# A STUDY OF THE EFFECT OF MILK UPON THE BACTERIAL FLORA OF THE INTESTINAL TRACT.

# A STUDY OF THE EFFECT OF MILK UPON THE BACTERIAL FLORA OF THE INTESTINAL TRACT.

THESIS

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By

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# INTRODUCTION

Problems arising in connection with the high death rate among children in the United States have long been a worthy field for investigational and social welfare work.

It has been shown that of the 2,500,000 babies born each year in the United States, something like fourteen per cent or 350,000 die during the first year. This death rate as has been pointed out (35) is over seven times as high as that of the British soldiers engaged in the world war.

This great number of deaths is mainly due to two general causes: (1) neglect due to ignorance and (2) intestinal disorders. The first of these can be undoubtedly largely overcome by the efforts of social workers but the second must be controlled by the pediatrician who must have reliable data at hand in order to develop a rational system of treatment.

While clinical experience has shown the desirability of breast feeding over artificial feeding, definite data is still lacking in respect of the precise effects of artificial milk foods upon the intestinal flora of young children.

Tissier (1), Moro (2), Logan (3), Kendall (4), and others found that the organism named by Tissier as Bacillus bifidus communis was predominant in the feces of breast-fed infants, while Bacillus acidophilus was more predominant in the feces of some bottle-fed infants. These two organisms, being able to produce large amounts of acid in the presence of carbohydrates, were considered as fermentative organisms to distinguish them from putrefactive types.

It was thus thought that normal infants had a predominance of fermentative organisms in their feces and that these in turn produced sufficient acid to inhibit the growth of toxin producing organisms, which are believed to be the cause of many intestinal disorders among infants.

Porter, Morris and Myers (5) have recently shown that the above assumption is not altogether true. They found that the fecal flora of normal infants consisted of both fermentative and putrefactive organisms and that neither predominated, but that in the case of infants suffering with intestinal disorders that the putrefactive organisms predominated.

This project was thus undertaken primarily with a view to determine the type of bacterial flora existing in the feces, when various milk diets were

fed, and secondarily to determine whether the guinea pig could be utilized to advantage in this line of research.

HISTORICAL REVIEW OF LITERATURE

Escherich (6) was perhaps the first investigator to study the fecal flora of both breast and bottle-fed infants. He employed both aerobic and anaerobic culture methods in his investigation and in his list of the commonly encountered bacteria of the intestinal tract, we find some of the most well known organisms completely discribed.

This investigator noticed that the majority of the bacteria discernible in the freshly passed feces were gram-positive and that his pure cultures derived from feces were for the most part gram-negative.

Schmidt (7), a student of Escherich, claimed that he could bring about this change by growing them in media containing fat. This, however, was disproved by Lehman and Neumann (8) and finally by Escherich (9) who published a paper in which he shows the fallacy of Schmidt's observations.

This problem of these gram staining bacteria

remained unsolved until Tissier (10) and Moro (11) took up the investigation. These investigators used special media and methods and as a result contributed two new organisms to the intestinal flora.

Tissier isolated an anaerobe to which he gave the name Bacillus bifidus communis. Moro's organism was aerobic and was given the name of Bacillus acidophilus because of its ability to grow in relatively large amounts of free acid. Both of these organisms were gram-positive bacilli, morphologically similar to those found in the stools of normal nurslings.

Finkelstein (12) working independently and using different media isolated organisms similar to and probably identical with the organisms isolated by Tissier and Moro.

A controversy then arose as to the relative predominance of B. bifidus and B. acidophilus. Tissier claimed that B. bifidus was the dominant bacillus in normal breast-fed infants and that Moro's organism occurred only in the feces of infants suffering from intestinal disorders and in normal bottle-fed infants.

Moro re-investigated the subject a few years later and found that B. bifidus was the dominant organism of normal breast-fed infants, but that B. acidophilus was also represented in the feces of such

infants though in fewer numbers.

Moro's view has now been substantiated by the researches of Cahn (13), Rodella (14), Coppolina (15), Jacobson (16), and others and it is definitely established that this view is correct.

Johannesen of Christiania in 1897 was probably the first to use acid broth as a medium for isolating bacteria which can grow in relative large amounts of free acid. About a year later, Bruno Heymann made use of dextrose broth to which acetic acid was added. This medium which is known as Heymann's medium is one of the best that has been used for the isolation of aciduric bacteria (17).

Among the first investigators to study the effect of diet on the types of intestinal flora were Herter and Kendall (18) who found in their experiments on kittens and monkeys that an abrupt change in diet from a dominant protein to a milk and sugar diet is followed by an alteration of physiological conditions in three distinct ways: (1) in the nature of the intestinal flora, (2) in the putrefactive products of feces and urine, (3) in the clinical conditions. These authors suggest that the change in intestinal flora is due to the addition of carbohydrates rather than a diminution in protein.

About the same time that Herter and Kendall did their work, MacNeal, Latzer and Kerr (19) made a study of the fecal bacteria of healthy men.

Noguchi (20) published a paper "Pleomorphism and Pleobiosis of Bacillus Bifidus Communis" in which he draws the conclusion that B. bifidus has an aerobic phase in which it closely resembles B. mesentericus fusicus and that the one source of the organism in the feces of breast-fed infants is the breast of the mother where it occurs as an organism of the skin.

Kendall (17), in 1910 published a paper, "Observation On Aciduric Bacteria" suggesting that there are two types of aciduric organisms: "The true or obligate aciduric bacteria of which B. acidophilus is the best known member; and the facultative aciduric bacteria which may include various organisms".

One year later Kendall (21) published a paper "Activity of Bacteria In Intestinal Tract", in which he concluded that by the feeding of lactose and dextrose the intestinal flora may be temporarily changed from that of toxin producing bacteria to that of fermentative bacteria. However, the after-feeding will be the determining factor as to whether the fermentative flora will remain.

In the researches of Logan (22) on "The In-

testinal Flora of Infants and Children", we find the findings of Moro (2) confirmed; namely, that in the flora of breast-fed infants the aciduric group is predominant and is strictly of the type called B. bifidus and that in the artificially fed infants the place of the B. bifidus is largely taken by the facultative aerobe, B. acidophilus. The cases on both breast and bottle showed a halfway stage between the two types of flora.

Cohendy (23) who fed four patients for an extended period on milk curdled with B. bulgaricus, concluded that this organism became readily established in the intestine and that it persisted there for a considerable time after the subject had ceased taking the fermented milk. This was said to be especially true if a diet was adopted containing suitable carbohydrates for the ingested organism. It is stated that the growth of these bacteria took place in the upper two-thirds of the colon.

In a later paper the same writer shows that intestinal putrefaction as indicated by the excretion of ethereal sulphates in the urine was materially reduced by the addition of sour milk to the diet, and that this reduction may be reasonably attributed to the disinfection of the large intestine, prolonged

after the ingestion of sour milk was discontinued. This may be taken as an indication that the growth of bacteria continued after introduction ceased.

Belonovsky (24) studied the effect of the Bacillus bulgaricus on the intestinal flora of mice. In his experiment several lots of mice were fed on a basic ration of sterilized grain and water and to the ration of two of his lots were added milk cultures of B. bulgaricus. Mice fed on this ration forty-five days showed this organism in the feces fifteen days after the last feeding. With animals fed the culture for four months, it was present for four weeks after the last feeding.

On the other hand, Herter (25) found that in the digestive tract of a monkey killed after feeding for two weeks on milk soured with B. bulgaricus, this organism was abundant in the upper part of the small intestine only. In the lower part of the small intestine and in the large intestine, B. bulgaricus was present in only moderate numbers as compared with other bacteria.

Heinemann and Hefferan (26) found an organism answering to the description of B. bulgaricus in ordinary milk in feces of cows, horses, and man, and in soil, grains, and pickles. They consider that it

is normally present in small numbers in the digestive tract and suggested that it may cause pathological conditions under certain circumstances.

In 1915 Rettger (27) published a paper, "The Influence of Milk Feeding on Mortality and Growth, and On the Character of the Intestinal Flora", in which he says "Practically the same results were obtained whether sweet or sour milk was fed and no difference could be observed in the relative value of ordinary sour milk and of the so-called bulgaricus product. Hence, the unique properties of this food exists in the milk as such rather than in any milk acids or milk bacteria that may be present". He also concludes that milk and lactose diet exert a very important influence on the character of the intestinal bacteria, especially in white rats and chickens. Within a few days after the ingestion of milk on lactose, a transformation of the flora takes place, the usual mixed flora giving away to a more simplified flora in which B. acidophilus and B. bifidus are prominent.

