control rats subsisting on the basic diet alone, were killed and careful examinations made of the contents of the caecum and colon for the presence of reducing substances and the corresponding predominant bacterial types.

The contents of both the caecum and colon of each rat, in amounts ranging from one half to one gram, were placed in large test tubes containing twenty cubic centimeters of water and broken glass and shaken until a fairly homogeneous suspension was obtained. The tubes were then heated in a boiling water bath for ten minutes to hasten the extraction of the carbohydrate from the suspended particles. After thorough centrifugation the supernatant fluid was filtered twice. In

CHART 28

Curves indicating average percentages of B. bulgaricus and B. acidophilus appearing in fecal specimens from rats fed on a diet containing lactose and B. bulgaricus

CHART 29

Graphs indicating average percentages of *B. bulgaricus* and *B. acidophilus* appearing in different parts of the intestine of rats fed on a diet containing lactose and *B. bulgaricus*

executing the tests for reducing substances two cubic centimeters of the filtrate were added to five cubic centimeters of Benedict's solution, the mixture boiled for one minute, and allowed to remain in the refrigerator for ten to twelve hours before taking the final readings.* The bacteriological tests were carried out in the usual manner.

The feces and the fecal material from the caecum and colon of the lactose- and dextrin-fed rats gave positive reduction tests with the Benedict solution, and revealed a completely transformed flora dominated almost entirely by *B. acidophilus*. On the other hand, the samples from the rats which received maltose, sucrose or glucose gave the same results as those of the control animals which received the basal

* In the testing for lactose heating with dilute acid was resorted to for inversion of the sugar.

60 TRANSFORMATION OF THE INTESTINAL FLORA

diet only. There was no reduction of Benedict's solution and the intestinal flora was of the usual mixed type. (See Table 23.)

According to the above results neither lactose nor dextrin is completely absorbed from the intestine of albino rats when two grams are fed daily. In other experiments, reduction was obtained with the fecal specimens of rats which received one gram of lactose or dextrin daily together with one cubic centimeter of *B. acidophilus* suspension. The reduction tests were not as pronounced, however, as in the experiments described here.

The demonstration of incomplete disappearance of lactose and dextrin before they reach the colon offers some foundation to the avowed assumption that these carbohydrates favor the proliferation of *B. acidophilus* in the lower intestine because they serve as pabulum which is readily available to this organism for energy requirement and thus establish for it an optimum environment. Maltose, sucrose and glucose disappear from the intestine before they reach the ileo-caecal valve, and therefore do not establish a favorable environment for the aciduric group of organisms.

TABLE 23

THE RELATION OF DIET TO REDUCING CARBOHYDRATES AND TO THE BACTERIAL FLORA IN FECAL MATERIAL FROM THE CAECUM AND COLON OF RATS

RAT	DIET IN GRAMS	PERIOD OF FEEDING	REDUCTION OF BENEDICT'S SOLUTION	PER CENT OF BACIDOPH.
55	CONTROL	15 Days	—	4
56	Bread 10 Beef 3			1
57	Bread 10 Beef 3	"	+	98
58	Lactose 2			96
59	Bread 10 Beef 3	"	—	2
60	Maltose 2			3
61	Bread 10 Beef 3	"	+	97
62	Dextrin 2			99
63	Bread 10 Beef 3	"	—	2
64	Sucrose 2			6
65	Bread 10 Beef 3	"	—	5
66	Dextrose 2			4

There appears to be, then, a definite correlation between the rate of absorption in the alimentary canal of a utilizable carbohydrate and its tendency to effect, when administered per os, a transformation of the intestinal flora from the ordinary complex type to one in which the dominating organism is *B. acidophilus*. This conclusion is further strengthened by the results of the following observations.

RELATION OF HYDROGEN ION CONCENTRATION TO THE CHARACTER OF THE INTESTINAL FLORA

As has been stated in the historical résumé of this paper, the possible relation of acidity of the intestinal tract to the prevailing flora has been a subject of much debate. Metchnikoff's theory underlying the alleged implantation of *B. bulgaricus* rests largely on the assumption that this organism exerts its remedial influence on the host because of the acid production which it causes within the enteric tract, particularly the large intestine.

In the present study the hydrogen ion concentrations of the feces and of the contents of the intestine at different levels were determined partly by the Sørensen indicator method as modified by Henderson and Palmer (1912-1913) and partly with the aid of the colorimetric standards prepared according to the description of Clark and Lubs (1917). In preparing the fecal extracts for the determinations the general plan first suggested by Howe and Hawk (1912) and later employed by Nelson and Williams (1916-1917) was followed, with the exception that 500 milligrams of the fresh feces in place of two grams, and that twenty cubic centimeters of practically neutral water instead of fifty cubic centimeters of N/2 sodium sulphate, were employed.

The filtered fecal extracts were usually colored light yellow to brown, and gave very little if any sediment upon standing. Contrary to the experiences of Howe and Hawk, comparatively sharp readings could be made. Four color indicators were used, namely brom thymol blue, brom cresol purple, methyl red and sodium alizarine sulphonate, the first two being chosen because they offer more distinguishable shades of color within the same range of pH than does para-nitrophenol which was used by Nelson and Williams (1916-1917). Owing to the wide color range of sodium alizarine sulphonate (4.7 to 8.0), preliminary tests were made with this agent to determine the narrow ranges within which the reactions fell, and thus to facilitate the more exact determinations made with the other indicators. Brom thymol blue and brom cresol purple often serve as excellent checks for each other.

Altogether 146 hydrogen ion concentration determinations were made. The results of some of the individual tests are given in Table 24. The regular bacteriological examinations and reduction tests with

Benedict's solution were made in connection with the hydrogen ion determinations.

The results show clearly that, while the hydrogen ion concentration varies normally within certain limits, the figures for the rats harboring a simplified flora strongly dominated by *B. acidophilus* run almost parallel with those of the animals having the usual mixed flora. The hydrogen ion concentration limits remain practically the same.

It was found also, in association with the above tests, that the feces of the lactose- and dextrin-fed rats contained reducing carbohydrates and a corresponding flora consisting almost entirely of *B. acidophilus*, thus further emphasizing the observed relationship existing between incomplete absorption of the carbohydrates and simplification of the flora through dextrin and lactose feeding.

CONCLUSIONS

Diet is a controlling factor in the regulation of bacterial activities in the intestinal canal of albino rats.

B. acidophilus of Moro is a common inhabitant of the alimentary tract of the white rat.

Lactose and dextrin, when given in sufficient amounts, bring about a marked transformation of the intestinal flora in which *B. acidophilus* assumes particular prominence and may even completely supplant all other types of bacteria. Maltose, glucose and sucrose, on the other hand, have very little or no tendency to induce such a change.

The same results are obtained when one gram of lactose or dextrin is reinforced with one cubic centimeter of the standard *B. acidophilus* suspension as by the daily feeding of two grams of either of these carbohydrates alone in conjunction with the basal diet of bread and meat. In both instances the bacterial transformation to the simple aciduric type is practically complete.

The daily administration of two cubic centimeters of the *B. acidophilus* suspension (nephelometer five) without the addition of the carbohydrates also leads to a profound change in the character of the flora of the white rat, and an implantation of *B. acidophilus* to the extent of 90 to 99 per cent of the total flora is effected.

The simple character of the new flora persists so long as one or another of the above diets or preparations is continued, but reverts gradually to the normal or usual mixed type within five or six days after a return to the basal diet.

B. bulgaricus, which is not an intestinal organism, is unable under any circumstance to establish itself in the alimentary canal of the albino rat. Even the daily administration of five cubic centimeters of the *B. bulgaricus* suspension exerts no influence on the bacterial population of the intestine.

There appears to be a definite relation between the rate of absorption or digestion in the alimentary canal of a utilizable carbohydrate and its tendency to effect a transformation. Only those carbohydrates which reach the caecum and colon unchanged have a transforming influence.

The process of elimination of the ordinary mixed flora and the establishment of *B. acidophilus* apparently does not depend on any changes in the hydrogen ion concentration within the intestine.

III. FEEDING AND IMPLANTATION EXPERIMENTS WITH HUMAN SUBJECTS

THE following experiments followed closely upon those which were conducted on white rats and which form the subject matter of the preceding chapter. Very little difficulty was experienced in procuring volunteers, most of whom were graduate students and assistants in the laboratory. Thus far in the investigation seventeen human subjects have been employed of which fifteen were apparently normal and two had a long history of intestinal disturbances. Altogether 600 stools were examined. Most of the subjects served in more than one experiment, bringing the total number of individuals up to forty-six.

The attempts to duplicate the results obtained with white rats were in a very large measure successful. The investigation resolved itself into numerous phases in almost all of which the chief aim was to establish a *Bacillus acidophilus* flora in the intestinal tract, either by the administration, in conjunction with the ordinary daily diet, of special carbohydrates, namely lactose and dextrin, or of living and viable cultures of *B. acidophilus* in whey broth or in milk, or of a combination of either of these carbohydrates with the living cultures.

Following a brief period of preliminary observation, and after several bacteriological examinations of the stools had been made, the subjects entered upon a period of daily administration of the above-mentioned test materials, which usually extended over ten days, and in some instances twenty and thirty days. The feces were collected in paraffined paper containers, and immediately brought to the laboratory where they were placed in the refrigerator, and examined on the same day. Daily examinations were made, the technique being essentially the same as that employed in connection with the feeding experiments on rats except that the fecal suspensions for the inoculation of whey agar for plating and of the Veillon tubes were made up to match tubes ten to twelve of the nephelometer scale, instead of eight, and that the bacteria on the Gram-stained slides were counted and the numbers of the different types given in terms of percentage. The greater turbidity was found desirable owing to the decidedly smaller number of viable bacteria present in human as compared with rat feces.

The various tables indicate a fairly close correlation between the results obtained through cultural procedures and the direct counts of the Gram-stained films prepared from the fecal suspensions. The

cultural tests furnish the more reliable information as to the actual bacterial activities within the alimentary canal, owing partly to the uncertainty of identification of the more important organisms by morphology alone, and partly to the fact that an examination of the Gram-stained film can offer no clue regarding the viability of the bacteria. The relative number of viable bacteria in human feces, as compared with the total bacterial content, is extremely small, as has been shown by various investigators. McNeal, Latzer and Kerr (1909) have estimated it at about one to three thousand. The absence in some instances of complete correlation between the results of the direct and indirect methods of determination of intestinal flora is in all probability due to an unusually large proportion of dead bacteria.

BASAL DAILY DIET

In order to determine the influence of the ordinary daily diet on the types of bacteria commonly developing within the alimentary canal the stools of subjects C and I were examined for ten and fifteen days respectively. Throughout the observation period these individuals were found to harbor the ordinary complex flora. (See Tables 25 and 26.) *B. acidophilus* was present in extremely small numbers. Subjects A and D, who had been consuming as part of their regular daily diet one half to one quart of milk daily for weeks and perhaps months prior to the experiments, were instructed to continue the use of a quart of milk daily for ten days. These subjects carried a relatively high proportion of *B. acidophilus*, and the amount of gas production in the Veillon tubes was considerably less than in the tubes of C and I. Upon the removal of the milk from the daily regimen the percentage of *B. acidophilus* dropped gradually until there was a complete reversion of the fecal flora to the ordinary mixed type as seen in subjects C and I. (See Tables 27 and 28, and Chart 30.)

The examinations of the stools of subjects A and D supported the claims of Hull and Rettger and others that *B. acidophilus* is a normal inhabitant of the intestinal tract of man. We have had no difficulty in later experiments to demonstrate again and again this important and fundamental fact. Under ordinary conditions of diet, however, the organism is present in very small numbers, and requires a dietary stimulus or bacterial reinforcement to incite it to increased activity.

The ordinary daily diets of the various subjects employed in this investigation have been well suited for studying the simplifying influence of the various test agents, inasmuch as they encourage the development of the so-called putrefactive and gas-producing organisms, and only in a very slight degree or not at all *B. acidophilus*. With such diets as the starting points, the administration of the various test materials which have a transforming influence in the direction of simplification of flora

should make possible an easy recognition of change in the bacterial types.

No efforts have been made in any phase of the present investigation on man to prescribe a definite diet. It was assumed that if changes in the character of the intestinal flora can be brought about without following any definite or limited dietary scheme, it should be relatively much easier to control the bacterial types by limiting the diet, as for example by reducing the amount of protein food consumed.

TABLE 25—SUBJECT C

ORDINARY DAILY DIET

<i>Days After Initial Administration of Diet</i>	<i>Per Cent of B. Acidoph.</i>	<i>Gram-Stained Films from Fecal Suspensions</i>					
		<i>Veillon Tubes</i>				<i>Total Organisms</i>	
		<i>—1—</i>		<i>—2—</i>		<i>Per Cent Gram-Positive</i>	<i>Gram-Positive Organisms</i>
		<i>Gas</i>	<i>B. Acidoph.</i>	<i>Gas</i>	<i>B. Acidoph.</i>		
1	0	+++	—	+++	—	15	85
2	2	+++	—	+++	—	14	86
3	3	+++	—	+++	—	21	79
4	0	+++	—	+++	—	24	76
5	1	+++	—	+++	—	30	70
6	4	+++	—	+++	—	28	62
7	6	+++	—	+++	—	25	75
8	12	+++	—	+++	—	32	68
9	6	+++	—	+++	—	32	68
10	4	+++	—	+++	—	38	62
						<i>Per Cent Gram-Negative</i>	<i>Gram-Positive Organisms</i>
							<i>Per Cent Rods</i>
							<i>Per Cent Cocci</i>

TABLE 26—SUBJECT I

ORDINARY DAILY DIET

1	2	+++	—	+++	—	23	77	10	13
2	1	+++	—	+++	—	26	74	12	14
3	0	+++	—	+++	—	41	59	14	27
4	6	+++	—	+++	—	37	63	12	25
5	1	+++	—	+++	—	30	70	10	20
6	3	+++	—	+++	—	31	69	12	19
7	0	+++	—	+++	—	28	72	8	20
8	4	+++	—	+++	—	34	66	12	22
9	0	+++	—	+++	—	27	73	9	18
10	2	+++	—	+++	—	36	64	14	22
11	2	+++	—	+++	—	29	71	11	18
12	0	+++	—	+++	—	35	65	10	25
13	0	+++	—	+++	—	24	76	8	16
14	4	+++	—	+++	—	33	67	12	21
15	0	+++	—	+++	—	38	62	14	24

TABLE 27—SUBJECT A

ORDINARY DAILY DIET, INCLUDING SWEET MILK 1000 cc.

<i>Days After Initial Administration of Diet</i>	<i>Per Cent of B. Acidoph.</i>	<i>Gram-Stained Films from Fecal Suspensions</i>							
		<i>Veillon Tubes</i>				<i>Total Organisms</i>		<i>Gram-Positive Organisms</i>	
		<i>—1—</i>		<i>—2—</i>		<i>Per Cent Gram-Positive</i>	<i>Per Cent Gram-Negative</i>	<i>Per Cent Rods</i>	<i>Per Cent Cocci</i>
		<i>Gas</i>	<i>B. Acidoph.</i>	<i>Gas</i>	<i>B. Acidoph.</i>				
1	12	+++	—	++	—	32	68	12	20
2	30	+++	+	++	+	38	62	20	18
3	78	++	++	+	++	60	40	40	20
4	65	++	+	+	+	52	48	34	18
5	80	+	++	+	++	65	35	50	15
6	85	+	+++	—	+++	62	38	48	14
7	75	+	++	—	++	64	36	46	18
8	86	+	+++	—	+++	55	45	30	25
9	60	++	+	+	+	58	42	32	26
10	72	++	++	+	++	54	46	35	19

ORDINARY DAILY DIET WITHOUT MILK

11	70	++	+	+	+	46	54	28	18
12	22	+++	—	++	—	40	60	16	24
13	12	+++	—	++	—	38	62	18	20
14	3	+++	—	+++	—	36	64	16	20
15	2	+++	—	+++	—	35	65	16	19

TABLE 28—SUBJECT D

ORDINARY DAILY DIET, INCLUDING SWEET MILK 1000 cc.

1	10	+++	—	+++	—	21	79	8	13
2	5	++++	—	+++	—	30	70	18	12
3	3	++++	—	+++	—	32	68	15	17
4	6	+++	—	++	—	27	73	12	15
5	12	+++	—	+++	—	34	66	16	18
6	18	+++	—	++	—	38	62	17	21
7	42	+++	—	++	—	48	52	29	19
8	62	++	+	+	+	54	46	32	22
9	69	++	+	++	+	51	49	31	20
10	78	+	++	+	++	58	42	36	22

ORDINARY DAILY DIET WITHOUT MILK

11	8	+++	—	++	—	35	65	12	23
12	10	+++	—	++	—	34	66	15	19
13	18	+++	—	++	—	31	69	10	21
14	12	++++	—	+++	—	30	70	10	20
15	2	++++	—	+++	—	28	72	12	16

CHART 30

CARBOHYDRATE FEEDING

In all of the carbohydrate ingestion experiments on man either lactose or dextrin was employed. These substances were administered three times daily in 100 gram quantities, except when the total amount per day was only 150 grams, in which case they were consumed in one dose. The lactose was taken in the form of a heavy aqueous suspension, while the dextrin was used in 50 per cent solution prepared by boiling the dextrin in water. Owing to its unpleasant taste the dextrin was flavored with a little orange juice. These carbohydrates were taken between meals.

TRANSFORMING INFLUENCE OF LACTOSE

This sugar was administered to six persons, and the results were fairly uniform. The ingestion of 300 grams of lactose, in addition to the ordinary daily diet, by subjects A, C, D and L effected a radical transformation of the fecal flora within four to eight days, from the usual mixed type to one dominated almost entirely by *B. acidophilus*. The essentially aciduric flora persisted as long as the use of lactose was continued. (See Tables 29 to 32, and Chart 31.) It will be seen, however (Table 33 and Chart 31), that the flora of subject B, a sturdy individual weighing almost 200 pounds, failed to respond to the influence of 300 grams of lactose taken daily for ten days, whereas the ingestion of 400 grams of the sugar brought about a complete simplification of the intestinal flora within four days.

The use of 150 grams of lactose daily by subject I (Table 34 and Chart 32) resulted in an appreciable multiplication of *B. acidophilus*, but there was no radical suppression of the other types of intestinal bacteria.

The simplification of the intestinal flora was characterized by an enormous relative and actual increase in the numbers of *B. acidophilus* appearing in the stools, and the almost complete suppression and elimination of the other bacterial types ordinarily present. After the expiration of the first four days following the initial administration of the carbohydrate, the characteristic colonies of *B. acidophilus* were found to dominate in the whey agar plates and at times were practically the only colonies present. The Veillon tubes very strikingly indicated a gradual diminution in the numbers of gas-producing organisms. These observations were further confirmed by the increase in the relative numbers of Gram-positive rods in the Gram-stained slides.

TRANSFORMING INFLUENCE OF DEXTRIN

The ingestion of 300 grams of dextrin exerted a marked transforming action on the intestinal flora, stimulating the proliferation of *B.*

TABLE 29—SUBJECT A

ORDINARY DAILY DIET + LACTOSE 300 GMS.

Days After Initial Administration of Diet	Per Cent of B. Acidoph.	Gram-Stained Films from Fecal Suspensions							
		Veillon Tubes				Total Organisms		Gram-Positive Organisms	
		—1—		—2—		Per Cent Gram-Positive	Per Cent Gram-Negative	Per Cent Rods	Per Cent Cocci
		Gas	B. Acidoph.	Gas	B. Acidoph.				
1	6	+++	—	+++	—	38	62	16	22
2	62	+++	++	++	+	46	54	30	16
3	60	+++	++	++	+	48	52	36	12
4	92	+	+++	+	+++	68	32	51	17
5	99	+	+++	—	+++	78	22	62	16
6	94	+	+++	—	+++	80	20	68	12
7	92	+	+++	—	+++	84	16	70	14
8	98	—	+++	—	+++	88	12	76	12
9	99	—	+++	—	+++	85	15	72	13
10	97	—	+++	—	+++	86	14	76	10

TABLE 30—SUBJECT C

ORDINARY DAILY DIET + LACTOSE 300 GMS.

1	0	+++	—	+++	—	25	75	10	15
2	0	+++	—	+++	—	28	72	12	16
3	2	+++	—	+++	—	34	66	14	20
4	21	+++	—	+++	—	40	60	18	28
5	30	+++	+	++	+	42	58	29	13
6	28	+++	+	++	+	41	59	27	14
7	62	+++	+	++	+	43	57	30	13
8	95	++	+++	+	+++	62	38	50	12
9	89	+	+++	+	+++	66	34	52	14
10	91	+	+++	—	+++	65	35	51	14

TABLE 31—SUBJECT D

ORDINARY DAILY DIET + LACTOSE 300 GMS.

1	0	+++	—	+++	—	30	70	12	18
2	32	+++	+	++	+	52	48	40	12
3	84	+	+++	+	+++	64	36	48	16
4	92	—	+++	—	+++	72	28	58	14
5	90	—	+++	—	+++	70	30	52	18
6	99	—	+++	—	+++	87	13	72	15
7	94	+	+++	—	+++	88	12	78	10
8	99	—	+++	—	+++	90	10	78	12
9	94	—	+++	—	+++	86	14	72	14
10	99	—	+++	—	+++	89	11	73	16

CHART 31

acidophilus within three days to such an extent as to considerably overshadow all of the other bacterial types. (See Table 35.) Similar to the results of lactose feeding, 90 to 99 per cent of the colonies on the whey agar plates were those of *B. acidophilus*. *Acidophilus* colonies were very abundant also in the Veillon tubes in which there was no gas production. Direct examinations of the Gram-stained slides offered

TABLE 32—SUBJECT L

ORDINARY DAILY DIET + LACTOSE 300 GMS.

Days After Initial Administration of Diet	Per Cent of <i>B. Acidoph.</i>	Gram-Stained Films from Fecal Suspensions							
		Veillon Tubes				Total Organisms		Gram-Positive Organisms	
		—1—		—2—		Per Cent Gram-Positive	Per Cent Gram-Negative	Per Cent Rods	Per Cent Cocci
		Gas	<i>B. Acidoph.</i>	Gas	<i>B. Acidoph.</i>				
1	0	+++	—	+++	—	26	74	10	16
2	0	+++	—	+++	—	38	62	14	24
3	0	+++	—	+++	—	35	65	16	19
4	2	+++	—	+++	—	36	64	15	21
5	0	+++	—	+++	—	38	62	18	20
6	15	+++	—	+++	—	40	60	22	18
7	37	+++	+	+++	+	44	56	24	20
8	70	+++	+	++	+	62	38	48	14
9	89	+++	++	++	+	68	32	50	18
10	87	++	++	+	++	69	31	52	17

TABLE 33—SUBJECT B

ORDINARY DAILY DIET + LACTOSE 300 GMS.

1	0	+++	—	+++	—	34	66	12	22
2	0	+++	—	+++	—	30	70	10	20
3	6	+++	—	+++	—	42	58	16	26
4	1	+++	—	+++	—	32	68	15	17
5	18	+++	—	+++	—	30	70	14	16
6	3	+++	—	+++	—	38	62	18	20
7	26	+++	—	+++	—	44	56	20	24
8	12	+++	—	+++	—	48	52	26	22
9	10	+++	—	+++	—	40	60	22	18
10	8	+++	—	+++	—	46	54	28	18

ORDINARY DAILY DIET + LACTOSE 400 GMS.									
11	16	+++	—	+++	—	45	55	25	20
12	48	+++	+	++	+	68	32	40	28
13	60	+++	+	++	+	64	36	42	22
14	86	++	+++	+	+++	70	30	50	20
15	89	+	+++	—	+++	72	28	54	18

TABLE 34—SUBJECT I

ORDINARY DAILY DIET + LACTOSE 150 GMS.

Days After Initial Administration of Diet	Per Cent of <i>B. Acidoph.</i>	Gram-Stained Films from Fecal Suspensions							
		Veillon Tubes				Total Organisms		Gram-Positive Organisms	
		—1—		—2—		Per Cent Gram-Positive	Per Cent Gram-Negative	Per Cent Rods	Per Cent Cocci
		Gas	<i>B. Acidoph.</i>	Gas	<i>B. Acidoph.</i>				
1	0	+++	—	+++	—	27	73	12	15
2	18	+++	—	+++	—	34	66	16	18
3	46	+++	+	++	+	30	70	15	15
4	38	+++	+	+++	+	42	68	24	18
5	40	+++	+	+	+	40	60	25	15
6	49	++	+	++	+	42	58	28	14
7	66	++	+	+	+	49	51	38	11
8	58	+	+	+	+	54	46	36	18
9	60	++	+	+	+	45	55	27	18
10	68	++	++	+	++	50	50	36	14

CHART 32

Curve indicating percentage of *B. acidophilus* appearing in fecal specimens from human subjects.

Diet

Ordinary daily diet

Lactose 150 gms.

Ordinate—Per cent of *B. acidophilus*.

Abscissa—Number of days after administration of diet.

additional evidence of transformation of the bacterial types. Gram-positive acidophilus-like rods held prominence and at times their number reached as high as 83 per cent of the total number of organisms counted. The simple character of the flora tended to remain permanent as long as the ingestion of the dextrin was continued. However, within five days after the last dextrin intake the fecal flora gradually reverted to the usual complex type. (See Chart 33.)

TABLE 35—SUBJECT C

ORDINARY DAILY DIET + DEXTRIN 300 GMS.

<i>Days After Initial Administration of Diet</i>	<i>Per Cent of B. Acidoph.</i>	<i>Gram-Stained Films from Fecal Suspensions</i>							
		<i>Veillon Tubes</i>				<i>Total Organisms</i>		<i>Gram-Positive Organisms</i>	
		<i>—1—</i>		<i>—2—</i>		<i>Per Cent Gram-Positive</i>	<i>Per Cent Gram-Negative</i>	<i>Per Cent Rods</i>	<i>Per Cent Cocci</i>
		<i>Gas</i>	<i>B. Acidoph.</i>	<i>Gas</i>	<i>B. Acidoph.</i>				
1	10	+++	—	+++	—	20	80	8	12
2	30	+++	++	++	+	32	68	15	17
3	96	+	+++	—	+++	78	22	60	18
4	98	+	+++	—	+++	81	19	64	17
5	98	—	+++	—	+++	80	20	70	10
6	99	—	+++	—	+++	85	15	73	12
7	99	—	+++	—	+++	88	12	80	8
8	98	+	+++	—	+++	82	18	62	20
9	99	—	+++	—	+++	92	8	81	11
10	99	—	+++	—	+++	90	10	83	7

ORDINARY DAILY DIET

11	70	+	+++	—	++	60	40	48	12
12	56	+++	++	+	+	42	58	32	10
13	40	+++	+	++	+	46	54	28	18
14	8	+++	—	+++	—	30	70	10	20
15	2	+++	—	+++	—	22	78	10	12

TABLE 36—SUBJECT F

ORDINARY DAILY DIET + DEXTRIN 200 GMS.

1	26	+++	—	+++	—	28	72	16	12
2	20	+++	—	+++	—	36	64	20	16
3	18	+++	—	+++	—	30	70	18	12
4	21	+++	—	++	—	44	56	28	16
5	30	+++	+	++	+	40	60	25	15
6	25	+++	+	+++	+	38	62	20	18
7	20	+++	—	+++	—	45	55	22	23
8	10	+++	—	+++	—	33	67	15	18
9	15	+++	—	++	—	34	66	12	22

CHART 33

The administration of 200 grams of dextrin to subject F exercised a slight transforming effect (see Table 36), whereas 150 grams of the same carbohydrate to subject I resulted in much greater stimulation of the development of *B. acidophilus* (Table 37). Idiosyncrasies of this sort on the part of the human subject were observed in the lactose feeding also. While the ingestion of either 150 or 200 grams of dextrin encouraged the proliferation of *B. acidophilus*, there was no marked suppression of other types of bacteria vegetating in the intestinal canal.

The rise in the *B. acidophilus* curve as the result of the feeding of 300 grams of dextrin daily is at least as sharp as in the administration of the same amount of lactose, reaching its maximum on the third or the fourth day. Dextrin has been found as effective a transforming agent as lactose (Chart 33). The decidedly objectionable odor and taste of the dextrin solution will of necessity stand in the way of its practical use, however, even though other substances like orange juice may be employed to partly obscure the taste and odor.

TABLE 37—SUBJECT I

ORDINARY DAILY DIET + DEXTRIN 150 GMS.

Days After Initial Administration of Diet	Per Cent of <i>B. Acidoph.</i>	Gram-Stained Films from Fecal Suspensions							
		Veillon Tubes				Total Organisms		Gram-Positive Organisms	
		—1—		—2—		Per Cent Gram-Positive	Per Cent Gram-Negative	Per Cent Rods	Per Cent Cocci
		Gas	<i>B. Acidoph.</i>	Gas	<i>B. Acidoph.</i>				
1	4	+++	—	+++	—	30	70	12	18
2	10	+++	—	++	—	35	65	18	17
3	28	+++	—	++	—	34	66	20	14
4	60	++	+	+	+	52	48	38	14
5	42	++	+	+	+	50	50	32	18
6	88	—	+++	—	++	68	32	54	14
7	62	++	+	+	+	60	40	45	15
8	84	—	+++	—	+++	64	36	51	13
9	86	—	++	—	++	62	38	50	12
10	80	+	++	+	++	59	41	42	17

ORDINARY DAILY DIET									
11	46	+	++	+	++	42	58	30	12
12	10	+++	—	++	—	31	69	20	11
13	8	+++	—	+++	—	37	63	18	19
14	10	+++	—	+++	—	33	67	16	17
15	1	+++	—	++	—	32	68	14	18

TABLE 38—SUBJECT A

ORDINARY DAILY DIET + *B. ACIDOPHILUS* 300 CC.
(WHEY BROTH CULTURE, NEPH. 5)

1	0	+++	—	+++	—	38	62	12	38
2	80	++	+	+	+	52	48	40	12
3	85	—	+++	—	+++	56	44	42	14
4	80	+	+++	—	+++	50	50	40	10
5	90	—	+++	—	+++	75	25	68	7
6	90	—	+++	—	+++	80	20	72	8
7	90	—	+++	—	+++	84	16	78	6
8	90	—	+++	—	+++	88	12	80	8
9	92	—	+++	—	+++	86	14	78	8
10	94	—	+++	—	+++	85	15	76	9

The experiments have demonstrated conclusively that both lactose and dextrin, when administered in 300 gram quantities or more daily, exert a most profound influence on the character of the intestinal flora, transforming the latter from the ordinary mixed type to one dominated by *B. acidophilus*.