According to Rettger's studies the ingestion of large numbers of foreign bacteria does not of itself bring about an elimination or displacement of the common intestinal microorganisms.

Sisson (28) found in his work with puppies that the type of organisms occurring at the duodemun, ileum, cecum and rectum are in all instances similar and that one cannot speak of a characteristic local flora occurring in these regions. He also disagrees with previous investigators in that feeding puppies with cow's milk mixed with high percentages of sucrose and lactose does not cause characteristic changes in the intestinal flora at any level.

In direct contrast to Sisson's work is the work of Torrey (29) who found that the intestinal flora is changed by the feeding of carbohydrate diets and that it is not only shown in the feces but may be shown at the different levels of the intestines. Torrey suggests as a reason for Sisson's failure to observe a change in the flora, that the media he used was not suited to the growth of the aciduric type that is found when a high carbohydrate diet is fed.

Porter, Morris and Myers (30) found in their work with infants suffering with intestinal disorders that these disorders were always accompanied by a predominance of putrefactive organisms and that by a change to a high carbohydrate diet that these putrefactive bacteria could be replaced with more of the fermentative bacteria, thus relieving a large amount of the disorder. These investigators also found that

in the feces of a normal infant neither putrefactive nor fermentative bacteria predominated but that they occurred in about equal numbers.

## METHOD OF INVESTIGATION

The literature reviewed has not revealed any data or studies of the intestinal flora produced by the feeding of modified and fermented milks (except milk inoculated with B. bulgaricus) often fed to infants.

This investigation was designed to cover the following points: (1) a study of the intestinal flora of infants on various diets, (2) a study of the intestinal flora of guinea pigs fed on various modified milks with particular attention to raw and pasteurized milk.

The samples of feces studied were obtained and handled in the following manner: (I) those from infants were obtained through the courtesy of the Sparrow Hospital, Lansing, Michigan and were collected by means of a copper wire containing a loop in one end; standardized with a No. 2 lath nail. This wire was inserted into a test tube or small bottle through the cotton plug and sterilized before being taken to the hospital. The nurse in attendance at the time

excreta was passed would fill the loop of the wire from a representative portion of the feces and then place the wire and plug in the bottle. The bottle was then taken to the laboratory where proper dilutions were made by addition of sterile saline solution to the bottle containing the specimen. (No effort was made to secure quantitative results).

From the above suspension the following plates were poured:

(1) Endo plates that gave an insight into the type of aerobic bacteria which develop rather characteristically on this media such as B. coli, B. proteus, B. mesentericus and Streptococci.

(2) Liver glucose agar plates + 4 to phenolphthalein, incubated aerobically (31). This media offers a very good means of differentiating the aciduric bacteria. It also has the advantage of suppressing Streptococci and to a large extent B. coli.

(3) Liver glucose blood agar plates + 1 to phenolphthalein, incubated anaerobically for the cultivation of B. bifidus (31).

(4) Liver glucose agar plates + 1 to phenolphthalein, incubated anaerobically for cultivation of such spore bearing bacteria that might not develop on the other media (31).

(5) Litmus lactose agar plates, incubated aerobically for the per cent of acid producers that develop on this medium.

(6) Gelatin plates, incubated aerobically for the per cent of organisms producing liquefaction.

(7) Fermentation tubes of dextrose, lactose, saccharose and litmus milk were also inoculated and the percentage of gas recorded, likewise the physical appearance of the litmus milk, such as reduction and production of red and blue color.

(8) Acetic acid broth having acidities of N/20, N/10 and N/5, respectively were inoculated and incubated for the isolation of aciduric bacteria.

All cultures with the exception of the fermentation tubes were incubated for 72 hours and all but the gelatin plates at 37°C. Fermentation tubes were incubated for 48 hours at 37°C. and gelatin plates were incubated at 20°C. for 72 hours.

Novy's jars and the pyrogallic acid-caustic soda methods were used in obtaining anaerobic conditions.

(II) Fecal specimens from the guinea pigs were collected in the morning at intervals of every few days. Beaver-board, which had been scrubbed with bichloride of mercury was placed in the cages

as a false floor for the deposit of the feces. The pigs being closely watched until a deposit was made, when a portion was immediately collected and placed in a test tube containing about 10 cc. of physiological salt solution. After a suspension of the feces had been made, appropriate dilutions were prepared from the suspensions and the same media used as was used in the case of infants feces.

## EXPERIMENTAL DATA

### (1) Results of Infant Study.

The study of the fecal flora of infants was undertaken in order that the writer might test the media to be used as well as familiarize himself with the normal flora of healthy infants.

Table No. I gives the results of the studies made on the fecal flora of infants.

| Date | Cas I | 10/1 | 10/1 |
|---|---|---|---|

From the table it will be noticed that Cases I, II, V, and VI were fed from the breast. In these four cases the writer found a strongly gram-positive flora with B. bifidus predominating both in the stained film and the cultures. Other gram-positive organisms present were B. acidophilus, Staphlococcus pyogenes, and B. proteus. In Case I, Staphlococcus pyogenes aureus and Bact. welchii were quite prominent. This case, having a syphilitic history, was troubled to a great extent with constipation, senna and oil being given in order to produce bowel movement.

Case III which received Mellin's Food showed a gram-positive flora composed of B. acidophilus, B. bifidus and Streptococcus lacticus. This case also showed a very high fermentative flora.

Case IV was fed both from the breast and bottle. The latter (hospital formula) consisted of 1 oz. of milk sugar, 2 oz. of top milk, 11 oz. of water, and 1 oz. of lime water dissolved by adding the milk sugar to boiling water after which the top milk and lime water were added and stirred well. This formula was fed at the rate of 1 to 2 oz. every 2 1/2 hours.

The flora in Case IV was very similar to that of Case III except Streptococcus lacticus was

not so much in evidence and the percentage of fermentative organisms was lower.

It will be noticed from the table that the early specimens collected after birth showed little or no growth upon the culture media. This was in every case the meconium and in most cases it was doubtful whether the child had had the breast before motions.

The writer also observed that the three phases of bacterial infection of infants as mentioned by Kendall (4) are quite distinct, that is, (1) a sterile period in which the meconium is practically sterile for about 24 hours, (2) "Period of mixed infection" which occurrs about the third day, due probably to the presence of food in the alimentary canal. The organisms found during this period are _B. subtilis_, _B. coli_, _B. proteus_, _B. mesentericus_, etc. (3) "Period of Transition" in which _B. coli_ diminishes in number but does not entirely disappear; _B. bifidus_ becomes predominant and _B. acidophilus_ also appears.

The media used in identifying the organisms found were as follows: plain agar, dextrose agar, litmus lactose agar, blood agar, gelatin, plain milk, litmus milk, lactose, dextrose saccharose, mannit, and maltose broths in fermentation tubes as well as the special media mentioned on page 12.

## (2) Morphological and Cultural Characteristics of the Aciduric Group

*Bacillus bifidus*, the dominant organism in the stools of breast-fed infants, morphologically is a long thin rod, frequently slightly curved with tapering ends occurring singly in pairs or groups with the long axis parallel. Typically gram-positive, but under certain conditions may appear as gram-positive granules in otherwise gram-negative rods. Culturally, *B. bifidus* is an obligate anaerobe, fermentative in character, producing lactic acid but no gas. Grows best on glucose blood agar (Torrey 31) upon which its colonies are visible in 24 hours but more distinctive after 48 hours incubation as globular opaque colonies 1 to 3 mm. in diameter, buff to reddish brown in color.

*Bacillus acidophilus* is described by Moro (32) and by Finkelstein (33) as a somewhat pleiomorphic bacillus of varying length which may occur singly or in pairs, chain formation not being uncommon in artificial media. The organism forms no spores or capsules and is typical gram-positive, although in old cultures it may appear gram-negative.

*B. acidophilus* may be isolated direct from suspected material in N/20, N/10 and N/5 acetic acid dextrose broth by a series of transfers. Probably the

best solid medium for the growth of this organism is glucose liver agar + 4 to phenolphthalein and containing 0.2 per cent sodium oleate according to the procedure of Torrey (31). Most strains of _B_. _acidophilus_ form on this medium flat, dingy colonies with a serrated edge, although a few produce a round convex smooth edged colony. This organism like _B_. _bifidus_ is also fermentative in character but produces no gas.