IMPLANTATION EXPERIMENTS WITH LIVING CULTURES
OF *BACILLUS ACIDOPHILUS*

B. acidophilus was administered in the form of whey broth cultures which were incubated for forty-eight hours at 37° C., and reduced, when necessary, to five of the McFarland turbidity scale. Four different strains of the organism were employed; they were isolated originally from human stools. In some instances the subjects were given their own strains in conjunction with others.

TABLE 39—SUBJECT B

ORDINARY DAILY DIET + *B. ACIDOPHILUS* 300 cc.
(WHEY BROTH CULTURE, NEPH. 5)

Days After Initial Administration of Diet	Per Cent of <i>B. Acidoph.</i>	Gram-Stained Films from Fecal Suspensions						Gram-Positive Organisms	
		Veillon Tubes				Total Organisms			
		—1—		—2—		Per Cent Gram-Positive	Per Cent Gram-Negative	Per Cent Rods	Per Cent Cocci
		Gas	<i>B. Acidoph.</i>	Gas	<i>B. Acidoph.</i>				
1	0	+++	—	+++	—	26	74	8	18
2	52	+++	+	+	+	40	60	27	13
3	60	+	++	—	+	44	56	25	19
4	90	—	+++	—	+++	72	28	61	11
5	90	—	+++	—	+++	70	30	62	8
6	90	+	+++	—	+++	76	24	67	9
7	90	—	+++	—	+++	81	19	74	7
8	90	+	+++	—	+++	80	20	72	8
9	90	+	+++	—	+++	82	18	74	8
10	92	—	+++	—	+++	88	12	70	18

TABLE 40—SUBJECT C

ORDINARY DAILY DIET + *B. ACIDOPHILUS* 300 cc.
(WHEY BROTH CULTURE, NEPH. 5)

1	0	+++	—	+++	—	42	58	25	17
2	0	+++	—	++	—	51	49	38	13
3	50	+++	+	++	+	55	45	40	15
4	70	++	++	+	++	60	40	48	12
5	70	++	++	+	++	68	32	52	16
6	85	+	++	—	++	82	18	66	16
7	80	+	++	—	++	80	20	68	12
8	90	+	++	—	++	88	12	79	9
9	92	—	+++	—	+++	87	13	80	7
10	90	+	+++	—	+++	86	14	72	14

In the attempts to implant *B. acidophilus* in the absence of special carbohydrates five subjects received 300 cubic centimeters each of the whey broth cultures, in addition to their usual daily diets, for a period of ten days. The bacterial culture was taken in one dose, usually before the noon luncheon.

Within two to four days after the first administration of the viable living bacteria, *B. acidophilus* colonies were observed in both the whey agar plates and in the Veillon tubes in appreciable numbers. The Gram-stained slides gave additional evidence of the simplification of the intestinal flora. Gram-positive rods resembling *B. acidophilus* constituted 75 to 80 per cent of the total bacterial count. This transformation reached its maximum on the fourth to sixth day, and the simple flora persisted as long as the *B. acidophilus* cultures were administered. (See Tables 38 to 42, and Chart 34.) *B. acidophilus* dominated the fecal flora to practically the same extent as when 300 grams of lactose or dextrin were fed. (See Chart 34.)

The conclusion may be drawn from the above experiments that *B. acidophilus* is capable of establishing itself in the intestinal tract of man when administered per os without accompanying special carbohydrates. In each of the subjects it became the predominating organism in the enteric tract, almost wholly suppressing or eliminating all of the other viable bacterial types. The results warrant the assumption that the organism in question is able to accommodate itself to the environmental conditions of the human intestine, and that positive implantation can be effected. This assumption has been strengthened further by the results of subsequent experiments.

SIMULTANEOUS USE OF THE SPECIAL CARBOHYDRATES AND *BACILLUS ACIDOPHILUS*

The purpose of these experiments was to determine whether half or even less of the transforming doses of lactose and the whey broth cultures of *B. acidophilus* can effect a marked simplification of the intestinal flora in man when these agents are administered together. Accordingly, five human subjects whose flora was known to be of the usual complex type were given, in addition to their regular daily diet, 150 grams of lactose and 150 cubic centimeters of the *acidophilus* cultures.

The results are summarized in Tables 43 to 47. They show a pronounced change in the character of the intestinal flora, with a strong predominance of *B. acidophilus* as the outstanding feature. A comparison of these results with those obtained with the subjects who had received only 150 grams of lactose reveals striking differences. Following the combined feeding the development of *B. acidophilus* was so pronounced that, barring one exception, within four to six days after

the beginning of the experiment it constituted 90 to 99 per cent of the viable intestinal organisms. Subject H failed to respond very readily, but after the sixth day the transformation was practically complete, as indicated by the agar plates and Veillon tubes.

In every instance when simplification of bacterial types was effected there was a sharp diminution of gas production in the Veillon tubes and eventually complete or almost complete absence of gas. Furthermore, the Gram-stained films revealed a picture in which 60 to 80 per cent of the organisms were acidophilus-like rods.

TABLE 41—SUBJECT D

ORDINARY DAILY DIET + B. ACIDOPHILUS 300 CC.
(WHEY BROTH CULTURE, NEPH. 5)

<i>Days After Initial Administration of Diet</i>	<i>Per Cent of B. Acidoph.</i>	<i>Gram-Stained Films from Fecal Suspensions</i>					
		<i>Veillon Tubes</i>				<i>Total Organisms</i>	
		<i>—1—</i>		<i>—2—</i>		<i>Per Cent Gram-Positive</i>	<i>Gram-Positive Organisms</i>
		<i>Gas</i>	<i>B. Acidoph.</i>	<i>Gas</i>	<i>B. Acidoph.</i>		
1	50	+++	+	+	+	64	36
2	60	++	+	+	+	62	38
3	90	+	+++	—	+++	72	28
4	90	—	+++	—	+++	85	15
5	98	—	+++	—	+++	92	8
6	93	—	+++	—	+++	90	10
7	90	+	+++	—	+++	88	12
8	95	—	+++	—	+++	94	6
9	95	—	+++	—	+++	90	10
10	92	—	+++	—	+++	93	7

TABLE 42—SUBJECT E

ORDINARY DAILY DIET + B. ACIDOPHILUS 300 CC.
(WHEY BROTH CULTURE, NEPH. 5)

1	0	+++	—	+++	—	15	85	6	9
2	0	+++	—	+++	—	22	78	12	10
3	0	+++	—	+++	—	30	70	18	12
4	60	++	+	+	+	45	55	35	10
5	70	+	++	+	++	52	48	40	12
6	84	+	+++	—	+++	68	32	57	11
7	99	—	+++	—	+++	83	17	78	5
8	95	—	+++	—	+++	88	12	72	16
9	98	—	+++	—	+++	84	16	75	9
10	96	—	+++	—	+++	82	18	70	12

82 TRANSFORMATION OF THE INTESTINAL FLORA

These results are in perfect harmony with those obtained in the feeding of albino rats, and may be summarized in a few words. In the combined feeding of lactose and whey broth cultures of *B. acidophilus* the same degree of transformation of intestinal flora may be obtained with half of the amounts of the lactose and cultures as when either of these agents is administered separately. Thus, in man the ingestion of 150 grams of lactose and 150 cubic centimeters of the whey broth daily results within the course of a few days in a profound transformation of the intestinal flora in which *B. acidophilus* becomes the dominating organism, with no reversion to the usual mixed type until several days after the use of the test materials has been discontinued (Chart 35).

TABLE 43—SUBJECT F

ORDINARY DAILY DIET + LACTOSE 150 GMS., *B. ACIDOPHILUS* 150 CC.
(WHEY BROTH CULTURE, NEPH. 5)

Days After Initial Administration of Diet	Per Cent of <i>B. Acidoph.</i>	Gram-Stained Films from Fecal Suspensions							
		Veillon Tubes				Total Organisms		Gram-Positive Organisms	
		—1—		—2—		Per Cent Gram-Positive	Per Cent Gram-Negative	Per Cent Rods	Per Cent Cocci
		Gas	<i>B. Acidoph.</i>	Gas	<i>B. Acidoph.</i>				
1	25	+++	—	+++	—	35	65	15	20
2	68	++	+	+	+	38	62	25	13
3	92	+	+++	—	+++	74	26	62	12
4	90	+	+++	—	+++	72	28	65	7
5	90	+	+++	—	+++	75	25	66	9
6	98	—	+++	—	+++	81	19	70	11
7	95	+	+++	—	+++	78	22	68	10
8	95	+	+++	—	+++	76	24	62	14
9	90	+	+++	+	+++	80	20	68	12
10	60	++	++	+	++	73	27	60	13

TABLE 44—SUBJECT G

ORDINARY DAILY DIET + LACTOSE 150 GMS., *B. ACIDOPHILUS* 150 CC.
(WHEY BROTH CULTURE, NEPH. 5)

1	90	+	+++	+	+++	64	36	45	19
2	80	+	+++	+	+++	68	32	50	18
3	95	—	+++	—	+++	74	26	62	12
4	85	+	+++	—	+++	70	30	56	14
5	85	+	+++	—	+++	72	28	60	12
6	98	—	+++	—	+++	82	18	74	8
7	90	+	+++	—	+++	78	22	69	9
8	98	+	+++	—	+++	80	20	70	10
9	80	+	+++	—	+++	76	24	68	8
10	94	—	++++	—	+++	83	17	72	11

CHART 35

Curves indicating percentage of
Bacillus appearing in fecal
specimens from human subjects.

Diet
Ordinary daily diet
Lactase 150gms
Bacillus 150c.c.
(Whey broth culture Neph. 5.)

Ordinates - Percent of *Bacillus*
Abscissae - Number of days after
administration of diet.

TABLE 45—SUBJECT H

ORDINARY DAILY DIET + LACTOSE 150 GMS., B. ACIDOPHILUS 150 CC.
(WHEY BROTH CULTURE, NEPH. 5)

Days After Initial Administration of Diet	Per Cent of B. Acidoph.	Gram-Stained Films from Fecal Suspensions							
		Veillon Tubes				Total Organisms		Gram-Positive Organisms	
		—1—		—2—		Per Cent Gram-Positive	Per Cent Gram-Negative	Per Cent Rods	Per Cent Cocci
		Gas	B. Acidoph.	Gas	B. Acidoph.				
1	0	+++	—	+++	—	30	70	12	18
2	0	+++	—	+++	—	32	68	10	22
3	0	+++	—	+++	—	36	64	15	21
4	0	+++	—	+++	—	35	65	18	17
5	2	+++	—	+++	—	40	60	21	19
6	70	+++	+	++	+	52	48	42	10
7	90	+	+++	—	+++	66	34	42	24
8	95	+	+++	—	+++	70	30	62	8
9	80	+	+++	—	+++	73	27	60	13
10	92	—	+++	—	+++	77	23	66	11

TABLE 46—SUBJECT I

ORDINARY DAILY DIET + LACTOSE 150 GMS., B. ACIDOPHILUS 150 CC.
(WHEY BROTH CULTURE, NEPH. 5)

1	2	+++	—	+++	—	31	69	10	21
2	20	+++	—	++	—	32	68	15	17
3	34	+++	+	++	+	35	65	20	15
4	90	++	+++	—	+++	64	36	48	16
5	90	+	+++	—	+++	60	40	48	12
6	93	+	+++	—	+++	71	29	62	9
7	95	—	+++	—	+++	70	30	60	10
8	95	—	+++	—	+++	86	14	68	18
9	90	—	+++	—	+++	81	19	70	11
10	93	—	+++	—	+++	85	15	73	12

TABLE 47—SUBJECT K

ORDINARY DAILY DIET + LACTOSE 150 GMS., B. ACIDOPHILUS 150 CC.
(WHEY BROTH CULTURE, NEPH. 5)

1	0	+++	—	+++	—	36	64	12	24
2	6	+++	—	+++	—	34	66	14	20
3	18	+++	—	+++	—	38	62	18	20
4	98	+	+++	—	+++	68	32	54	14
5	99	—	+++	—	+++	85	15	76	9
6	99	—	+++	—	+++	77	23	70	7
7	99	—	+++	—	+++	79	21	73	6
8	99	—	+++	—	+++	85	15	75	10
9	99	—	+++	—	+++	88	12	76	12
10	99	—	+++	—	+++	86	14	80	6

TRANSFORMING INFLUENCE OF MILK CULTURES OF *BACILLUS ACIDOPHILUS* ON THE INTES- TINAL FLORA

In the more recent experiments on intestinal implantation *B. acidophilus* was administered in the form of pure milk cultures. The strains which were employed were the same as in the preparation of the whey agar cultures previously described. Since *B. acidophilus* is primarily of intestinal origin, and not a milk-borne organism, many difficulties were encountered in the preparation of the sour milk. However, within three weeks the strains employed were by frequent and liberal transfers from milk to milk adapted to the new environment, so that ten cubic centimeters of inoculum added to one liter of sterile milk effected distinct acidification and creamy coagulation within twelve to fifteen hours at 35 to 37° C. A full description of the method of acclimating *B. acidophilus*, and of preparing and utilizing the sour milk culture will be found in one of the following chapters.

The *B. acidophilus* milk was given to quite a number of subjects, of whom some received 500 cubic centimeters and others one liter daily, in addition to the regular diets. The milk cultures were taken as a rule in 500 cubic centimeter quantities, and between meals. Occasionally the daily amount was divided and the portions taken at definite intervals.

Subject A whose intestinal flora was easily simplified by any and all of the transforming agents employed throughout the investigation, responded readily to the consumption of 500 cubic centimeters of the acidophilus milk per day (Table 48). *B. acidophilus* soon made its appearance and in a very short time became the dominant and practically the sole viable organism occurring in the stools. After the first two days of sour milk feeding no gas was produced in the Veillon tubes, and the stained slide showed a sharp increase in the Gram-positive rods of the aciduric type, which soon reached 75 to 80 per cent of the total number of fecal organisms counted. After the discontinuance of the sour milk feeding the flora gradually reverted to the ordinary mixed type. This return required about five days.

Again, the ingestion of the same amount of the milk culture (500 cubic centimeters) daily by subjects F and G exerted only a slight transforming influence on the fecal flora. (See Tables 49 and 50, and Chart 36.) The whey agar plates and Veillon tubes indicated a moderate multiplication of *B. acidophilus*, and the stained films showed the Gram-positive rods to be outnumbered by the other types of intestinal organisms. However, when one liter of the acidophilus milk was taken daily by these same subjects the complex intestinal flora was practically completely reduced in a few days to the aciduric type. (See Tables 51 and 52, and Chart 37.) Abundant proliferation of *B. acidophilus* was clearly evident after four to six days, and its complete predominance

86 TRANSFORMATION OF THE INTESTINAL FLORA

over the other intestinal organisms was maintained as long as the sour milk consumption was continued.

Similar results were obtained with subjects B, H, M, O and P, who received one liter of the milk culture daily in addition to the ordinary diet. The change in the intestinal flora of these individuals was quite uniform, the ingestion of the acidophilus milk causing in each case such a marked development of the *B. acidophilus* type as to overshadow completely all of the other viable organisms ordinarily present. (See Tables 53 to 57, and Charts 37 and 38.) It is of particular interest to note that the dominating *B. acidophilus* flora of subjects H and O, who received the milk cultures for twenty and thirty days respectively, remained unchanged during the entire milk-feeding period, without any apparent tendency whatever to revert to the original mixed flora. Following the discontinuance of the milk consumption the homogeneous

CHART 36

character of the simplified flora gave way to the usual heterogeneous mixture.