(3) _Result of Experimental Work With Guinea Pigs_

The experimental work which follows was conducted with guinea pigs. Pigs were selected of approximately the same age and size and were all fed a basic diet of sterilized bran as a concentrate and carrots which had been peeled and washed in a solution of 1-1000 bichloride of mercury as a succulent food. To this was added the experimental foods.

(4) _The Effect of Feeding Raw Skim Milk Upon the Intestinal Flora of the Guinea Pig_.

Two pigs were fed fresh raw skim milk for a period of 45 days. The fecal flora of these pigs were studied at intervals of every 7 days. The results of these studies are shown in Table II.

Table II

Showing Results of Feeding Fresh Raw Skim Milk Upon Fecal Flora of the Guinea Pig

| | | |
|---|---|---|
| Diet | Bran | 35 gr. |
| | Carrots | 100 gr. |
| | Raw Milk | 100 cc. |

| Case | I | | | | | | | | | II | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Feeding period in days. | B | 1 | 3 | 7 | 10 | 17 | 24 | 31 | 45 | B | 1 | 3 | 7 | 10 | 17 | 24 | 31 | 45 |
| B. acidophilus | - | - | +- | + | + | + | + | + | + | - | - | - | +- | +- | + | + | + | + |
| B. bifidus | - | - | - | - | - | +- | +- | +- | +- | - | - | - | - | - | +- | +- | F- | +- |
| B. bulgaricus | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| B. coli | ++ | ++ | ++ | + | + | +- | - | +- | +- | ++ | ++ | + | + | + | - | - | +- | +- |
| B. l. aerogenes | + | + | + | +- | - | - | - | - | - | + | + | +- | +- | +- | - | - | - | - |
| B. mesentericus vulgatus | + | +- | +- | - | - | - | - | - | +- | +- | - | - | - | - | - | - | - | - |
| B. proteus vulgaris | ++ | ++ | + | + | +- | - | +- | +- | +- | + | + | +- | - | - | - | - | - | - |
| B. pyocyaneus | - | +- | - | - | - | - | - | - | - | + | + | + | +- | - | - | - | - | - |
| B. subtilis | ++ | ++ | + | + | +- | +- | +- | +- | +- | ++ | ++ | ++ | + | + | + | + | - | - |
| Staphlococcus pyogenes aureus | ++ | + | + | + | + | +- | ++* | +- | +- | + | + | + | + | - | - | - | +- | +- |
| Staphlococcus pyogenes albus | - | - | - | - | - | - | - | - | - | + | + | +- | - | - | - | - | - | - |
| Streptococcus lacticus | - | - | +- | + | + | + | + | ++ | + | - | - | +- | +- | + | + | + | + | + |
| Streptococcus pyogenes | - | +- | +- | + | +- | + | + | + | + | + | + | + | + | + | + | + | +- | +- |
| Other bacteria | +- | +- | - | - | +- | +- | - | - | - | + | +- | - | - | BME +- | - | SF +- | - | - |
| % Gas in dextrose broth | 30 | 35 | 30 | 10 | 10 | 5 | 0 | 5 | 5 | 25 | 20 | 20 | 5 | T | 0 | 0 | 5 | 5 |
| % Gas in lactose broth | 25 | 25 | 23 | 7 | 5 | T | 0 | T | 5 | 18 | 15 | 15 | 5 | 0 | 0 | 0 | T | T |
| % Gas in Saccharose broth | 10 | 10 | 8 | T | T | 0 | 0 | 0 | 0 | 15 | 10 | 10 | T | 0 | 0 | 0 | 0 | 0 |
| Action on Litmus milk | SA P | A P | A P | AC | AC | AC | AC | AC | AC | AP | AP | AC | AC | AC | AC | AC | AC | AC |
| % Gelatin liquefiers | 23 | 21 | 16 | 14.3 | 11.4 | 9.3 | 15* | 3 | 6 | 30 | 26 | 33.3 | 3 | 0 | 0 | 0 | 1.2 | 2.1 |
| % Acid producers | 4 | 3 | 2 | 11.6 | 16.6 | 19.2 | 33 | 30 | 32 | 21.5 | 23 | 19.5 | 7 | 22.2 | 20 | 27 | 31 | 36 |
| % Alkaline and Inert | 73 | 76 | 82 | 74.1 | 72 | 71.5 | 52 | 67 | 62 | 48.5 | 51 | 47.2 | 90 | 77.8 | 80 | 73 | 67.8 | 61.9 |
| N/5 Acetic acid broth | - | - | - | +- | +- | +- | +- | +- | +- | - | - | - | - | - | - | +- | +- | ?- |
| N/10 Acetic acid broth | - | - | - | +- | + | + | + | + | + | - | - | +- | +- | +- | +- | + | + | + |
| N/20 Acetic acid broth | - | - | - | + | ++ | ++ | ++ | ++ | ++ | - | - | +- | + | + | + | + | + | + |
| Yeasts | - | - | - | +- | +- | + | + | +- | +- | - | - | - | - | - | +- | - | + | + |

* High percentage of liquefiers due to Staphlococcus aureus

The Symbols used in the tables are listed below.

++ = Very numerous

\+ = Many

+- = Few

\- = None found

SA = Slightly acid

P = Peptonized

T = Trace

A = Acid

C = Coagulation

B.ME = B. Edematis maligni

SF = Spore former

B = Before receiving milk

BW = Bact. welchii

MT = Micrococcus tetragenus

HD = Hemolytic diplococcus

Dip = Diplococci

G+B = Gram-positive bacilli

A brief study of Table II will first reveal that the feeding of raw milk to these pigs resulted in a change of flora within three days; second that the new flora consisted largely of organisms which are capable of decomposing carbohydrates rather than those which attack and break down proteins. It will likewise be observed that while bacteria which are able to ferment carbohydrates were present in the greater number, that they were not gas producers, and that the gas producers originally present were greatly diminished.

The number of organisms capable of liquefying gelatin decreased in Case I from 23% to 6%, while those in Case II decreased from 30% to 2.1%. Those capable of fermenting carbohydrates during the same period increased in Case I from 4% to 32%, and in Case II from 21.5% to 36%, thus showing some individual variations.

The per cent of fermentative bacteria shown in the table and in the tables which are to follow were determined by adding together the percentages of those which developed as acid colonies on litmus lactose agar and those belonging to the aciduric group, as shown on the special glucose liver agar used.

## (5) Effect of Feeding Pasteurized Milk Upon the Intestinal Flora of the Guinea Pig

As was previously mentioned in this paper, these pigs were fed raw skim milk for a period of 45 days. They were then transferred to milk which had been pasteurized at 145°F. for 30 minutes and cooled to approximately 50°F.

The results obtained from the study of the fecal flora from pasteurized skim milk is shown in Table No. III.

Table III
Showing Results of Feeding Pasteurized Milk Upon the Fecal Flora of the Guinea Pig

| Diet | |
|---|---|
| | Bran------------------------35 gr. |
| | Carrots-------------------100 gr. |
| | Past. Milk----------------100 cc. |