The application of pure milk cultures of *B. acidophilus* as compared with the use of special carbohydrates like lactose and dextrin, and of whey agar cultures, either with or without the carbohydrates, has very

TABLE 48—SUBJECT A

ORDINARY DAILY DIET + *B. ACIDOPHILUS* MILK 500 cc.

Days After Initial Administration of Diet	Per Cent of <i>B. Acidoph.</i>	Gram-Stained Films from Fecal Suspensions							
		Veillon Tubes				Total Organisms		Gram-Positive Organisms	
		—1—		—2—		Per Cent Gram-Positive	Per Cent Gram-Negative	Per Cent Rods	Per Cent Cocci
		Gas	<i>B. Acidoph.</i>	Gas	<i>B. Acidoph.</i>				
1	80	+	+++	—	++	42	58	28	14
2	99	—	+++	—	+++	68	32	50	18
3	99	—	+++	—	+++	70	30	54	16
4	99	—	+++	—	+++	84	16	72	12
5	99	+	+++	—	+++	82	18	78	4
6	99	—	++++	—	+++	90	10	80	10
7	98	—	++++	—	+++	86	14	78	8
8	99	—	++++	—	++++	85	15	74	11
9	96	—	++++	—	+++	87	13	75	12
10	98	—	+++	—	+++	82	18	71	11

ORDINARY DAILY DIET

11	80	+	+++	—	++	72	28	60	12
12	64	++	++	+	++	61	39	50	11
13	40	+++	+	++	+	40	60	23	17
14	15	+++	—	+++	—	42	58	20	22
15	6	+++	—	+++	—	38	62	14	24

TABLE 49—SUBJECT F

ORDINARY DAILY DIET + *B. ACIDOPHILUS* MILK 500 cc.

1	4	+++	—	+++	—	30	70	5	25
2	2	++++	—	+++	—	35	65	15	20
3	5	+++	—	+++	—	38	62	16	22
4	18	+++	—	+++	—	36	64	15	21
5	10	+++	—	+++	—	32	68	10	22
6	15	++++	—	+++	—	30	70	10	20
7	8	+++	—	+++	—	40	60	15	25
8	10	+++	—	+++	—	37	63	14	23
9	14	+++	—	+++	—	36	64	16	20
10	2	++++	—	+++	—	30	70	12	18

great advantages, which will be dwelt on at greater length in another chapter. Of special significance is the very marked and almost immediate transforming power on the intestinal bacteria which such milk culture possesses. Furthermore, the milk can be prepared by trained specialists and technicians with comparatively little expense and with

TABLE 50—SUBJECT G

ORDINARY DAILY DIET + B. ACIDOPHILUS MILK 500 CC.

Days After Initial Administration of Diet	Per Cent of B. Acidoph.	Gram-Stained Films from Fecal Suspensions							
		Veillon Tubes				Total Organisms		Gram-Positive Organisms	
		—1—		—2—		Per Cent Gram-Positive	Per Cent Gram-Negative	Per Cent Rods	Per Cent Cocci
		Gas	B. Acidoph.	Gas	B. Acidoph.				
1	68	+++	+	++	+	40	60	22	18
2	10	++++	—	+++	—	30	70	13	17
3	8	+++	—	+++	—	33	67	15	18
4	6	++++	—	+++	—	35	65	15	20
5	12	+++	—	++	—	38	62	16	22
6	10	+++	—	+++	—	35	65	14	21
7	6	++++	—	+++	—	30	70	12	18
8	15	+++	—	+++	—	42	58	15	27
9	30	+++	+	++	—	44	56	18	26
10	20	+++	—	++	—	36	64	15	21

TABLE 51—SUBJECT F

ORDINARY DAILY DIET + B. ACIDOPHILUS MILK 1000 CC.

1	4	++++	—	+++	—	37	63	18	19
2	10	++++	—	+++	—	35	65	20	15
3	20	+++	—	++	—	38	62	22	16
4	8	+++	—	+++	—	41	59	23	18
5	65	+	++	+	++	58	42	38	20
6	78	+	++	+	++	64	36	42	22
7	90	+	+++	—	+++	80	20	60	20
8	94	—	+++	—	+++	78	22	65	13
9	88	+	+++	—	+++	76	24	64	12
10	92	—	+++	—	+++	75	25	60	15

ORDINARY DAILY DIET

11	60	++	++	+	+	68	32	49	19
12	28	+++	—	++	—	62	38	40	22
13	4	++++	—	+++	—	44	56	26	18
14	10	++++	—	+++	—	40	60	16	24
15	2	++++	—	+++	—	33	67	12	21

CHART 37

90 TRANSFORMATION OF THE INTESTINAL FLORA

a high degree of certainty of obtaining a uniform product which is decidedly palatable and wholesome, to say the least.

It may be of interest here to state that subjects O and P were persons who presented a history of intestinal disturbances. O had been subject to most obstinate constipation requiring regular use of drugs to relieve

TABLE 52—SUBJECT G

ORDINARY DAILY DIET + B. ACIDOPHILUS MILK 1000 cc.

<i>Days After Initial Administration of Diet</i>	<i>Per Cent of B. Acidoph.</i>	<i>Gram-Stained Films from Fecal Suspensions</i>							
		<i>Veillon Tubes</i>				<i>Total Organisms</i>		<i>Gram-Positive Organisms</i>	
		<i>—1—</i>		<i>—2—</i>		<i>Per Cent Gram-Positive</i>	<i>Per Cent Gram-Negative</i>	<i>Per Cent Rods</i>	<i>Per Cent Cocci</i>
		<i>Gas</i>	<i>B. Acidoph.</i>	<i>Gas</i>	<i>B. Acidoph.</i>				
1	92	++	+++	+	+++	61	39	40	21
2	75	++	++	+	++	66	34	42	24
3	52	++	++	+	++	60	40	44	16
4	64	++	++	+	++	58	42	42	16
5	78	++	+++	+	++	64	36	48	16
6	95	+	+++	—	+++	72	28	60	12
7	92	—	+++	—	+++	80	20	65	15
8	96	—	+++	—	+++	85	15	72	13
9	94	—	+++	—	+++	82	18	70	12
10	90	—	+++	—	+++	88	12	76	12

ORDINARY DAILY DIET

11	60	+	++	+	++	70	30	57	13
12	32	+++	+	++	+	62	38	40	22
13	46	+++	+	++	+	57	43	38	19
14	4	+++	—	+++	—	41	59	20	21
15	6	+++	—	+++	—	35	65	16	19

TABLE 53—SUBJECT B

ORDINARY DAILY DIET + B. ACIDOPHILUS MILK 1000 cc.

1	78	+++	+	++	+	44	56	28	16
2	88	++	+++	+	+++	53	47	38	15
3	94	+	+++	+	+++	69	31	52	17
4	98	—	+++	—	+++	78	22	62	16
5	90	—	+++	—	+++	75	25	60	15
6	96	+	+++	—	+++	82	18	70	12
7	98	—	+++	—	+++	86	14	75	11
8	97	—	+++	—	+++	80	20	70	10
9	98	—	++++	—	++++	87	13	78	9
10	99	—	++++	—	+++	84	16	76	8

CHART 38

Curves indicating percentage of *B. acidophilus* appearing in fecal specimens from human subjects.

Diet

Ordinary daily diet

B. acidophilus milk 1000 c.c.

Ordinates—Per cent of *B. acidophilus*.

Abscissae—Number of days after administration of diet.

TABLE 54—SUBJECT H

ORDINARY DAILY DIET + B. ACIDOPHILUS MILK 1000 CC.

<i>Days After Initial Administration of Diet</i>	<i>Per Cent of B. Acidoph.</i>	<i>Gram-Stained Films from Fecal Suspensions</i>							
		<i>Veillon Tubes</i>				<i>Total Organisms</i>		<i>Gram-Positive Organisms</i>	
		<i>—1—</i>		<i>—2—</i>		<i>Per Cent Gram-Positive</i>	<i>Per Cent Gram-Negative</i>	<i>Per Cent Rods</i>	<i>Per Cent Cocci</i>
		<i>Gas</i>	<i>B. Acidoph.</i>	<i>Gas</i>	<i>B. Acidoph.</i>				
1	0	+++	—	++	—	28	72	10	18
2	4	+++	—	++	—	35	65	12	23
3	1	+++	—	++	—	37	63	15	22
4	82	+	++	+	++	54	46	35	19
5	72	++	++	++	++	60	40	40	20
6	78	+	++	+	++	55	45	38	17
7	88	+	+++	—	+++	64	36	48	16
8	92	—	+++	—	+++	75	25	60	15
9	98	—	+++	—	+++	78	22	68	10
10	98	—	+++	—	+++	76	24	65	11
11	92	—	+++	—	+++	70	30	58	12
12	94	+	+++	—	+++	80	20	65	15
13	98	+	+++	—	+++	82	18	66	16
14	95	—	+++	—	+++	84	16	68	16
15	96	—	+++	—	+++	85	15	66	19
16	98	—	+++	—	+++	84	16	70	14
17	98	—	+++	—	+++	86	14	75	11
18	96	—	+++	—	+++	80	20	70	10
19	96	—	+++	—	+++	83	17	74	9
20	92	—	+++	—	+++	78	22	65	13

ORDINARY DAILY DIET

21	84	+	+++	—	+++	66	34	52	14
22	70	+	++	+	++	68	32	48	20
23	72	++	++	+	++	65	35	44	21
24	48	+++	+	++	+	52	48	28	24
25	30	+++	+	++	+	45	55	25	20

TABLE 55—SUBJECT M

ORDINARY DAILY DIET + B. ACIDOPHILUS MILK 1000 CC.

1	62	+++	++	++	++	64	36	38	26
2	96	++	+++	+	+++	84	16	66	18
3	80	++	+++	+	+++	80	20	55	25
4	88	—	+++	—	+++	82	18	60	22
5	94	—	+++	—	+++	88	12	70	18
6	96	—	+++	—	+++	94	6	78	16
7	95	—	+++	—	+++	85	15	72	13
8	94	+	+++	—	+++	90	10	75	15
9	98	—	+++	—	+++	92	8	84	8
10	96	—	+++	—	+++	90	10	86	4

the condition for the time being. P, on the other hand, suffered from chronic diarrhea acquired while he was residing in the tropics. The stools were of the most offensive odor.

Both of these subjects responded readily to the acidophilus milk administration, in so far as implantation of *B. acidophilus* is concerned, as is seen in the accompanying tables (56 and 57). What is of further interest is that subject O presented a specimen of stool for examination every day but one during the thirty-five or more days that he took the acidophilus milk. These specimens were from all appearances normal.

TABLE 56—SUBJECT O

ORDINARY DAILY DIET + *B. ACIDOPHILUS* MILK 1000 cc.

Days After Initial Administration of Diet	Per Cent of <i>B. Acidoph.</i>	Gram-Stained Films from Fecal Suspensions							
		Veillon Tubes				Total Organisms		Gram-Positive Organisms	
		—1—		—2—		Per Cent Gram-Positive	Per Cent Gram-Negative	Per Cent Rods	Per Cent Cocci
		Gas	<i>B. Acidoph.</i>	Gas	<i>B. Acidoph.</i>				
1	86	+++	++	++	++	39	61	22	17
2	92	+++	++	++	++	65	35	41	24
3	96	+	+++	+	+++	68	32	50	18
4	98	+	+++	—	+++	72	28	58	14
5	98	—	+++	—	+++	75	25	56	19
6	96	—	+++	—	+++	70	30	54	16
7	96	—	+++	—	+++	74	26	60	14
8	98	—	+++	—	+++	80	20	70	10
9	94	+	+++	—	+++	78	22	66	12
10	96	—	+++	—	+++	72	28	64	8
11	98	—	+++	—	+++	76	24	65	11
12	98	+	+++	—	+++	82	18	70	12
13	99	—	+++	—	+++	85	15	78	7
14	90	+	+++	—	+++	84	16	74	10
15	96	—	+++	—	+++	83	17	75	8
16	95	—	+++	—	+++	78	22	65	13
17	98	+	+++	—	+++	80	20	72	8
18	95	+	+++	—	+++	82	18	70	12
19	96	+	+++	—	+++	85	15	75	10
20	90	—	+++	—	+++	74	26	68	6
21	90	—	+++	—	+++	76	24	66	10
22	98	—	+++	—	+++	78	22	70	8
23	93	—	+++	—	+++	80	20	68	12
24	96	—	+++	—	+++	88	12	81	7
25	94	—	+++	—	+++	84	16	74	10
26	98	—	+++	—	+++	85	15	78	7
27	92	—	+++	—	+++	83	17	74	9
28	98	—	+++	—	+++	88	12	80	8
29	99	—	+++	—	+++	90	10	80	10
30	95	—	+++	—	+++	85	15	76	9

94 TRANSFORMATION OF THE INTESTINAL FLORA

The subject claimed to have been improved in his general condition. Within three or four days after presenting himself, a change was noted in the stool of subject P from the diarrheal to what appeared to be a normal specimen, and a complete disappearance of the offensive odor. During the entire period of observation this individual expressed himself strongly as having been very much benefited by the acidophilus milk. These two cases are too few, however, to be of much significance.

TABLE 57—SUBJECT P

ORDINARY DAILY DIET + *B. ACIDOPHILUS* MILK 1000 cc.

Days After Initial Administration of Diet	Per Cent of <i>B. Acidoph.</i>	Gram-Stained Films from Fecal Suspensions							
		Veillon Tubes				Total Organisms		Gram-Positive Organisms	
		—1—		—2—		Per Cent Gram-Positive	Per Cent Gram-Negative	Per Cent Rods	Per Cent Cocci
		Gas	<i>B. Acidoph.</i>	Gas	<i>B. Acidoph.</i>				
1	12	+++	—	+++	—	22	78	8	14
2	80	++	++	+	++	38	62	28	10
3	92	+	+++	—	+++	64	36	50	14
4	94	+	+++	—	+++	79	21	64	15
5	98	—	+++	—	+++	75	25	65	10
6	96	—	+++	—	+++	74	26	66	8
7	93	—	+++	—	+++	76	24	66	10
8	90	+	+++	—	+++	72	28	60	12
9	96	—	+++	—	+++	78	22	68	10
10	98	—	+++	—	+++	84	16	75	9
ORDINARY DAILY DIET									
11	82	+	+++	—	+++	80	20	68	12
12	60	++	++	+	++	67	33	50	17
13	52	++	++	+	++	48	52	36	12
14	50	++	++	+	++	45	55	30	15
15	30	++	++	+	++	42	58	22	20
16	22	+++	+	++	—	34	66	18	16
17	12	+++	—	++	—	35	65	15	20

USE OF *BACILLUS ACIDOPHILUS* MILK REINFORCED WITH LACTOSE OR DEXTRIN

The influence of the special carbohydrates as supplement to the milk cultures of *B. acidophilus* was studied by adding 100 grams of either lactose or dextrin to 500 cubic centimeters of the milk product, mixing the two well by shaking in a large flask and administering them in one

or two feedings (usually one) between meals to subjects who had failed to respond very appreciably to the 500 cubic centimeters of *B. acidophilus* milk alone. In the single dextrin ingestion experiment the dextrin was added to the milk in the form of a 50 per cent aqueous solution.

The results are given in the following tables (58 to 64). In every instance the combination exerted a marked transforming influence on the intestinal flora with a resultant simplification of bacterial types which was comparable with the changes effected by 300 grams of the lactose or dextrin or by 1000 cubic centimeters of the acidophilus milk alone. (See Charts 39 and 40.) There was an almost complete absence of gas-producing organisms in the Veillon tubes, and the Gram-stained slides presented a bacterial picture in which *B. acidophilus*-like rods were strikingly prominent. These observations were well supported by the colonies on the whey agar plates. The discontinuance of the use of lactose or dextrin and the milk cultures by subjects F and G after feeding periods of twenty and ten days respectively resulted in the return of the original mixed flora within five to ten days.