| Case | I | | | | | | | II | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Feeding period in days | 2 | 5 | 10 | 17 | 24 | 31 | 45 | 2 | 5 | 10 | 17 | 24 | 31 | 45 |
| B. acidophilus | + | + | +- | +- | +- | +- | +- | + | + | + | +- | +- | +- | +- |
| B. bifidus | +- | - | - | +- | - | - | - | +- | +- | - | - | - | - | - |
| B. bulgaricus | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| B. coli | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| B. L. Aerogenes | + | + | +- | +- | +- | +- | +- | +- | +- | +- | +- | +- | +- | +- |
| B. mesentericus vulgatus | - | - | +- | - | - | - | +- | - | - | - | +- | - | - | - |
| B. proteus vulgaris | - | +- | +- | +- | +- | +- | +- | - | - | +- | +- | +- | +- | +- |
| B. pyocyaneus | - | - | - | +- | - | - | +- | - | +- | - | - | - | - | - |
| B. subtilis | + | + | +- | +- | +- | + | +- | +- | +- | +- | +- | +- | +- | +- |
| Staphlococcus pyogenes aureus | + | + | + | ++ | ++ | ++ | ++ | + | + | + | ++ | ++ | ++ | ++ |
| Staphlococcus pyogenes albus | - | - | - | - | +- | - | - | - | - | - | - | - | - | - |
| Streptococcus lacticus | +- | +- | +- | +- | - | - | - | + | +- | +- | +- | +- | - | - |
| Streptococcus pyogenes | + | + | + | ++ | ++ | ++ | ++ | + | + | + | + | + | + | + |
| Other bacteria | SF +- | SF +- | SF +- | SF + | SF + | SF + | SF + | +- | +- | SF +- | SF +- | SF +- | SF + | SF +- |
| % Gas in dextrose broth | 20 | 20 | 15 | 15 | 10 | 10 | 15 | 20 | 15 | 15 | 20 | 20 | 15 | 20 |
| % Gas in lactose broth | 10 | 15 | 10 | 10 | 8 | 5 | 10 | 20 | 15 | 10 | 10 | 15 | 10 | 15 |
| % Gas in Saccharose broth | 10 | 10 | 10 | 5 | T | T | 5 | 10 | 10 | 5 | 10 | 10 | 10 | 10 |
| Action on litmus milk | AC | AC | AC | AP | AP | AP | AP | AC | AC | AC | AC | AP | AP | AP |
| % Gelatin liquefiers | 1 | 3.3 | 5 | 7 | 10 | 11.9 | 13 | 2 | 2.6 | 3.9 | 5 | 10 | 10.9 | 11.7 |
| % Acid producers | 40 | 18 | 6 | 6 | 7 | 8.3 | 7.9 | 30 | 20 | 16.6 | 11.5 | 7.7 | 9 | 6.7 |
| % Alkaline and inert | 59 | 78.7 | 89 | 87 | 83 | 79.8 | 78.8 | 68 | 67.4 | 79.5 | 83.5 | 82.3 | 80.1 | 82.6 |
| N/5 Acetic acid broth | +- | - | - | - | - | - | - | +- | - | - | - | - | - | - |
| N/10 Acetic acid broth | + | +- | +- | +- | +- | +- | +- | + | + | +- | +- | +- | +- | +- |
| N/20 Acetic acid broth | + | + | + | + | + | + | + | + | + | + | + | + | +- | +- |
| Yeasts | - | +- | - | - | - | - | - | + | + | +- | +- | +- | - | - |

From Table No. III it will be observed that when pasteurized milk was fed, the per cent of fermentative organisms decidedly decreased and particularly true was this of the *Streptococcus lacticus* group. It will likewise be observed that the putrefactive organisms increased slightly upon this diet. This possibly may have been due to the fact that the *Streptococcus lacticus* group was greatly decreased during pasteurization while the putrefactive group, which is more resistant, (many of them being spore formers) was not decreased to such an extent, thus making it easier for them to gain a foothold.

(6) Effect of Feeding Sterilized Milk Inoculated With Streptococcus lacticus Upon the Intestinal Flora of the Guinea Pig.

As a direct contrast to feeding pasteurized milk in which the *Streptococcus lacticus* group is greatly decreased. Sterilized skim milk which was freshly inoculated with a pure culture of *Streptococcus lacticus* was fed for a period of 45 days to another group of pigs. The results of which are recorded in Table IV.

Table IV

Showing Results of Feeding Streptococcus lacticus in Milk Upon the Fecal Flora of the Guinea Pig.

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Diet | Bran------------------35 gr.<br>Carrots-----------------100 gr.<br>Milk + Strept. lact.----100 cc. | | | | | | | | | | | | | |
| Case | III | | | | | | | IV | | | | | | |
| Feeding period in days | B | 3 | 10 | 17 | 24 | 31 | 45 | B | 3 | 10 | 17 | 24 | 31 | 45 |
| B. acidophilus | - | - | + | + | + | + | + | - | - | + | + | + | + | + |
| B. bifidus | - | - | - | +- | +- | - | - | - | - | +- | - | - | +- | - |
| B. bulgaricus | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| B. coli | ++ | ++ | + | +- | +- | + | + | ++ | ++ | + | + | +- | + | + |
| B. l. aerogenes | + | +- | +- | +- | - | +- | +- | +- | +- | +- | +- | - | +- | +- |
| B. mesentericus vulgatus | - | - | +- | - | - | - | - | - | - | - | - | - | - | - |
| B. proteus vulgaris | + | + | +- | +- | - | +- | +- | + | + | +- | + | +- | +- | +- |
| B. pyocyaneus | - | - | +- | - | +- | - | +- | +- | +- | - | - | - | - | - |
| B. subtilis | + | + | +- | +- | +- | +- | +- | + | + | +- | - | - | - | - |
| Staphlococcus pyogenes aureus | ++ | ++ | +- | +- | +- | ++ | + | + | + | ++ | ++ | + | + | + |
| Staphlococcus pyogenes albus | + | - | - | - | - | +- | +- | | | | | | | |
| Streptococcus lacticus | - | - | + | + | ++ | ++ | + | - | +- | +- | + | ++ | + | ++ |
| Streptococcus pyogenes | + | + | + | +- | +- | + | + | | + | + | +- | +- | +- | + |
| Other bacteria | BW + | SP +- | - | +- | SF +- | BW +- | BW +- | MT + | MT + | SF + | - | - | - | - |
| % Gas in Dextrose broth | 35 | 30 | 10 | 10 | T | 30 | 12 | 25 | 25 | 10 | 10 | T | 25 | 25 |
| % Gas in Lactose broth | 30 | 30 | 5 | 5 | T | 20 | 10 | 15 | 10 | 10 | 10 | T | 20 | 15 |
| % Gas in Saccharose broth | 15 | 10 | T | T | 0 | 15 | 7 | 5 | 5 | T | T | 0 | 10 | 10 |
| Action on litmus milk | AP | AP | AC | AC | AC | AP | AP | AP | AC | AC | AC | AC | AC | AC |
| % Gelatin liquefiers | 24 | 22 | 12.5 | 10 | 3.9 | 20 | 11.5 | 25 | 33 | 12.8 | 9.7 | 6.7 | 3.7 | 4 |
| % Acid producers | 14 | 24 | 40 | 57.7 | 63.7 | 20 | 36.7 | 15.6 | 22 | 40 | 48.3 | 50 | 59 | 67 |
| % Alkaline and inert | 62 | 54 | 47.5 | 32.3 | 24 | 60 | 51.8 | 50.4 | 45 | 42 | 42 | 43.3 | 37.5 | 29 |
| N/5 Acetic acid broth | - | - | - | +- | +- | - | - | - | - | +- | +- | +- | +- | +- |
| N/10 Acetic acid broth | - | - | +- | ++ | ++ | +- | +- | - | +- | + | + | + | +- | + |
| N/20 Acetic acid broth | +- | +- | + | ++ | ++ | + | +- | +- | + | ++ | ++ | ++ | + | ++ |
| Yeast | - | - | - | - | - | - | - | - | - | - | - | - | +- | +- |

Table V.

Showing Results of Feeding Streptococcus lacticus in Milk Upon the Fecal Flora of the Guinea Pig

| Diet | Bran---------------------35 gr.<br>Carrots-----------------100 gr.<br>Milk + Strept. lact.----100 cc. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Case | VII | | | | VIII | | | | | | | |
| Feeding period in days | B | 3 | 10 | Died of pneumonia | B | 3 | 10 | 17 | 24 | 31 | 45 | |
| B. acidophilus | - | +- | + | | - | - | +- | | + | + | + | |
| B. bifidus | - | - | - | | - | - | - | | +- | - | - | |
| B. bulgaricus | - | - | - | | - | - | - | | - | - | - | |
| B. coli | ++ | + | +- | | ++ | ++ | + | | +- | +- | +- | |
| B. l. aerogenes | + | +- | - | | +- | +- | +- | | +- | - | - | |
| B. mesentericus vulgatus | +- | - | - | | - | - | - | | +- | - | - | |
| B. proteus vulgaris | + | + | +- | | + | + | + | | +- | + | +- | |
| B. pyocyaneus | - | - | - | | +- | +- | +- | Plates broken on this date | - | - | +- | |
| B. subtilis | + | + | - | | + | + | + | | - | - | - | |
| Staphlococcus pyogenes aureus | + | + | + | | + | + | + | | + | +- | +- | |
| Staphlococcus pyogenes albus | +- | - | - | | - | - | +- | | - | - | - | |
| Streptococcus lacticus | - | +- | - | | - | +- | + | | ++ | ++ | ++ | |
| Streptococcus pyogenes | + | + | + | | + | + | + | | + | +- | +- | |
| Other bacteria | SF + | SF F | - | | SF + | SF + | SF +- | | - | SF +- | SF +- | |
| % Gas in Dextrose broth | 30 | 20 | 10 | | 20 | 50 | 25 | | 15 | 5 | 5 | |
| % Gas in Lactose broth | 25 | 20 | 5 | | 20 | 40 | 15 | | 10 | T | T | |
| % Gas in Saccharose broth | 10 | 10 | T | | 12 | 25 | 12 | | 5 | 0 | 0 | |
| Action on litmus milk | AP | AC | AC | | AP | AP | AC | | AC | AC | AC | |
| % Gelatin liquefiers | 46 | 42 | 13.9 | | 50 | 12.6 | 20 | | 15 | 10 | 7.7 | |
| % Acid producers | 4.8 | 8 | 27.8 | | 15 | 25 | 38.3 | | 45 | 50 | 69.2 | |
| % Alkaline and inert | 49.2 | 50 | 41.7 | | 35 | 37.6 | 58.3 | | 40 | 40 | 23.1 | |
| N/5 Acetic acid broth | - | - | - | | - | - | - | | +- | +- | + | |
| N/10 Acetic acid broth | - | +- | +- | | +- | +- | + | | + | + | ++ | |
| N/20 Acetic acid broth | +- | + | + | | + | + | ++ | | ++ | ++ | ++ | |
| Yeast | - | - | - | | | | | | | | | |

Table No. IV shows a decided increase in the Streptococcus lacticus group and also in the number of B. acidophilus present. However, in Case III it does not show the reduction of putrefactive organisms that might be expected, while in Case IV, the results are decisive.

As Bact. welchii persisted quite consistently in the feces of Case III and as that case did not respond to the feeding of Streptococcus lacticus as did Case IV. It was decided to try the experiment on two more pigs which had a very high percentage of putrefactive organisms to commence with. The results as shown in Table No. V correspond quite closely with Case IV in Table No. IV. Therefore, it may be said that the flora of the milk consumed has a temporary effect on the fecal flora of the guinea pig.

### (7) The Effect of Feeding Sterilized Skim Milk Inoculated With Bacillus bulgaricus Upon the Intestinal Flora of the Guinea Pig.

As many pediatrists find that feeding milk inoculated with Bacillus bulgaricus to infants and children suffering with intestinal disorders is benificial and in that there have been many recent attempts to establish this organism in the intestinal tract without success, it was deemed advisable to feed

the organism to guinea pigs and study its effect on the intestinal flora.

Sterilized milk which had been inoculated with a pure culture of B. bulgaricus was fed for a period of 90 days, after which the B. bulgaricus was discontinued. Upon being discontinued, specimens were collected every day to determine how long after feeding the organism could be isolated from the feces when suitable food, such as plain milk was fed to the subject.

The flora resulting from feeding milk inoculated with Bacillus bulgaricus is shown in Table No. VI.

Table VI

Showing Results of Feeding B. bulgaricus in Milk Upon Fecal Flora of Guinea Pig

| | |
|---|---|
| Diet | Bran----------------------35 gr.<br>Carrots------------------100 gr.<br>Milk + B. bulgaricus-----100 cc. |

| Case | V | | | | | | | VI | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Feeding period in days | B | 3 | 10 | 17 | 24 | 31 | 45 | B | 3 | 10 | 17 | 24 | 31 | 45 |
| B. acidophilus | - | + | ++ | ++ | ++ | + | + | - | - | + | ++ | ++ | ++ | ++ |
| B. bifidus | - | - | +- | +- | +- | +- | +- | - | - | +- | +- | +- | +- | +- |
| B. bulgaricus | - | - | + | + | ++ | ++ | ++ | - | - | +- | + | ++ | ++ | ++ |
| B. coli | ++ | + | +- | +- | - | - | - | ++ | + | +- | - | - | - | - |
| B. l. aerogenes | +- | +- | - | - | - | - | - | +- | +- | - | - | - | - | - |
| B. mesentericus vulgatus | - | - | - | - | - | - | - | +- | - | - | - | - | - | - |
| B. proteus vulgaris | + | + | +- | +- | +- | - | - | + | +- | +- | - | - | - | - |
| B. pyocyaneus | +- | +- | - | +- | +- | - | - | +- | + | +- | +- | +- | - | - |
| B. subtilis | + | + | - | - | - | - | - | + | +- | - | - | - | - | - |
| Staphlococcus pyogenes aureus | + | + | + | +- | +- | - | - | + | + | +- | +- | +- | +- | +- |
| Staphlococcus pyogenes albus | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Streptococcus lacticus | - | - | - | +- | - | - | - | - | - | - | - | - | - | - |
| Streptococcus pyogenes | ++ | + | + | + | + | +- | +- | ++ | + | +- | +- | +- | +- | +- |
| Other bacteria | SF + | SF + | - | - | - | - | - | SF + | SF +- | - | - | - | - | MT +- |
| % Gas in Dextrose broth | 20 | 20 | 5 | 5 | T | T | T | 20 | 15 | 5 | 0 | 0 | 0 | 0 |
| % Gas in Lactose broth | 18 | 15 | T | T | 0 | 0 | 0 | 20 | 10 | T | 0 | 0 | 0 | 0 |
| % Gas in Saccharose broth | 10 | 10 | T | T | 5 | 10 | 10 | 10 | 5 | 5 | 5 | 5 | 5 | 5 |
| Action on litmus milk | AP | AP | AC | AC | AC | AC | AC | AP | AC | AC | AC | AC | AC | AC |
| % Gelatin liquefiers | 24 | 21 | 6 | 5 | 4.3 | 0 | 0 | 44 | 12.5 | 11.2 | 14.3 | 8 | 0 | 0 |
| % Acid producers | 14 | 16 | 40 | 44 | 56.1 | 55 | 53.8 | 11 | 25 | 27 | 41.3 | 49 | 77.6 | 69.4 |
| % Alkaline and inert | 62 | 63 | 54 | 51 | 49.6 | 45 | 46.2 | 45 | 64.5 | 61.8 | 55.6 | 43 | 32.4 | 30.6 |
| N/5 Acetic acid broth | - | - | +- | +- | +- | + | + | - | - | +- | +- | +- | + | + |
| N/10 Acetic acid broth | - | - | + | + | + | ++ | ++ | - | + | + | + | + | ++ | ++ |
| N/20 Acetic acid broth | +- | +- | ++ | ++ | ++ | ++ | ++ | +- | ++ | ++ | ++ | ++ | ++ | ++ |
| Yeast | - | - | - | - | - | + | + | - | - | +- | + | - | - | - |

While the organism was being fed to the milk, the writer was able to isolate them from the feces in large numbers. The writer is aware that the above is contrary to the results of Rettger, who fed the organism to chickens.

In the past, there has been considerable controversy as to the differentiation between B. bulgaricus and B. acidophilus, which may have resulted in B. bulgaricus not being found in the feces of chickens.

The writer has found that while the growth of B. bulgaricus is very similar to B. acidophilus on liver glucose agar as used, the colonies can be readily picked because they are much thicker and have a more thread-like edge. The writer further believes that B. bulgaricus can be readily distinguished from B. acidophilus by its lack of ability to ferment maltose (34).

The presence of B. bulgaricus in the feces of Case V continued for a period of 17 days after ingestion of the organism. In Case VI, it was continued in the feces for a period of only 13 days, thus showing that it could not be permanently established in the intestinal tract of the guinea pig which corresponds with the investigations of Herter, Kendall, Rahe, Rettger, Torrey and others.

Table No. VI further shows that _B_. _bulgaricus_ milk increased the fermentative flora to a greater extent than did the milk alone as shown in Table No. II. This might seem to suggest that the organism itself exerted an influence upon the flora. To determine whether this was or was not the case, the following experiment was conducted.

(8) _Effect of Feeding Sterile Water Inoculated With B. bulgaricus on the Intestinal Flora of Guinea Pig._

_Bacillus bulgaricus_ was cultured on liver glucose agar, washed off in sterile water and fed to Cases XI and XII for a period of 45 days. The flora produced by this diet did not show any appreciable difference or change from the normal flora of the pigs. In just one instance was the writer able to isolate _B_. _bulgaricus_ from the feces which suggests that the organism dies off in the intestinal tract when no suitable carbohydrate is fed. Table No. VII shows the results of the feeding of _B_. _bulgaricus_ in water.