These observations are of particular interest in that they emphasize the necessity of accompanying *B. acidophilus* administration with suitable carbohydrate and hence the value of the dextrin and lactose in a

CHART 40

Curve indicating percentage of *B. acidophilus* appearing in fecal specimens from human subjects.

Diet

Ordinary daily diet

B. acidophilus milk 500 c.c.

Dextrin 100 gms.

Ordinate—Per cent of *B. acidophilus*.

Abscissa—Number of days after initial administration of diet.

TABLE 58—SUBJECT F

ORDINARY DAILY DIET + B. ACIDOPHILUS MILK 500 CC., LACTOSE 100 GMS.

Days After Initial Administration of Diet	Per Cent of <i>B. Acidoph.</i>	Gram-Stained Films from Fecal Suspensions							
		Veillon Tubes				Total Organisms		Gram-Positive Organisms	
		—1—		—2—		Per Cent Gram-Positive	Per Cent Gram-Negative	Per Cent Rods	Per Cent Cocci
		Gas	<i>B. Acidoph.</i>	Gas	<i>B. Acidoph.</i>				
1	30	+++	—	++	—	31	69	16	15
2	45	+++	+	++	+	37	63	12	15
3	42	+++	+	++	+	35	65	23	12
4	78	++	++	+	++	42	58	28	14
5	86	++	+++	+	+++	48	52	35	13
6	90	+	+++	—	+++	50	50	38	12
7	94	—	+++	—	+++	64	36	50	14
8	88	+	+++	—	+++	65	35	48	17
9	96	—	+++	—	+++	60	40	48	12
10	92	+	+++	—	+++	70	30	56	14
11	90	—	+++	—	+++	68	32	55	13
12	84	+	+++	—	+++	55	45	40	15
13	89	+	+++	—	+++	63	37	56	7
14	95	+	+++	—	+++	72	28	58	14
15	15	+	+++	—	+++	69	31	53	16
16	76	+	++	+	++	66	34	52	14
17	92	+	+++	—	+++	67	33	57	10
18	85	+	+++	+	+++	58	42	46	12
19	82	+	+++	—	+++	56	44	42	14
20	87	+	+++	—	+++	53	47	40	13

ORDINARY DAILY DIET

21	62	+	++	+	++	38	62	26	12
22	48	++	++	+	++	40	60	25	15
23	32	+++	+	++	+	34	66	20	14
24	16	+++	—	++	—	46	54	26	20
25	6	+++	—	++	—	39	61	18	21

TABLE 59—SUBJECT G

ORDINARY DAILY DIET + B. ACIDOPHILUS MILK 500 CC., LACTOSE 100 GMS.

1	42	+++	+	++	+	37	63	18	19
2	84	+	+++	+	+++	55	45	42	13
3	92	—	+++	—	+++	60	40	48	12
4	96	—	+++	—	+++	76	24	62	14
5	90	—	+++	—	+++	70	30	60	10
6	95	—	+++	—	+++	68	32	56	12
7	98	—	+++	—	+++	72	28	64	8
8	99	—	++++	—	++++	82	18	72	10

TABLE 60—SUBJECT G

ORDINARY DAILY DIET + B. ACIDOPHILUS MILK 500 CC., DEXTRIN 100 GMS.

Days After Initial Administration of Diet	Gram-Stained Films from Fecal Suspensions								
	Per Cent of <i>B. Acidoph.</i>	Veillon Tubes				Total Organisms		Gram-Positive Organisms	
		—1—		—2—		Per Cent Gram-Positive	Per Cent Gram-Negative	Per Cent Rods	Per Cent Cocci
		Gas	<i>B. Acidoph.</i>	Gas	<i>B. Acidoph.</i>				
1	82	+++	++	++	++	54	46	38	16
2	90	+	+++	+	+++	68	32	54	14
3	90	++	+++	—	+++	70	30	52	18
4	98	++	+++	—	+++	88	12	78	10
5	98	—	+++	—	+++	86	14	74	12
6	96	—	+++	—	+++	89	11	75	14
7	99	—	+++	—	+++	90	10	82	8
8	99	—	+++	—	+++	85	15	76	9
9	99	—	+++	—	+++	84	16	72	12
10	99	—	+++	—	+++	88	12	81	7

ORDINARY DAILY DIET

11	96	—	+++	—	+++	80	20	68	12
12	84	+	+++	+	+++	78	22	60	18
13	80	++	++	+	++	71	29	50	21
14	82	++	++	+	+	70	30	52	18
15	72	++	++	+	+	62	38	40	22
16	70	+++	+	++	+	60	40	44	16
17	62	+++	+	++	+	45	55	30	15
18	48	+++	+	+++	+	38	62	18	20
19	30	+++	—	+++	—	31	69	15	16
20	6	+++	—	+++	—	33	67	12	21

TABLE 61—SUBJECT H

ORDINARY DAILY DIET + B. ACIDOPHILUS MILK 500 CC., LACTOSE 100 GMS.

1	28	+++	—	+	—	45	55	27	18
2	46	++	+	+	+	42	58	28	14
3	92	+	+++	+	+++	65	35	50	15
4	96	—	+++	—	+++	73	27	61	12
5	94	—	+++	—	+++	75	25	65	10
6	92	+	+++	—	+++	78	22	66	12
7	93	—	+++	—	+++	70	30	60	10
8	98	—	+++	—	+++	84	16	76	8
9	95	—	+++	—	+++	80	20	68	12
10	96	—	+++	—	+++	82	18	66	16

98 TRANSFORMATION OF THE INTESTINAL FLORA

flora-transforming dietary. There can be no doubt that the most successful implantation of *B. acidophilus* is accomplished through the combined use of the lactose or dextrin and *B. acidophilus* cultures, which condition may be met by the use of sufficient acidophilus milk alone.

TABLE 62—SUBJECT N

ORDINARY DAILY DIET + *B. ACIDOPHILUS* MILK 500 CC., LACTOSE 100 GMS.

Days After Initial Administration of Diet	Per Cent of <i>B. Acidoph.</i>	Gram-Stained Films from Fecal Suspensions							
		Veillon Tubes				Total Organisms		Gram-Positive Organisms	
		—1—		—2—		Per Cent Gram-Positive	Per Cent Gram-Negative	Per Cent Rods	Per Cent Cocci
		Gas	<i>B. Acidoph.</i>	Gas	<i>B. Acidoph.</i>				
1	7	+++	—	++	—	33	67	12	21
2	28	+++	—	++	—	35	65	16	19
3	90	+	++	+	++	63	37	50	13
4	98	+	++	—	++	76	24	62	14
5	96	—	++	—	++	82	18	70	12
6	98	+	++	—	++	80	20	72	8
7	99	—	+++	—	+++	88	12	76	12
8	99	—	+++	—	+++	85	15	74	11
9	96	—	++	—	++	86	14	76	10
10	98	—	+++	—	++	83	17	75	8

TABLE 63—SUBJECT Q

ORDINARY DAILY DIET + *B. ACIDOPHILUS* MILK 500 CC., LACTOSE 100 GMS.

1	4	+++	—	++	—	31	69	10	21
2	35	++	+	++	+	38	62	15	23
3	68	++	++	+	++	52	48	38	14
4	78	+	++	—	++	57	43	44	13
5	96	—	++	—	++	64	36	52	12
6	92	—	++	—	++	71	29	62	9
7	98	—	++	—	++	75	25	65	10
8	98	—	++	—	++	73	27	66	7
9	95	—	++	—	++	70	30	60	10
10	96	+	++	—	++	72	28	58	14
11	92	+	++	—	++	69	31	58	11
12	98	—	++	—	++	78	22	68	10
13	96	—	++	—	++	74	26	65	9
14	90	+	++	—	++	70	30	58	12
15	95	—	++	—	++	80	20	72	8

TABLE 64—SUBJECT R

ORDINARY DAILY DIET + *B. ACIDOPHILUS* MILK 500 CC., LACTOSE 100 GMS.

Days After Initial Administration of Diet	Per Cent of <i>B. Acidoph.</i>	Gram-Stained Films from Fecal Suspensions					
		Veillon Tubes		Total Organisms		Gram-Positive Organisms	
		—1—	—2—	Per Cent Gram-Positive	Per Cent Gram-Negative	Per Cent Rods	Per Cent Cocci
		Gas	<i>B. Acidoph.</i>				
1	0	+++	—	24	76	8	16
2	2	+++	—	32	68	12	20
3	72	++	++	48	52	29	19
4	85	+	+++	53	47	36	17
5	83	+	+++	62	38	48	14
6	87	+	+++	65	35	50	15
7	96	—	+++	72	28	62	10
8	94	—	+++	70	30	62	8
9	98	—	+++	78	22	68	10
10	96	+	+++	84	16	72	12
11	90	+	+++	70	30	63	7
12	94	+	+++	73	27	62	11
13	97	—	+++	82	18	74	8
14	88	+	+++	75	25	66	9
15	96	—	+++	76	24	68	8

ATTEMPTS TO IMPLANT *BACILLUS BULGARICUS* IN MAN

In the two following experiments *B. bulgaricus* was administered in the form of milk cultures which were prepared with the same strains that were employed in previous experiments on white rats. The method of preparing these sour milk cultures of *B. bulgaricus* was identical with the procedure followed for the *B. acidophilus* milk.

Two subjects were chosen who had been particularly susceptible to the administration of *B. acidophilus* milk, 500 cubic centimeters of this product being sufficient to bring about a very profound change in the intestinal flora. The *B. bulgaricus* feeding was continued for ten days, and the daily amount consumed was 1000 cubic centimeters, taken twice a day between meals in 500 cubic centimeter portions. This was immediately followed by a five day period in which the subjects were given neither *B. bulgaricus*, *B. acidophilus* nor special carbohydrates. Following this control period, milk cultures of *B. acidophilus* were administered to the same two individuals, but instead of 1000 cubic centimeters being used, only 500 cubic centimeters were consumed daily.

100 TRANSFORMATION OF THE INTESTINAL FLORA

The ingestion of the large amounts of *B. bulgaricus* milk offered no encouragement for the development of *B. bulgaricus* in the intestine. (See Tables 65 and 66.) At no time could this organism be recovered from the feces. On the other hand, an appreciable increase took place in the numbers of *B. acidophilus*, and a partial transformation of the intestinal flora was effected. The character and extent of the transformation were the same as when these same subjects (A and D) consumed 1000 cubic centimeters of ordinary daily milk along with the ordinary diet. (See Tables 27 and 28.) The milk as such stimulated the growth of *B. acidophilus*. In fact these two individuals showed some response to even as little as 500 cubic centimeters of plain milk.

Within five days after the discontinuance of the *B. bulgaricus* milk *B. acidophilus* gradually disappeared and the usual complex flora again asserted itself. The results obtained in this series of experiments with human subjects are in full accord with those of Hull and Rettger (1917) and of our own on white rats (pages 11-64), and lead us to the conclusion that *B. bulgaricus* is unable to adjust itself to the conditions prevailing in the intestine of both man and the albino rat, and that so-called implantation of this organism in the intestine is impossible, at least under the conditions of the experiments thus far conducted. (See Chart 41.)

After having demonstrated the inability of *B. bulgaricus* to implant

CHART 41

Curves indicating percentage of *B. acidophilus* and *B. bulgaricus* appearing in fecal specimens from human subjects.

Diet—Ordinary daily diet, *B. bulgaricus* milk 1000 c.c.

B. acidophilus —————

B. bulgaricus - - - - -

Ordinates—Per cent of organisms.

Abscissae—Number of days after administration of diet.

TABLE 65—SUBJECT A

ORDINARY DAILY DIET + B. BULGARICUS MILK 1000 cc.

Days After Initial Administration of Diet	Per Cent of <i>B. Acidoph.</i>	Gram-Stained Films from Fecal Suspensions								
		Veillon Tubes				Total Organisms		Gram-Positive Organisms		Per Cent of <i>B. Bulgar.</i>
		—1—		—2—		Per Cent Gram-Positive	Per Cent Gram-Negative	Per Cent Rods	Per Cent Cocci	
		Gas	<i>B. Acidoph.</i>	Gas	<i>B. Acidoph.</i>					
1	18	+++	—	++	—	32	68	15	17	0
2	40	+++	+	++	+	55	45	35	20	0
3	15	+++	—	++	—	58	42	36	22	0
4	5	+++	—	+++	—	50	50	38	12	0
5	29	+++	—	++	—	52	48	33	19	0
6	38	+++	+	++	+	60	40	39	21	0
7	36	+++	+	+++	+	65	35	48	17	0
8	30	+++	+	+++	+	62	48	40	22	0
9	32	+++	+	++	+	64	36	44	20	0
10	26	+++	+	++	+	66	34	45	21	0

ORDINARY DAILY DIET

11	16	++++	—	+++	—	46	54	30	16	0
12	15	++++	—	+++	—	42	58	28	14	0
13	10	++++	—	+++	—	39	61	21	10	0
14	0	++++	—	+++	—	32	68	10	22	0
15	4	++++	—	+++	—	30	70	12	18	0

TABLE 66—SUBJECT D

ORDINARY DAILY DIET + B. BULGARICUS MILK 1000 cc.

1	12	++++	—	++	—	40	60	21	19	0
2	32	+++	+	++	+	42	58	28	14	0
3	46	+++	+	++	+	38	62	26	12	0
4	30	+++	+	+++	+	55	45	39	16	0
5	28	+++	+	++	+	48	52	30	18	0
6	36	+++	+	++	+	66	34	38	28	0
7	35	+++	+	++	+	62	38	40	22	0
8	32	+++	+	++	+	64	36	42	22	0
9	30	+++	+	++	+	68	32	45	23	0
10	37	+++	+	++	+	60	40	40	20	0

ORDINARY DAILY DIET

11	22	++++	—	+++	—	41	59	26	15	0
12	8	++++	—	+++	—	46	54	24	22	0
13	0	++++	—	+++	—	44	56	25	19	0
14	1	++++	—	+++	—	45	55	25	20	0
15	3	++++	—	+++	—	43	57	29	14	0

102 TRANSFORMATION OF THE INTESTINAL FLORA

itself within the intestinal tract of these human subjects (A and D), the same individuals received 500 cubic centimeters of *B. acidophilus* milk for twenty and ten days respectively in place of the 1000 cubic centimeters of *B. bulgaricus* milk. Within one to three days an almost complete simplification of intestinal flora took place in both subjects, as was shown by the whey agar plates, Veillon tubes and Gram-stained films of the fecal suspensions. (See Tables 67 and 68, and Chart 42.)

TABLE 67—SUBJECT A

ORDINARY DAILY DIET + *B. ACIDOPHILUS* MILK 500 CC.

Days After Initial Administration of Diet	Per Cent of <i>B. Acidoph.</i>	Gram-Stained Films from Fecal Suspensions					
		Veillon Tubes				Total Organisms	Gram-Positive Organisms
		1		2		Per Cent Gram-Positive	Per Cent Gram-Negative
		Gas	<i>B. Acidoph.</i>	Gas	<i>B. Acidoph.</i>		
1	80	++	+	—	+	62	38
2	92	+	+++	—	+++	69	31
3	99	—	+++	—	+++	72	28
4	99	—	+++	—	+++	80	20
5	99	—	+++	—	+++	78	22
6	99	—	+++	—	+++	85	15
7	94	—	+++	—	+++	88	12
8	98	—	+++	—	+++	82	18
9	98	—	+++	—	+++	90	10
10	92	+	+++	—	+++	84	16
11	99	—	+++	—	+++	80	20
12	95	+	+++	—	+++	83	17
13	90	—	+++	—	+++	88	12
14	98	—	+++	—	+++	80	20
15	92	—	+++	—	+++	85	15
16	96	—	+++	—	+++	87	13
17	99	—	+++	—	+++	90	10
18	99	—	+++	—	+++	80	20
19	96	—	+++	—	+++	86	14
20	98	—	+++	—	+++	88	12

ORDINARY DAILY DIET

21	90	—	+++	—	+++	82	18	66	16
22	82	+	+++	—	+++	77	23	58	19
23	70	+	++	+	++	68	32	50	18
24	44	++	++	+	++	60	40	34	26
25	40	++	+	++	+	39	61	19	20
26	45	++	+	++	+	42	58	24	18
27	27	+++	—	++	—	30	70	14	16
28	30	+++	—	+++	—	34	66	18	16
29	12	+++	—	+++	—	35	65	15	20
30	8	+++	—	+++	—	36	64	12	24

CHART 42

Curves indicating percentage of *B. acidophilus* appearing in fecal specimens from human subjects.

Diet

Ordinary daily diet

B. acidophilus milk 500 c.c.

Ordinates—Per cent of *B. acidophilus*.

Abscissae—Number of days after administration of diet.

104 TRANSFORMATION OF THE INTESTINAL FLORA

The typical mixed flora again appeared within ten days after discontinuance of the acidophilus milk.

NOTE.—In the search for *B. bulgaricus* in the whey agar plates of the fecal suspensions from the subjects receiving milk cultures of *B. bulgaricus*, several fluffy colonies resembling *B. bulgaricus* and *B. acidophilus* were fished from each set of plates. For identification the maltose test advocated by Rahe (1914) and studied at some length by us was utilized. None of the transplants from the plates failed to utilize maltose with acid production, and in every other respect there was lack of evidence of the presence of *B. bulgaricus*.

TABLE 68—SUBJECT D

ORDINARY DAILY DIET + *B. ACIDOPHILUS* MILK 500 CC.

Days After Initial Administration of Diet	Per Cent of <i>B. Acidoph.</i>	Gram-Stained Films from Fecal Suspensions							
		Veillon Tubes				Total Organisms		Gram-Positive Organisms	
		—1—		—2—		Per Cent Gram-Positive	Per Cent Gram-Negative	Per Cent Rods	Per Cent Cocci
		Gas	<i>B. Acidoph.</i>	Gas	<i>B. Acidoph.</i>				
1	44	++	+	++	+	41	59	22	19
2	88	+	+++	+	+++	63	37	51	12
3	96	—	+++	—	+++	60	40	48	12
4	90	—	+++	—	+++	67	33	56	11
5	98	—	++++	—	+++	82	18	68	14
6	92	—	+++	—	+++	84	16	75	9
7	96	—	+++	—	+++	85	15	72	13
8	98	—	+++	—	+++	88	12	76	12
9	99	—	++++	—	++++	86	14	78	8
10	99	—	+++	—	+++	90	10	76	14
ORDINARY DAILY DIET									
11	90	+	+++	—	+++	78	22	66	12
12	85	+	+++	—	+++	70	30	56	14
13	80	+	+++	+	+++	62	38	40	22
14	42	++	+	+	+	46	54	30	16
15	45	++	+	+	+	34	66	16	18
16	28	+++	—	+	—	40	60	23	17
17	20	+++	—	+	—	42	58	20	22
18	14	+++	—	++	—	35	65	15	20
19	15	+++	—	+++	—	36	64	12	24
20	5	+++	—	+++	—	31	69	14	17

The following two confirmatory experiments were carried on with pure cultures of *B. bulgaricus*. Subjects F and H each received 300 cubic centimeters of a whey broth culture of the given organism daily for ten days, in addition to the usual diet.

The ingestion of the very large numbers of *B. bulgaricus* brought about no apparent change in the intestinal flora. It was impossible to recover *B. bulgaricus* from the plates, which were free from colonies of this general type except an occasional *B. acidophilus* colony. There was no reduction whatever in the amount of gas production in the Veillon tubes, and the Gram-stained slides were typical of the usual flora of so-called "normal" individuals. The results are shown in Tables 68a and 68b, and Chart 42a. These results are in accord with those obtained with white rats, and fully corroborate the findings in the *B. bulgaricus* milk feeding experiments on man.

TABLE 68A—SUBJECT F

ORDINARY DAILY DIET + *B. BULGARICUS* 300 CC.
(WHEY BROTH CULTURE, NEPH. 5)

Days After Initial Administration of Diet	Per Cent of <i>B. Bulgar.</i>	Gram-Stained Films from Fecal Suspensions							
		Veillon Tubes				Total Organisms		Gram-Positive Organisms	
		—1—		—2—		Per Cent Gram-Positive	Per Cent Gram-Negative	Per Cent Rods	Per Cent Cocci
		Gas	<i>B. Bulgaricus</i>	Gas	<i>B. Bulgaricus</i>				
1	0	+++	—	+++	—	16	84	6	10
2	0	+++	—	+++	—	18	82	5	13
3	0	+++	—	+++	—	24	76	8	16
4	0	+++	—	+++	—	22	78	7	15
5	0	+++	—	+++	—	25	75	8	17
6	0	+++	—	+++	—	28	72	10	18
7					No Specimen				
8	0	+++	—	+++	—	35	65	12	23
9					No Specimen				
10	0	+++	—	+++	—	38	62	10	28

TABLE 68B—SUBJECT H

ORDINARY DAILY DIET + *B. BULGARICUS* 300 CC.
(WHEY BROTH CULTURE, NEPH. 5)

1	0	+++	—	+++	—	15	85	3	12
2	0	+++	—	+++	—	12	88	4	8
3	0	+++	—	+++	—	21	79	7	14
4	0	+++	—	+++	—	32	68	15	17
5	0	+++	—	+++	—	23	77	10	13
6	0	+++	—	+++	—	30	70	12	18
7	0	+++	—	+++	—	25	75	9	16
8	0	+++	—	+++	—	33	67	13	20
9	0	+++	—	+++	—	20	80	6	14
10	0	+++	—	+++	—	36	64	17	19

CHART 42A

Curves indicating percentage of *B. bulgaricus* appearing in fecal specimens from human subjects.

Diet

Ordinary daily diet

B. bulgaricus 300 c.c.

(Whey broth culture Neph. 5.)

Ordinates—Per cent of *B. bulgaricus*.

Abscissae—Number of days after administration of diet.

INCOMPLETE ABSORPTION OF LACTOSE FROM THE INTESTINE

In the feeding experiments with rats a direct correlation was established between the transforming influence of lactose and dextrin on the intestinal flora and the presence of a reducing substance in the large intestine and in the feces. It was assumed, therefore, that these carbohydrates owed their *Bacillus acidophilus*-stimulating influence to their incomplete absorption from the enteric tract. This view was strengthened by our failure to determine the presence of reducing substances after the feeding of the other carbohydrates, glucose, maltose and sucrose, which lacked the power of in any way effecting a change in the flora.

Since lactose and dextrin so readily effect a simplification of the intestinal flora in man also, attempts were made to demonstrate their presence in the stools of subjects receiving them. The experiments on lactose alone have been carried to completion, and are presented here.

The stools of four subjects (A, C, D and L) who received 300 grams, and of a fifth who took 400 grams, of lactose daily for ten days, in addition to the daily diet, were studied at some length. Besides the usual bacteriological examinations for the determination of type dis-

tribution, tests were made for the presence of reducing substances, the technique being the same as that employed in the experiments with white rats (page 58), with the exception that three grams of the human feces were employed and added to fifty cubic centimeters of dilution water.

The results are shown in the following table (69). In this table are included also the results obtained with two control subjects, that is persons who were not receiving the special carbohydrate. The stools from the subjects taking lactose gave an unmistakable reduction with Benedict's solution, and harbored a flora almost completely dominated by *B. acidophilus*, while those of the controls possessed no reducing properties and contained the usual mixed flora. The conclusion is drawn, therefore, that in man, as well as in rats, the lactose, when ingested in sufficient amounts to transform the flora, is not completely absorbed before it reaches the large intestine, and that it serves as particularly utilizable pabulum for the development of *B. acidophilus* in the large intestine, where the activities of intestinal organisms are greatest. Hence, an optimum environment is created in the lower intestine for *B. acidophilus*. This correlation between the simplifying property of lactose and its incomplete absorption from the enteric tube must be viewed as of considerable significance.

TABLE 69

THE RELATION OF DIET TO REDUCING CARBOHYDRATES AND TO THE BACTERIAL FLORA IN
FECAL SPECIMENS FROM HUMAN SUBJECTS

Subject	Diet	Period of Feeding	Reduction of Benedict's Solution	Per Cent of <i>B. Acidophilus</i>
<i>Control</i>				
F	Ordinary Daily Diet	10 days	-	1
I		10 days	-	2
A	Ordinary Daily Diet Lactose 300 gms.	10 days	+	97
C		10 days	+	91
D		10 days	+	99
L		10 days	+	87
B	Ordinary Daily Diet Lactose 400 gms.	10 days	+	89

RELATION OF HYDROGEN ION CONCENTRATION TO THE CHARACTER OF THE INTESTINAL FLORA

In the feeding experiments with albino rats no definite relation could be established between the hydrogen ion concentration and implantation of *B. acidophilus* in the intestine. Similar attempts have been made more recently to determine whether the rapid development of aciduric organisms following lactose feeding is the result of increased acidity of the intestinal contents. The same methods were employed as in the work on the rats (page 61) except that for the hydrogen ion determination tests of human stools three grams of fecal material were suspended in fifty cubic centimeters of neutral water. The subjects included five individuals who received either 300 or 400 grams of lactose daily, five who took 150 grams of lactose and 150 cubic centimeters of whey broth culture of *B. acidophilus*, five who received 300 cubic centimeters of the broth culture, two that were given 1000 cubic centimeters of *B. acidophilus* milk daily, and two controls subsisting on the ordinary daily diet. A total of thirty-eight hydrogen ion concentration determinations was made. The bacteriological findings are presented here along with the pH figures (Table 70).

TABLE 70

THE RELATION OF DIET TO HYDROGEN ION CONCENTRATION AND BACTERIAL FLORA IN FECAL SPECIMENS FROM HUMAN SUBJECTS

Subject	Diet	Suspension of Feces 8 Days After First Feeding		Suspension of Feces 10 Days After First Feeding	
			Per Cent of B. Acidophilus		Per Cent of B. Acidophilus
		Ph		Ph	
	Control				
F	Ordinary Daily Diet	6.4	0	6.1	1
I		6.0	3	6.2	2
A	Ordinary Daily Diet Lactose 300 gms.	5.6	98	5.5	97
C		6.2	95	6.4	91
D		5.0	99	5.1	99
L		6.4	70	6.2	87

TABLE 70 (Continued)

Subject	Diet	Suspension of Feces 8 Days After First Feeding		Suspension of Feces 10 Days After First Feeding	
		Ph	Per Cent of <i>B. Acidophilus</i>	Ph	Per Cent of <i>B. Acidophilus</i>
B	Ordinary Daily Diet Lactose 400 gms.	6.0	86	6.2	89
F	Ordinary Daily Diet Lactose 150 gms. <i>B. Acidophilus</i> 150 cc. (Whey Broth Cult., Neph. 5)	6.2	95	6.0	60
G		6.0	98	6.1	94
H		6.4	95	6.2	92
I		6.5	95	6.2	93
K		6.0	99	6.4	99
A	Ordinary Daily Diet <i>B. Acidophilus</i> 300 cc. (Whey Broth Cult., Neph. 5)	5.9	90	6.0	94
B		6.5	90	6.4	92
C		6.0	90	6.6	90
D		5.8	95	5.6	92
E		6.0	95	6.8	96
F	Ordinary Daily Diet <i>B. Acidophilus</i> Milk 1000 cc.	6.4	94	6.2	92
G		6.2	96	6.0	90

No correlation exists between acidity and the prevalence of *B. acidophilus* in the stools, according to the results of these experiments. While there is an appreciable range within which the hydrogen ion concentrations vary, the differences and the extremes are no greater than

for the combined list of controls and subjects receiving the whey broth culture of *B. acidophilus*, except subject D and perhaps A whose figures were as low at 5.0 and 5.5 respectively. These two subjects were very susceptible to lactose feeding, requiring much less than 300 grams to effect a simplification of the flora. When they received as much as 300 grams daily the implantation of *B. acidophilus* was practically complete, and there was a tendency toward a diarrheal condition. There was a large remnant of lactose in the feces, and the conclusion is to be drawn that the excess of lactose in itself caused some intestinal irritation either directly or through the production of more acid than could be neutralized or absorbed as quickly as it is formed. However, these figures are well within the range of normal stools, as will be seen by comparison with the results of Howe and Hawk (1912) and Nelson and Williams (1916-1917). Certainly there can be no question of increased acidity in the stools of subjects who received 150 grams of lactose together with the 150 cubic centimeters of *B. acidophilus* culture, as compared with those who took no lactose at all.

IV. A FULL ACCOUNT OF THE PREPARATION OF *BACILLUS ACIDOPHILUS* MILK FOR HUMAN CONSUMPTION, AND OF ITS KNOWN PROPERTIES

Much time and effort have been given in recent years to the production of sour milk and sour milk products. Milk soured with *B. bulgaricus* powders or tablets and special preparations sold as koumiss have gained wide usage, especially in this country. Irrespective of the alleged therapeutic properties from the standpoint of bacterial implantation in the intestine, these preparations are of undoubted merit. They are a valuable food and serve as a substitute for ordinary milk for which many persons have little or no tolerance and which to many others is objectionable as such. Furthermore, these sour milk products, when they have been prepared successfully, are beverages which have attained considerable popularity.

Bacillus acidophilus milk bears some resemblances to sour milk prepared with pure cultures of *B. bulgaricus* or with bulgaricus powders or tablets. Coagulation of the casein takes place in both, due to acid production, and no gas is produced. There is an absence also of other products of the ordinary putrefactive type. Both are distinctly acid to the taste, and unless the *B. bulgaricus* milk has been incubated too long, they are palatable.

The two products differ in the following respects. The acidophilus milk never attains the degree of acidity in old culture as does the bulgaricus milk; in fact there is little or no danger of the former becoming too sour if the time of incubation is within reasonable range, which cannot be said of the other product. The acidophilus milk acquires a creamy, but never sticky or stringy, consistency, while the bulgaricus milk has a thicker and at times a more or less slimy character, depending largely, of course, on the particular strains of *B. bulgaricus* employed in the preparation. The acidophilus milk has a decidedly perceptible aroma and taste, which add very materially to its palatability and which are more or less absent from the bulgaricus milk.

The following method is employed in the preparation of the *Bacillus acidophilus* milk.

Sweet skimmed milk is sterilized by autoclaving at an extra pressure

of fifteen pounds for twenty to thirty minutes, or longer when more than 1000 cubic centimeters are sterilized in a container. For this purpose Pyrex Florence flasks have been employed in the present investigation. The cooled milk is inoculated with a pure culture, preferably mixed strains, of *B. acidophilus*, and incubated at 35 to 37° C. for twelve to twenty-four hours. The inoculum should be a milk culture not more than seventy-two hours old.