Table VII

Showing Result of Feeding B. bulgaricus in $H_2O$ Upon the Fecal Flora of the Guinea Pig

Diet:
Bran---------------------35 gr.
Carrots-----------------100 gr.
Water + B. bulgaricus---100 cc.

| Case | XI | | | | | | | XII | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Feeding period in days | B | 3 | 10 | 17 | 24 | 31 | 45 | B | 3 | 10 | 17 | 24 | 31 | 45 |
| B. acidophilus | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| B. bifidus | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| B. bulgaricus | - | - | - | - | - | +- | - | - | - | - | - | - | - | - |
| B. coli | ++ | ++ | + | + | + | + | + | ++ | ++ | + | + | + | +- | + |
| B. l. aerogenes | +- | +- | +- | - | - | +- | +- | +- | +- | ?- | - | +- | - | - |
| B. mesentericus vulgatus | - | - | - | - | - | - | - | +- | - | - | - | +- | - | - |
| B. proteus vulgaris | + | + | + | - | +- | +- | +- | + | +- | +- | +- | + | +- | +- |
| B. pyocyaneus | - | +- | - | +- | - | - | - | - | - | +- | - | - | +- | - |
| B. subtilis | + | + | - | - | - | - | - | + | + | - | - | - | - | - |
| Staphlococcus pyogenes aureus | + | + | + | + | + | + | + | + | + | +- | + | + | +- | + |
| Staphlococcus pyogenes albus | - | +- | - | - | +- | +- | - | - | - | +- | +- | +- | - | - |
| Streptococcus lacticus | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Streptococcus pyogenes | + | + | + | + | +- | +- | + | + | +- | + | +- | + | + | +- |
| Other bacteria | SF + | SF + | SF + | SF + | SF +- | SF + | SF + | SF + | SF + | SF + | SF + | SF + | + | + |
| % Gas in Dextrose broth | 30 | 20 | 20 | 10 | 20 | 20 | 10 | 35 | 30 | 20 | 20 | 10 | T | 10 |
| % Gas in Lactose broth | 25 | 20 | 10 | 10 | 10 | 10 | 5 | 20 | 20 | 10 | T | 5 | 0 | T |
| % Gas in Saccharose broth | 15 | 10 | 4 | T | T | 10 | T | 15 | 10 | 10 | T | T | 0 | T |
| Action on litmus milk | AP | AP | AP | AC | AC | AC | AC | AP | AP | AP | AC | AC | AC | AC |
| % Gelatin liquefiers | 19.4 | 21 | 17.5 | 11.9 | 13 | 10.7 | 8.9 | 15.5 | 7.3 | 23 | 12.1 | 9 | 12.8 | 7.3 |
| % Acid producers | 11.3 | 9.9 | 12.2 | 7.6 | 16.4 | 14.3 | 13.1 | 16.7 | 11.2 | 9.7 | 17 | 15.1 | 21 | 19.6 |
| % Alkaline and inert | 69.3 | 69.1 | 70.3 | 81.5 | 70.6 | 75 | 78 | 67.8 | 71.5 | 67.3 | 70.9 | 76.9 | 66.2 | 73.1 |
| N/5 Acetic acid broth | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| N/10 Acetic acid broth | - | - | - | - | - | - | - | - | - | - | - | +- | +- | +- |
| N/20 Acetic acid broth | +- | +- | + | +- | +- | +- | +- | - | +- | +- | + | + | + | + |
| Yeast | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

(9) Effect of Feeding Milk Plus Lactose Upon the Intestinal Flora of the Guinea Pig.

It having been definitely determined by the investigations of Kendall (17), Torrey (31), Rettger (27), and Porter, Morris, and Meyers (5) that the addition of lactose to any diet would change the flora from a putrefactive to a fermentative type. This experiment was added as a control or check on the other experiments conducted. The results are shown in Table No. VIII and correspond with the findings of the investigators mentioned above. The aciduric group consisting of *B. bifidus* and *B. acidophilus* predominating and almost completely crowding out all other forms.

Table VIII

Showing Results of Feeding Milk Plus 5% Lactose, Upon the Fecal Flora of the Guinea Pig

| | |
|---|---|
| Diet | Bran------------------35 gr.<br>Carrots----------------100 gr.<br>Milk + 5% Lactose------100 cc. |

| Case | IX | | | | | | | | X | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Feeding period in days | B | 3 | 10 | 17 | 24 | 31 | 45 | | B | 3 | 10 | 17 | 24 | 31 | 45 | |
| B. acidophilus | - | +- | + | + | + | + | + | | - | +- | + | + | + | + | + | |
| B. bifidus | - | - | +- | + | + | + | + | | - | - | +- | + | + | ++ | ++ | |
| B. bulgaricus | - | - | - | - | - | - | - | | - | - | - | - | - | - | - | |
| B. coli | ++ | ++ | +- | +- | +- | +- | +- | | ++ | + | + | +- | +- | +- | +- | |
| B. l. aerogenes | +- | +- | - | - | - | - | - | | +- | +- | - | - | - | - | - | |
| B. mesentericus vulgatus | + | +- | - | - | - | - | - | | +- | +- | Q- | - | - | - | - | |
| B. proteus vulgaris | + | +- | - | - | - | - | - | | +- | - | - | - | - | - | - | |
| B. pyocyaneus | - | - | - | - | - | - | - | | - | - | +- | +- | +- | - | - | |
| B. subtilis | + | + | - | - | - | - | - | | + | - | - | - | - | - | - | |
| Staphlococcus pyogenes aureus | + | + | +- | +- | +- | - | - | | + | + | +- | +- | +- | +- | - | |
| Staphlococcus pyogenes albus | - | - | - | ÷ | - | - | - | | +- | - | - | - | - | - | - | |
| Streptococcus lacticus | - | - | - | - | - | - | - | | - | - | - | - | - | - | - | |
| Streptococcus pyogenes | + | + | +- | +- | +- | ?- | +- | | + | + | + | +- | +- | - | +- | |
| Other bacteria | SF + | SF + | ± | - | - | - | - | | SF + | SF +- | - | - | - | - | - | |
| % Gas in Dextrose broth | 75 | 15 | T | 5 | T | T | T | | 30 | 20 | 10 | 5 | T | T | T | |
| % Gas in Lactose broth | 50 | 10 | 0 | T | 0 | 0 | T | | 20 | 5 | T | T | 0 | 0 | 0 | |
| % Gas in Saccharose broth | 30 | T | 0 | 0 | 0 | 0 | 0 | | 12 | T | T | T | 0 | 0 | 0 | |
| Action on litmus milk | AP | AP | AC | AC | AC | AC | AC | | AP | AC | AC | AC | AC | AC | AC | |
| % Gelatin liquefiers | 50 | 4 | 1 | 4 | 4.2 | 0 | 0 | | 30.3 | 5 | 5.6 | 1.3 | 1 | .4 | 0 | |
| % Acid producers | 5 | 20 | 30 | 36.5 | 46.6 | 44.7 | 51.9 | | 12.2 | 50 | 53 | 47.6 | 61.8 | 66.6 | 69.3 | |
| % Alkaline and inert | 45 | 76 | 69 | 69.5 | 49.2 | 56.3 | 48.1 | | 57.5 | 45 | 41.4 | 51.1 | 37.2 | 33 | 30.7 | |
| N/5 Acetic acid broth | - | - | +- | +- | +- | +- | + | | - | - | - | - | +- | + | + | |
| N/10 Acetic acid broth | - | +- | ++ | ++ | ++ | ++ | ++ | | - | +- | + | + | ++ | ++ | ++ | |
| N/20 Acetic acid broth | +- | + | ++ | ++ | ++ | ++ | ++ | | +- | ++ | ++ | ++ | ++ | ++ | ++ | |
| Yeast | | | | | | | | | | | | | | | | |

(10) Effect of Feeding Borden's Eagle Brand, Condensed Milk, Horlick's Malted Milk and Mellin's Food on the Intestinal Flora of the Guinea Pig.

These kinds of milk mentioned above being used in a great many instances as food for infants and especially so among the pediatrists of Lansing; it was considered of sufficient importance to determine the typical fecal flora resulting from their feeding.