It is at this point that special effort is required to obtain a product that is well soured and viable. Recently isolated strains of *B. acidophilus* are slow and ineffective. Therefore, it is necessary to employ strains which have been grown in milk for at least two or three weeks and which have been transplanted from milk to milk at frequent intervals, preferably every day. The amount of inoculum for each transplantation should be large, as compared with the usual procedure. For every 1000 cubic centimeters five to ten cubic centimeters of the inoculum are required to obtain maximum development within twenty-four hours' time. The transfers are made with sterile pipettes made from sections of glass tubing. These pipettes are drawn out at one end, but the opening must be large enough to admit coagulated milk. Platinum loops are useless for making the transfers.

As soon as the milk has undergone coagulation it is removed and placed in the refrigerator. It is important that the refrigerator compartment in which the milk is kept be clean and free from odors which may be absorbed. When kept under these conditions the milk changes very little in the course of a few days, but should be used very soon.

If it is desired to reenforce the milk with lactose, the sugar may be added at the time the milk is taken. It will be expedient to shake the lactose and milk well and allow the mixture to stand for thirty minutes or more in a cool place. This will allow a goodly portion at least of the sugar to dissolve and to reduce the gritty character of the lactose-enriched milk. For those who crave sugar the addition of the lactose greatly adds to the richness of the milk.

In the present investigation four strains of *B. acidophilus* have been employed for the production of acidophilus milk. These strains were isolated from human feces in October, 1919, and were grown on whey agar for five months, transfers being made three times a month. For the inoculation of the first milk tubes the four strains were combined. Transfers were made almost daily. At first it required five or six days, and at times even more than a week for the milk to show indications of acidity and coagulation. The coagulation time was gradually shortened until three weeks after the first milk culture was prepared, when coagulation was obtained within twenty-four hours. At the end of the fourth week of almost daily transfers the coagulation time was reduced to twelve to fifteen hours, where it stands at the present time, many months after the first milk inoculation.

Experiments are in progress to determine the influence of temperature on coagulation time. Most rapid growth and acid production take place at 35 to 37° C. Very satisfactory results are obtained also at 30°, though in a somewhat shorter period. At 20° slow coagulation occurs, while below this temperature little or no change takes place in the appearance of the milk during the first three or four days. It appears from these experiments as if incubation at 30° may be substituted for the 37° temperature, providing the organisms are quite viable in milk, as in the present instance.

Experiments are under way also to determine whether sterilization of the milk, prior to the inoculation, is absolutely necessary. Instead of heating the milk in the autoclave it is heated in a double boiler or in a flask deeply submerged in boiling water for forty-five minutes to one hour. This treatment does not destroy spores and hence the milk is not rendered sterile. Inoculation with *B. acidophilus* was followed by incubation of duplicate samples at different temperatures, 20°, 30° and 37° C. Control flasks, that is flasks which were heated in the same way as the others, were incubated without being inoculated with *B. acidophilus*, and incubated beside the others. All of the flasks were examined at twelve hour intervals when whey agar plates were poured and Veillon tubes were inoculated from the different flasks.

Within twenty-four hours the milk flasks that had received the *B. acidophilus* inoculum and were incubated at 30 and 37° underwent the characteristic acidophilus coagulation, and there was no indication by odor or physical appearance that any putrefactive action was in progress. These observations were fully corroborated by the plates and Veillon tubes, which contained pure or practically pure cultures of *B. acidophilus*. The control flasks, on the other hand, gave marked evidence of putrefaction within twenty-four hours. There was partial coagulation and later a very extensive liquefaction of the casein, the liquid appearing watery, accompanied by offensive odors. Similar changes took place in the corresponding flasks incubated at 20° C., but they were very much slower.

These experiments indicate that, while *B. acidophilus* does not produce as much acid in milk as do *B. bulgaricus* and *Streptococcus lacticus*, it nevertheless protects the milk against undesirable decomposition by so-called putrefactive organisms, and if viable and present at the outset in considerable numbers, it should render milk safe against decomposition harmful to the consumer. Whether boiled milk may be substituted for the sterilized and the same satisfactory product can be obtained has not yet been determined. Boiled or incompletely sterilized milk must be inoculated immediately, however, before any bacterial change has taken place. Hence, from the practical standpoint, sterilization will be necessary to obtain constant and reliable results.

V. METHODS EMPLOYED IN THE ROUTINE EXAMINATION OF FECES

No phase of bacteriology has been more seriously handicapped by the lack of adequate methods than the study of intestinal micro-organisms. The most recent contribution is the work of Morris, Porter and Meyer (1919), who advocate the technique employed by them in their bacteriological examinations of children's stools. While these methods may be of considerable value in the hands of trained technicians, and for certain purposes, they have not appeared practical and sufficiently direct to be of material help in the present investigation. It has been our aim to employ the simplest technique that is consistent with our purpose, namely to determine at frequent intervals the relative numbers of *B. acidophilus*-like bacteria as compared with the total of all other organisms, and to follow the progress of gas-producing organisms as they increase or decrease in numbers in the fecal material examined. The following methods were adopted early in this work and have constituted a large part of the daily routine examinations. They are presented at some length here because they have proven themselves practical and in a high degree satisfactory.

THE USE OF WHEY AGAR PLATES

At the outset 1 per cent glucose agar was employed for plating, etc. This was soon discarded in favor of whey agar which by a series of comparative tests was found to be superior in every way to the other media under consideration. Many difficulties were at first encountered, however, in the preparation of clear whey from skimmed milk. These difficulties were eliminated as soon as the following method was perfected.

The skimmed milk is heated to 80 to 90° C., and five cubic centimeters removed to a test tube; while the test sample is still hot it is treated with 10 per cent solution of hydrochloric acid drop by drop until complete coagulation takes place. This is repeated two or three times. The calculated amount of acid required to coagulate all of the milk is thoroughly mixed with the milk, the temperature of which is still 80 to 90°, and the casein allowed to settle. This is followed by filtration,

at first through absorbent cotton and then paper, and neutralization of the filtrate with sodium hydroxide to pH 6.8-7.0. Before further filtering, 0.5 per cent peptone is added and the medium autoclaved for fifteen minutes at fifteen pounds extra pressure. The albuminous material is filtered off through paper, and the clear filtrate employed as such for whey broth or for the preparation of whey agar. To convert into whey agar add 1.2 per cent of standard shredded or powdered agar to the whey broth.

The agar plates were prepared by the usual dilution process. The first tube of liquefied whey agar was inoculated with a four millimeter platinum loop, a bi-convex loopful of the standardized fecal suspension (see pages 11-12) being employed. For further dilutions, three and five loopfuls of tubes one and two were transferred. The plates were incubated for forty-eight hours at 37° C. under aerobic conditions.

B. acidophilus colonies were recognized with the aid of the microscope, and their relative number as compared with all other colonies determined. The counting was facilitated by the use of an ocular which is marked off in small squares. There are two types of agar colonies of *B. acidophilus* designated by Horton and Rettger (1914) as X and Y. The former have a decidedly fuzzy appearance and resemble small agar colonies of *B. tetani*; these are indistinguishable from the colonies of *B. bulgaricus*. (See Plate II.) The Y type is small and round to spindle-shaped and is only partly fringed, at times appearing almost perfectly smooth.

When in some of the experiments there was any doubt as to whether the observed colonies were those of *B. acidophilus* or *B. bulgaricus* transfers were made into maltose (1 per cent) broth and the tubes incubated at 37° for at least forty-eight hours. *B. acidophilus* attacks the maltose and causes a decided acidity which is easily recognized by any of the ordinary tests. *B. bulgaricus* produces no such change. Morphological studies and milk coagulation tests were made also in some instances.

The study of whey agar plates gave the most valuable information in so far as the relative numbers of viable *B. acidophilus* are concerned, and in a most satisfactory way showed the changes that were taking place in the flora from the complex to the simple acidophilus phase, and vice versa. The results are given in the tables in terms of percentage.

VEILLON TUBES

The Veillon tube as described by Veillon and Zuber (1898) was employed, with the following modification. Instead of one end being permanently sealed, both ends were left open, and the tube was nothing more than a nine-inch section of one centimeter glass tubing. Previous

to filling, one end of the tube is tightly plugged with a rubber stopper. After filling, the other end is plugged with non-absorbent cotton.

The Veillon tubes are filled about twelve centimeters deep with the 1.2 per cent whey agar described above, and sterilized in the autoclave. The inoculation of the tubes was accomplished in the same way as the agar tubes that are used for immediate plating. It is of much importance to mix the agar and inoculum thoroughly, and this is done best by tilting the tubes back and forth somewhat vigorously, even though the cotton plug becomes soiled. The formation of gas bubbles must be avoided, however. The tubes are incubated for at least forty-eight hours at 37° C. The colonies are examined with the aid of a hand lens which magnifies at least four or five times.

These tubes are well adapted for the study of both aerobic and anaerobic organisms as well as the intermediate forms, as for example *B. bifidus*. Furthermore, they present an excellent index of the presence of gas-producing organisms found in the intestine, namely *B. coli* and *B. welchii*, and of their relative numbers. For this purpose alone they have been of inestimable value not only for the study of rat, but of human feces as well. Gas-producing bacteria in rat feces are usually relatively few, but in the stools of man they are as a rule very abundant and it was feared at first that the agar would be so thoroughly broken up as to make it impossible to study the individual colonies with any degree of satisfaction. This obstacle is encountered in the examination of ordinary (often termed "normal") feces of man, but in direct proportion to the transformation that is brought about in the intestinal flora by the agents which have been successfully employed in this work, the number of gas-producing bacteria correspondingly diminishes and often the tubes remain entirely free from gas in the course of the experiments.

Bacillus acidophilus is recognized in the Veillon tubes by the rather small fuzzy or sea-urchin-like colonies which develop particularly well in the upper and middle layers of the agar. Colonies of *B. bifidus* are small, disc-like and smooth, and are most abundant in the three to six centimeter layer which begins about one centimeter below the surface. The colonies of the strict anaerobes make their appearance in the depth of the agar. The different colonies may be reached for subculture study and microscopic examination by removing the rubber stopper, drawing or forcing the agar column out of the lower end of the tube, and fishing with a platinum wire or a very fine-pointed pipette, or by filing and breaking the tube in the immediate vicinity of the chosen colony. In the present work almost the entire emphasis was placed on *B. acidophilus* colonies and gas production. These are indicated in the various tables by the following signs: —, +—, +, ++, +++ and ++++, as has already been explained on page 12. (See Plate V.)

DIRECT MICROSCOPIC EXAMINATION OF FECAL
SUSPENSIONS

Films were prepared from the standardized suspensions of fecal specimens and stained by the Gram-method. The slides were counter-stained with dilute carbol fuchsin. Such slides are of considerable value in that they show the relative predominance of the different types of bacteria, and emphasize in particular the conspicuous large *Bacillus acidophilus*-like rods when they are present in any numbers. This method has serious limitations, however, in that it does not give a true picture of the viable organisms which are present in the intestinal tract. Many of the rods and cocci that are seen in these preparations are dead at the time of leaving the animal host, although they take one or the other of the stains. Furthermore, such dead bacteria may continue for considerable periods to be eliminated, owing to their being held back in the folds and on the inner walls of the intestine.

The results of the examination of stained films were recorded verbally in the tables summarizing the data from the rat-feeding experiments, while at the time the human subjects were employed they were recorded in terms of percentage of Gram-positive and Gram-negative organisms and Gram-positive rods and cocci. As will be seen in the different tables these results correlated definitely with those obtained by the use of whey agar plates and the Veillon tubes. The relative numbers of aciduric organisms as compared with all other organisms present in human feces were as a rule smaller by the direct than the culture methods, and in many instances very considerably smaller. In the examination of the rat feces, on the other hand, the direct count was in perfect agreement quantitatively with the results of the other two methods. In spite of the recognized limitations of this method, it has proven itself to be indispensable in the present investigation.

VI. GENERAL DISCUSSION AND SUMMARY

Under conditions of normal functioning the intestinal flora is fundamentally a physiological unit rather than a heterogeneous collection of adventitious microorganisms, and bears a definite relation to the diet of the host.

The present study has shown that the types of bacteria developing in the alimentary tract may be influenced in a noteworthy manner, not only through special alterations in the chemical composition of the diet, but also through the ingestion of living cultures of *B. acidophilus* with or without accompanying carbohydrates. Only two of the carbohydrates here employed, however, have the property of stimulating the aciduric bacterial type, namely lactose and dextrin.

Lactose and dextrin, when added in given amounts to the basal diet (bread and meat) of the white rats or when taken by men in addition to the usual daily diet, brought about a marked transformation of the intestinal flora within four to six days, and stimulated the development of *B. acidophilus* to such an extent that it largely or entirely dominated the flora, effecting an almost complete suppression of all other bacterial types commonly found in the enteric tract. The simple flora persisted as long as the feeding of these carbohydrates was continued. *B. bifidus* occasionally increased in numbers under these dietary conditions, but usually remained relatively obscure. It never became the dominant type.

These results are in harmony with those of certain other observers. Barker (1914) and Torrey (1915) claimed to have secured favorable results with the feeding of lactose and milk to typhoid fever patients. Torrey demonstrated that the administration of a high carbohydrate (lactose) diet tended to reduce the putrefying types of bacteria, and to favor the growth of organisms of the *B. acidophilus* type. Our results are in accord also with those of earlier investigations reported by Hull and Rettger (1917) and the more recent announcements of Torrey (1919), but they are diametrically opposed to those obtained by Sisson (1917) in similar feeding experiments with puppies. Sisson reported that a milk diet containing 10 to 15 per cent of lactose did not cause an intestinal flora essentially different from that following a diet of whole pasteurized milk. In fact, he failed to find any organisms of the *B. acidophilus* type in any portion of the alimentary canal.

In a short paper by Distaso and Schiller (1914) some feeding

experiments with white rats were reported which indicated that lactose and dextrin exercise a marked transforming power on the intestinal flora, bringing about a predominance of *B. bifidus*. These results are in agreement with our own in principle, but differ in that they placed chief emphasis upon the Tissier organism in their findings, whereas in our experiments *B. acidophilus* played the rôle of primary importance.

Hull and Rettger (1917), as the result of limited observations on this particular carbohydrate, reported that the administration of dextrin to albino rats did not cause any noticeable increase in the numbers of *B. acidophilus*. These findings are in disagreement with the results of dextrin feeding in the present investigation, and may have been due to the idiosyncrasies of the few rats that were used by Hull and Rettger in the dextrin feeding experiments.

Kendall's suggestion (1911) that the feeding of liberal amounts of lactose to infants may cause an abnormal or excessive development of *B. welchii*, and similar contentions of other observers, receive no support whatever from the present studies. While there can be no doubt that *B. welchii* ordinarily finds favorable conditions within the intestinal tract of man and of the albino rat, the feeding of lactose has at no time caused an increase in the numbers of this organism. On the contrary, there was always a marked reduction, and at times complete elimination of not only *B. welchii*, but of *B. coli* as well.

Following the administration of two grams of lactose or dextrin in association with the basic diet, *B. acidophilus* made its appearance in all parts of the intestine of white rats, even the duodenum. Whether the presence of this organism in appreciable numbers in the small intestine was due to the proliferation of bacilli already occurring there, or to an upward movement of bacilli from the large intestine must of course still remain undetermined.