The guinea pigs used in these experiments were first fed upon formulas recommended by the respective manufacturers for infants of one month of age and gradually increased until they were receiving the formulas recommended for infants of twelve months of age.

The type of flora and the relative numbers of the different organisms are shown in Tables No. IX, X, and XI respectively.

Table IX

Showing Results of Feeding Borden's Eagle Brand Condensed Milk Upon the Fecal Flora of the Guinea Pig

| | |
|---|---|
| Diet | Bran------------------------35 gr.<br>Carrots-------------------100 gr.<br>Borden's Condensed Milk---100 cc. |

| Case | XIII | | | | | | | | XIV | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Feeding period in days | B | 2 | 9 | 15 | 22 | 29 | | | B | 2 | 9 | 15 | 22 | 29 | | |
| B. acidophilus | +- | +- | + | + | + | + | | | +- | +- | + | + | + | + | | |
| B. bifidus | - | - | - | +- | - | - | | | - | - | +- | - | +- | - | | |
| B. bulgaricus | - | - | - | - | - | - | | | - | - | - | - | - | - | | |
| B. coli | ++ | + | +- | +- | +- | +- | | | ++ | + | +- | +- | +- | +- | | |
| B. l. aerogenes | +- | +- | - | - | - | - | | | + | +- | - | - | - | - | | |
| B. mesentericus vulgatus | - | - | - | +- | - | - | | | - | - | - | +- | - | - | | |
| B. proteus vulgaris | +- | +- | - | - | - | - | | | + | +- | - | +- | +- | - | | |
| B. pyocyaneus | - | - | - | - | - | - | | | +- | - | - | - | - | - | | |
| B. subtilis | + | + | - | - | - | - | | | + | + | - | - | - | - | | |
| Staphlococcus pyogenes aureus | + | + | +- | +- | +- | +- | | | + | + | +- | +- | +- | +- | | |
| Staphlococcus pyogenes albus | - | +- | - | - | - | - | | | +- | - | - | - | - | - | | |
| Streptococcus lacticus | + | + | ++ | ++ | ++ | ++ | | | ++ | + | ++ | ++ | ++ | ++ | | |
| Streptococcus pyogenes | + | +- | +- | +- | +- | +- | | | + | +- | +- | +- | +- | +- | | |
| Other bacteria | MT ? | MT + | - | SF + | - | - | | | SF + | SF + | - | - | - | - | | |
| % Gas in Dextrose broth | 40 | 20 | 5 | 12 | 10 | T | | | 20 | 10 | 5 | 8 | 10 | 10 | | |
| % Gas in Lactose broth | 30 | 10 | T | 5 | T | T | | | 10 | 8 | 0 | T | T | 5 | | |
| % Gas in Saccharose broth | 10 | T | T | T | T | 0 | | | 10 | T | 0 | 0 | T | T | | |
| Action on litmus milk | AC | AC | AC | AC | AC | AC | | | AC | AC | AC | AC | AC | AC | | |
| % Gelatin liquefiers | 11.2 | 7.3 | 3.7 | 4.1 | 6 | 2.7 | | | 8.8 | 7.5 | 1.9 | 3 | 10.9 | 6.3 | | |
| % Acid producers | 9.2 | 16.4 | 36.6 | 60 | 41 | 3.3 | | | 33 | 50 | 55 | 73 | 44 | 51 | | |
| % Alkaline and inert | 79.6 | 76.3 | 59.7 | 35.9 | 53 | 44.3 | | | 58.2 | 42.5 | 43.1 | 24 | 45.1 | 42.7 | | |
| N/5 Acetic acid broth | +- | +- | +- | +- | +- | +- | | | +- | +- | +- | +- | + | +- | | |
| N/10 Acetic acid broth | + | + | + | + | + | + | | | + | + | + | ++ | ++ | + | | |
| N/20 Acetic acid broth | ++ | ++ | ++ | ++ | ++ | ++ | | | ++ | ++ | ++ | ++ | ++ | ++ | | |
| Yeasts | - | - | - | - | - | - | | | - | - | - | - | - | | | |

Table X

Showing Results of Feeding Horlick's Malted Milk Upon Fecal Flora of Guinea Pigs

| Diet | Bran---------------------35 gr.<br>Carrots------------------100 gr.<br>Horlick's Malted Milk----100 cc. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Case | VII | | | | | | VIII | | | | | |
| Feeding period in days | B | 2 | 9 | 16 | 23 | 30 | B | 2 | 9 | 16 | 23 | 30 |
| B. acidophilus | - | +- | + | + | + | + | +- | + | + | + | + | + |
| B. bifidus | - | - | +- | +- | - | +- | - | - | ++ | - | - | - |
| B. bulgaricus | - | - | - | - | - | - | - | - | - | - | - | - |
| B. coli | ++ | +- | +- | +- | +- | +- | + | +- | - | +- | +- | +- |
| B. l. aerogenes | +- | - | - | - | - | - | +- | +- | - | +- | - | - |
| B. mesentericus vulgatus | - | - | - | - | - | - | - | - | - | - | - | - |
| B. proteus vulgaris | + | +- | - | - | - | - | +- | +- | - | - | - | - |
| B. pyocyaneus | +- | - | - | - | - | - | +- | - | - | +- | +- | - |
| B. subtilis | + | - | - | - | - | - | + | - | - | - | - | - |
| Staphlococcus pyogenes aureus | + | +- | +- | +- | +- | +- | +- | +- | +- | +- | ?- | +- |
| Staphlococcus pyogenes albus | +- | - | - | - | - | - | - | - | - | - | - | - |
| Streptococcus lacticus | - | + | ++ | ++ | ++ | ++ | + | + | ++ | ++ | ++ | ++ |
| Streptococcus pyogenes | + | +- | +- | +- | +- | +- | + | +- | +- | +- | +- | +- |
| Other bacteria | SF<br>+ | - | - | - | - | - | SF<br>+ | SF<br>+- | HD<br>+- | - | - | - |
| % Gas in Dextrose broth | 20 | 8 | 10 | 5% | T | T | 10 | 10 | T | 10 | 10 | 5 |
| % Gas in Lactose broth | 10 | T | 5 | T | 0 | 0 | 5 | T | 0 | 5 | T | 0 |
| % Gas in Saccharose broth | 10 | T | T | 0 | 0 | 0 | 5 | T | 0 | T | T | 0 |
| Action on litmus milk | AP | AC | AC | AC | AC | AC | AP | AC | AC | AC | AC | AC |
| % Gelatin liquefiers | 13.2 | 5.6 | 1.3 | 3.1 | 2.9 | 3.3 | 11.3 | 1.4 | 3. | 1.5 | 2. | .5 |
| % Acid producers | 8.3 | 25 | 40 | 80 | 62.2 | 61 | 29.1 | 42.2 | 38.9 | 45 | 44 | 30 |
| % Alkaline and inert | 78.5 | 79.4 | 58.7 | 16.9 | 34.9 | 35.7 | 59.6 | 56.4 | 58.1 | 53.5 | 54 | 69.5 |
| N/5 Acetic acid broth | - | - | - | - | - | +- | - | - | - | +- | +- | +- |
| N/10 Acetic acid broth | - | +- | + | + | + | + | - | - | + | + | + | + |
| N/20 Acetic acid broth | +- | + | ++ | ++ | ++ | ++ | +- | +- | ++ | ++ | ++ | ++ |
| Yeasts | - | - | - | - | - | - | - | - | - | - | | |