Maltose, saccharose and glucose appeared to exercise no transforming influence on the types of bacteria present in the intestinal tract of white rats. These results are in perfect agreement with those of Distaso and Schiller (1914) and Hull and Rettger (1917). Torrey (1919), however, claims to have obtained a moderate increase in the numbers of *B. acidophilus* as a result of sucrose feeding in dogs. The findings in the present investigation, which run almost parallel with those encountered in the bacteriological examinations of the fecal specimens of rats subsisting on the basal diet alone, emphasize the importance of the selection of special carbohydrates (lactose and dextrin) for favorable transformation of the intestinal flora, and not merely a carbohydrate, as is so often recommended.

The most plausible explanation of the favorable influence of lactose and dextrin feeding on the implantation of *B. acidophilus* is one which to a large extent rests upon the fact that they are not completely destroyed before reaching the large intestine. There they establish an optimum environment by serving as a readily available source of energy

for *B. acidophilus*. As this organism is normally present in the intestine, though usually in small numbers, and merely requires a stimulus to further development, which is furnished by the dextrin and lactose, the conditions for its development upon the administration of these carbohydrates become even more favorable than for any of the other intestinal organisms. This theory was advanced by Hull and Rettger (1917) who also reported the presence of a reducing substance in the feces of rats fed on lactose. The other carbohydrates used in this investigation, on the other hand, do not reach the large intestine, where bacterial activities are at their greatest, and hence are unable to exert any influence on the putrefactive processes going on here.

That the stimulating influence of lactose and dextrin is not due to acids produced from these substances is strongly indicated by the absence of increased hydrogen ion concentration in the fecal materials from the caecum and colon of rats and from the feces of men receiving these carbohydrates. This observation is in harmony with those of other investigators. Hirschler (1886) concluded that the presence of acid in the intestine is not in itself sufficient to prevent putrefaction. Winternitz (1892) claimed that lactose when fed to animals or man exerted an inhibitory influence on putrefaction, but that this action was due to the sugar itself, and not the lactic acid formed from it. Rovighi (1892) pointed out that the ingestion of lactic acid had only a very slight influence on intestinal putrefaction, and Fischer (1915) observed that acid played no part in inhibiting indol formation.

While the multiplication of *B. acidophilus* is stimulated to a greater or less extent, the transformation brought about by the feeding of one gram of lactose or dextrin to white rats or 150 grams to man seldom amounted to more than 50 per cent, and with a few exceptions in man, there was no radical suppression of other types of bacteria developing in the intestinal tract. In order to obtain complete transformation two grams of lactose are required for rats, and as a rule 300 grams for man. This is in accord with findings of Hull and Rettger (1917), who showed further that with increases of lactose beyond two grams daily *B. bifidus* tended more and more to acquire prominence in the feces of white rats, and that when as much as three grams was fed daily the Tissier organism assumed the chief rôle, in place of *B. acidophilus*.

The successful transformation brought about in rats by the combined action of one gram of lactose or dextrin and one cubic centimeter of *B. acidophilus* suspension, and of 150 grams of lactose and 150 cubic centimeters of culture in man offers a new avenue of approach to the intricate field of bacterial implantation within the intestinal tract.

What appears to us to be of no less significance is the demonstration in the present investigation that the administration of the acidophilus suspension alone, that is, without added carbohydrates, results in the implantation of *B. acidophilus* and suppression of the other bacterial

PLATE I

FIGURE 1

FIGURE 2

[See explanation of Plates on p. 125]

PLATE II

FIGURE 3

FIGURE 4

FIGURE 5

PLATE IV

FIGURE 6

FIGURE 7

PLATE V

FIGURE 8

PLATE VI

FIGURE 9

PLATE VII

FIGURE 10

PLATE VIII

FIGURE 11

types ordinarily present in the alimentary canal. It is of interest, too, to know that two cubic centimeters and 300 cubic centimeters respectively of the suspension, when fed daily to rats and to man, are sufficient to effect the transformation, and the maintenance of the simple flora.

Since the bacteria within the digestive tract procure their pabulum directly or indirectly from the diet consumed by the host, it is logical to assume that there is a definite relation between the chemical nature of the ingested food and the metabolic activities of the intestinal organisms. Many investigators have noted that marked changes in the intestinal flora follow sharp alterations in the diet; namely, that a proteolytic or putrefactive flora results from high protein feeding, and that a carbohydrate or fermentative flora develops when the protein intake is low and the diet consists largely of carbohydrates. However, the ordinary high-calory diets accomplish reformation only, and not a simplification of the intestinal flora, that is, a change from the putrefactive to the fermentative state of metabolism. As soon as the proper carbohydrate-protein ratio is disturbed the reformed intestinal tenants begin to sin and putrefactive metabolism again holds sway.

The harmful effects caused by, or associated with, pathogenic organisms of exogenous origin, such as *B. typhosus*, *B. dysenteriae*, *Ms. cholerae*, need no further comment. But it should be a matter of much concern that the so-called "normal" organisms of the intestinal canal may occasionally bring about decidedly abnormal conditions. Booker (1897) claimed that *Proteus vulgaris* and certain streptococci may elicit diarrheas in infancy. Bertrand (1914) found *Proteus vulgaris* in each of his fifty-five patients suffering from diarrhea. Schumburg (1902) reported the same organism as the cause of poisoning following the consumption of sausages. Klein (1896, 1898) concluded that *B. welchii* is an important factor in the causation of infantile diarrhea, and Tissier (1905) described cases of acute diarrheas caused by this organism. More recently Kendall and his associates (1911, 1913) and Smith (1913) gave expression to similar observations and views.

Senator (1868) declared that the decomposition of proteins within the intestinal canal under ordinary conditions results in the formation of substances toxic to the host. Bouchard (1884) elaborated the theory of auto-intoxication. Combe (1907) stated that food too rich in nitrogen will cause intestinal auto-intoxication. Metchnikoff published his views on the same subject, calling attention to a definite alleged relation between premature senility and intestinal putrefaction. He claimed that the proteolytic intestinal organisms are constantly producing substances which are absorbed by the host and which act as accumulative poisons. Metchnikoff proposed to fight these proteolytic bacteria on their own battlefield by the introduction of an antagonistic microbic army, the lactic acid bacilli, and thus ushered in a new and

interesting field of study, namely bacterial implantation within the digestive tract for therapeutic purposes.

According to Metchnikoff, the so-called lactic acid therapy consists essentially in administering per os living cultures of lactic acid bacilli, either in milk soured by them or as tabloids, or in pure culture together with some fermentable carbohydrate. The lactic acid bacilli ferment the carbohydrate in the alimentary canal, producing lactic acid and thus exerting a direct influence on the bacterial processes, particularly in the large intestine. Soured milk was considered to be of special merit in this method of treatment, particularly milk which was rendered acid with *B. bulgaricus*.

So general has the use of soured milk and of *B. bulgaricus* products become that the market is flooded with various commercial preparations, in the form of powders, tablets or liquid cultures or suspensions which contain as their active principle the organism which has been shown to be the most important souring agent in the sour milk products of the Orient, namely *B. bulgaricus*. Not only has it been claimed that beneficial results are obtained by the ingestion of milk which has been soured by *B. bulgaricus*, but that this organism itself, without the addition of milk or lactose exerts the same favorable influence by destroying or eliminating harmful bacteria from the intestinal tract, and thus preventing auto-intoxication.

Repeated attempts in the present investigation to establish *B. bulgaricus* in the alimentary canal of albino rats and of man through the administration per os of extremely large numbers of this organism were entirely unsuccessful. At no time in the study was *B. bulgaricus* recovered from the feces of man or the white rats or from any portion of the digestive tract of the rats, irrespective of whether the organism was fed alone or together with a utilizable carbohydrate. However, in all of the subjects which received the suspension of *B. bulgaricus* in connection with lactose or dextrin there followed a multiplication of *B. acidophilus*, and to the same extent as when similar amounts of the carbohydrates were fed without any addition of bacterial suspensions.

These results are in harmony with those of the following observers. Kulka (1914) demonstrated in his experiments upon man that the "*B. metchnikovi*," when introduced per os, cannot be recovered from the feces. Rahe (1915) failed to procure any evidence by feeding experiments that *B. bulgaricus* is capable of surviving in the lower intestine of man. Morse and Bowditch (1906) found that practically the same, and in some instances better, results were obtained with pasteurized as with raw acidified milk or buttermilk, and concluded that the action of the lactic acid bacteria is unimportant.

Although considerable doubt has been cast upon Metchnikoff's explanation of the merits of sour milk therapy, the fundamental principle of his arguments must in the main be accepted. That is, bacterial implantation with the concomitant transformation of the intestinal

flora is both desirable and possible. But it is possible only when living cultures of properly qualified organisms are employed, or when such specific diets are administered which stimulate and favor such a transformation. *B. bulgaricus* is incapable of accommodating itself to intestinal conditions. *B. acidophilus*, on the other hand, being of intestinal origin lends itself readily to implantation in the intestinal canal.

That the value of sour milk feeding does not lie in the lactic-acid-producing bacteria in the milk, nor in the acids which they produce, has been conclusively demonstrated by Rettger (1915) and his associates in their extensive experiments with several thousand chicks. Practically the same results were obtained whether sweet or sour milk was fed, and no difference could be observed in the growth- and health-stimulating properties of ordinary sour milk and of milk soured with *B. bulgaricus*.

The feeding of *B. bulgaricus*, without due regard to the use of milk, can have little or no importance attached to it. The beneficial results which have been attributed to yoghurt and other Oriental sour milk products have in all probability been due to the milk as such, rather than to the acid-producing bacteria which they contain.

Milk by virtue of its milk sugar stimulates proliferation of *B. acidophilus* in the intestine. This important fact was apparently overlooked by Metchnikoff and his pupils. What they observed as *B. bulgaricus* in the feces after the feeding of milk soured with *B. bulgaricus* was in reality not this organism, but *B. acidophilus*. That such an error of interpretation could have been made should not be at all surprising in view of the fact that these two organisms resemble each other so closely and are in fact indistinguishable in practically all of their various aspects. The marked resemblance of *B. bulgaricus* and *B. acidophilus* in cell morphology and orientation and in colony formation on lactose or whey agar plates is well shown in the accompanying photographs (Plates 1 and 2). It should be borne in mind that in many of the experiments conducted by Metchnikoff and his enthusiastic followers large quantities of milk were consumed, and hence the conditions for the development of *B. acidophilus* were more favorable, so that instead of the acclimatization of *B. bulgaricus*, as maintained by them, *B. acidophilus* gained prominence and was the organism seen by them in the feces. This assumption is strengthened by the announced observations of Belonowsky (1907b) that the intestinal flora of nursing mice was of the same character as that of the older mice receiving the *B. bulgaricus* milk culture.

It was demonstrated in the present investigation that the amount of lactose or dextrin required to cause practically complete simplification of the fecal flora could be reduced by one half, providing living cultures or suspensions of *B. acidophilus* were given along with the carbohydrate.

B. bifidus is also an inhabitant of the large intestine, and may be looked upon as a logical candidate for simplifying the flora, but this

organism is not so easily cultivated as *B. acidophilus*, and it has a much higher energy requirement, so that much larger amounts would be needed to establish it as the dominating flora than when the Moro bacillus is employed. However, both *B. bifidus* and *B. acidophilus* administration with a view to implantation has a thoroughly sound and logical basis, which cannot be said of *B. bulgaricus*.

Milk cultures of *B. acidophilus* have proven themselves to be particularly effective in transforming the intestinal flora of man. In some instances 500 cubic centimeters of a twelve to twenty-four hour culture were sufficient to bring about simplification with the desired elimination of the non-aciduric forms. A total of 1000 cubic centimeters taken daily in two applications in every instance brought about the transformation in the course of a very few days. Practically the same results were obtained when a daily amount of 500 cubic centimeters was reinforced with 100 grams of lactose.

B. acidophilus sour milk may be prepared readily, providing strains of the organism are employed which have been grown for some time in milk and which are adapted to this medium, and if transfers are made to the sterile milk with liberal amounts of the inoculum, preferably a young milk culture. When correctly prepared and cooled the milk is of a creamy consistence. It should be shaken, however, for some time, before using, to make it perfectly homogeneous and creamy in appearance. Furthermore, the product has a pleasing odor and is decidedly palatable. Aside from any therapeutic or medicinal properties which acidophilus milk may possess, it should gain wide use as a beverage alone, or as a food which has the nutritional properties of milk as such.

No definite claims are made at this time that whey broth and milk cultures of *B. acidophilus* have distinct therapeutic properties, although two subjects who presented a long history of serious intestinal disturbance were among the 17 subjects employed in the 45 milk-feeding experiments conducted on man, and appeared to respond favorably to the administration of 1000 cubic centimeters daily. These clinical cases were not sought at this time, but requested to be admitted to the experiments. We hesitate to discuss further the very satisfactory results that have been obtained, from every standpoint, with these two subjects until a large number of clinical cases have been under observation. Our real aim thus far has been to determine whether intestinal implantation of *B. acidophilus* in the white rat and in man can be accomplished, and under what conditions such implantation, whether temporary or relatively permanent, can be brought about. The experiments here recounted furnish the answers.

EXPLANATION OF PLATES

PLATE I

Fig. 1. *B. acidophilus*. Stained preparation from culture in glucose broth incubated forty-eight hours at 37° C. Note the pleomorphism of the organism. X 1000.

Fig. 2. *B. bulgaricus*. Stained preparation from pure culture in whey broth incubated forty-eight hours at 37° C. Note close similarity of the individual cells and chains of this and the preceding figure. X 1000.

PLATE II

Fig. 3. Forty-eight-hour colony of *B. acidophilus* on glucose agar incubated at 37° C. X 100.

Fig. 4. Forty-eight-hour colony of *B. bulgaricus* on whey agar incubated at 37° C. X 100. Note the similarity of the two colonies.

PLATE III

Fig. 5. Surface growth of *B. acidophilus* in a tube of whey agar grown aerobically at 37° C. for forty-eight hours. Note small discrete colonies. X 4.

PLATE IV

Fig. 6. Colonies on whey agar plates prepared from feces of human subject immediately preceding a period during which 1000 cc. of *B. acidophilus* milk was taken daily. Note absence of small irregular and fluffy colonies. X 40.

Fig. 7. Colonies on whey agar plates prepared from feces during the period in which 1000 cc. of *B. acidophilus* milk were taken daily. Note the dominating colonies of *B. acidophilus* which are characterized by their small size and very irregular border. X 40.

PLATE V

Fig. 8. Veillon tubes used in the present investigation, demonstrating their particular value in the determination of gas-producing organisms in the intestine of the white rat and of man. Note the gradual diminution of gas production from left to right in the series of six tubes. The gradations correspond to the different stages of transformation of the usual complex flora to the simple flora dominated by *B. acidophilus*. Natural size.

CAMERA LUCIDA DRAWINGS OF GRAM-STAINED FILMS OF
STANDARDIZED FECAL SUSPENSIONS FROM A
REPRESENTATIVE HUMAN SUBJECT

PLATE VI

Fig. 9. Film prepared immediately preceding the administration of *B. acidophilus* milk. Note relatively large number of Gram-negative organisms. X 1000.

PLATE VII

Fig. 10. Film prepared four days after the beginning of *B. acidophilus* milk feeding (1000 cc. daily). Note increase in the number of Gram-positive acidophilus-like rods. X 1000.

PLATE VIII

Fig. 11. Film prepared eight days after the initial feeding of *B. acidophilus* milk (1000 cc. daily). Note great preponderance of Gram-positive acidophilus-like rods. X 1000.

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134 TRANSFORMATION OF THE INTESTINAL FLORA

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