Table XI

Showing Results of Feeding Mellin's Food Upon the Fecal Flora of the Guinea Pig

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Diet | Bran------------------35 gr.<br>Carrots---------------100 gr.<br>Mellin's Food---------100 cc. | | | | | | | | | | | | | | | |
| Case | IX | | | | | | | | X | | | | | | | |
| Feeding period in days | B | 3 | 10 | 17 | 24 | 31 | | | B | 3 | 10 | 17 | 24 | 31 | | |
| B. acidophilus | +- | + | + | + | + | + | | | + | + | + | + | + | + | | |
| B. bifidus | - | - | +- | +- | +- | +- | | | +- | +- | +- | +- | +- | +- | | |
| B. bulgaricus | - | - | - | - | - | - | | | - | - | - | - | - | - | | |
| B. coli | ++ | + | + | +- | +- | +- | | | + | +- | +- | +- | +- | +- | | |
| B. l. aerogenes | +- | +- | +- | - | - | - | | | - | - | - | - | - | - | | |
| B. mesentericus vulgatus | +- | +- | - | - | - | - | | | - | - | - | - | - | - | | |
| B. proteus vulgaris | +- | +- | - | - | - | - | | | +- | - | - | - | - | - | | |
| B. pyocyaneus | - | - | - | - | - | - | | | - | - | - | - | - | - | | |
| B. subtilis | + | - | - | - | - | - | | | + | - | - | - | - | - | | |
| Staphlococcus pyogenes aureus | + | + | +- | +- | +- | +- | | | + | +- | +- | +- | +- | +- | | |
| Staphlococcus pyogenes albus | - | - | - | - | - | - | | | - | - | - | - | - | - | | |
| Streptococcus lacticus | - | +- | + | ++ | ++ | ++ | | | +- | + | ++ | ++ | ++ | ++ | | |
| Streptococcus pyogenes | +- | +- | +- | +- | +- | +- | | | + | +- | +- | +- | +- | - | | |
| | SF | | | Dip | Dip | Dip | | | G+B | | | | | | | |
| Other bacteria | +- | - | - | + | + | + | | | +- | +- | +- | +- | +- | - | | |
| % Gas in Dextrose broth | 20 | 30 | 20 | 5 | T | T | | | 10 | 5 | T | 5 | T | T | | |
| % Gas in Lactose broth | 10 | 20 | 10 | T | 0 | 0 | | | 5 | 0 | 0 | T | 0 | 0 | | |
| % Gas in Saccharose broth | 10 | 5 | T | T | 0 | 0 | | | T | 0 | 0 | 0 | 0 | 0 | | |
| Action on litmus milk | AP | AC | AC | AC | AC | AC | | | AP | AP | AC | AC | AC | AC | | |
| % Gelatin liquefiers | 3.7 | 4.1 | 1.7 | .5 | 0 | 0 | | | 2.1 | 7 | 1.9 | + | + | + | | |
| % Acid producers | 11.9 | 15.7 | 30 | 30 | 53 | 67 | | | 10. | 30 | 37 | 46.5 | 55.5 | 63 | | |
| % Alkaline and inert | 84.4 | 80.2 | 68.3 | 69.5 | 52 | 33 | | | 87.9 | 63 | 61.1 | 53.4 | 44.5 | 37 | | |
| N/5 Acetic acid broth | - | - | - | - | - | +- | | | - | - | - | +- | +- | +- | | |
| N/10 Acetic acid broth | +- | +- | +- | + | + | + | | | +- | +- | + | + | + | + | | |
| N/20 Acetic acid broth | + | + | ++ | ++ | ++ | ++ | | | + | + | ++ | ++ | ++ | ++ | | |
| Yeasts | +- | + | + | +- | +- | - | | | - | - | - | - | - | - | | |

From the foregoing tables it will be observed that the flora produced by the various milk diets are very similar, all of them coming under the fermentative type and, to a large extent, consisting of B. acidophilus and Streptococcus lacticus.

Since these kinds of milk all contain a large percentage of carbohydrates particularly maltose, the results obtained with Horlick's Malted Milk and Mellin's Food correlate with the previous work done by various investigators with this particular sugar.

(11) The Effect of Feeding Sterilized Milk Inoculated With Bacillus acidophilus Upon the Intestinal Flora of the Guinea Pig

The intestinal tract being the natural habitat of B. acidophilus, the writer considered it of interest to determine if it could be established in the intestinal tract of the guinea pig, which animal was found to contain few if any of this organism in the intestinal tract. Therefore, milk which had been sterilized was inoculated with a pure culture of B. acidophilis and fed to Cases XI and XII for a period of 45 days, during which time B. acidophilus became the predominant organism of the feces.

Table XII

Showing Results of Feeding B. Acidophilus Upon the Fecal Flora of Guinea Pig

| | |
|---|---|
| Diet | Bran---------------------35 gr.<br>Carrots------------------100 gr.<br>Milk + B. acidophilus----100 cc. |

| Case | XI | | | | | | | | XII | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Feeding period in days | B | 3 | 10 | 17 | 24 | 31 | | | B | 3 | 10 | 17 | 24 | 31 | | |
| B. acidophilus | - | ++ | ++ | ++ | ++ | ++ | | | - | ++ | ++ | ++ | ++ | ++ | | |
| B. bifidus | - | - | +- | +- | +- | +0 | | | - | - | - | +- | - | - | | |
| B. bulgaricus | - | - | - | - | - | - | | | - | - | - | - | - | - | | |
| B. coli | ++ | + | + | +- | +- | +- | | | ++ | ++ | +- | +- | +- | +- | | |
| B. l. aerogenes | +- | +- | - | - | - | - | | | +- | +- | - | - | - | - | | |
| B. mesentericus vulgatus | - | - | - | - | - | - | | | +- | - | + | - | - | - | | |
| B. proteus vulgaris | +- | +- | +- | - | - | - | | | +- | + | + | - | - | - | | |
| B. pyocyaneus | - | - | - | - | - | - | | | - | - | - | - | - | - | | |
| B. subtilis | - | - | - | - | - | - | | | - | - | - | - | - | - | | |
| Staphlococcus pyogenes aureus | + | + | +- | +- | +- | +- | | | + | + | +- | +- | +- | +- | | |
| Staphlococcus pyogenes albus | +- | - | - | - | - | - | | | - | +- | - | - | - | - | | |
| Streptococcus lacticus | - | - | - | +- | + | + | | | - | - | - | +- | +- | +- | | |
| Streptococcus pyogenes | + | +- | +- | +- | - | +- | | | + | + | +- | +- | +- | +- | | |
| Other bacteria | + | + | + | - | - | - | | | + | + | - | - | - | - | | |
| % Gas in Dextrose broth | 20 | 10 | 10 | T | T | T | | | 15 | 15 | 10 | T | T | 5 | | |
| % Gas in Lactose broth | 10 | T | T | 0 | 0 | 0 | | | 5 | 10 | 5 | 0 | 0 | 0 | | |
| % Gas in Saccharose broth | 10 | T | 0 | 0 | 0 | 0 | | | T | 10 | T | 0 | 0 | 0 | | |
| Action on litmus milk | AP | AC | AC | AC | AC | AC | | | AP | AC | AP | AC | AC | AC | | |
| % Gelatin liquefiers | 9.3 | 8.0 | 9. | 0 | 0 | 0 | | | 7.9 | 3.9 | 10 | 0 | 0 | 0 | | |
| % Acid producers | 12.7 | 43.8 | - | - | - | - | | | 17.2 | 39. | 20 | 27.5 | 13 | - | | |
| % Alkaline and inert | 78 | 48.2 | - | - | - | - | | | 74.9 | 57.1 | 70 | 72.5 | - | - | | |
| N/5 Acetic acid broth | - | +- | +- | +- | + | + | | | - | +- | +- | + | + | + | | |
| N/10 Acetic acid broth | + | + | + | ++ | ++ | ++ | | | + | + | + | ++ | ++ | ++ | | |
| N/20 Acetic acid broth | ++ | ++ | ++ | ++ | ++ | ++ | | | + | + | ++ | ++ | ++ | ++ | | |
| Yeasts | - | - | - | - | - | - | | | - | + | - | - | - | - | | |

After the 45 day period sterilized milk was fed without being inoculated with *B. acidophilus*. However, *B. acidophilus* remained the predominating organism during the next 45 days that the milk was fed. Subsequent to this, milk was also discontinued as a part of the diet and *B. acidophilus* almost immediately commenced to diminish until the 31st day was reached, at which time the writer failed to find them in the feces of the guinea pigs.

## DISCUSSION

From the experimental work done, the writer has shown that the fecal flora of the guinea pig may be influenced by diet. However, he does not believe that they meet the requirements of an experimental animal for this kind of work, because (1) their stools do not resemble closely enough those of the human, (2) they do not consume their food quickly nor cleanly, thereby making it difficult to keep sterile food from becoming contaminated, (3) it is difficult to obtain uncontaminated stools, (4) they are hard to maintain on a monotonous diet.

The data presented also suggest that it might be well to inoculate pasteurized milk with a pure culture of lactic acid bacteria before being

consumed as has been proposed in a recent patent (36).

If for any reason it should not prove desirable to inoculate pasteurized milk with some lactic acid bacteria, the writer would suggest that cow's milk, particularly in the summer and in case of intestinal disorders among children, be modified by the addition of lactose. Human milk contains almost four times as much lactose as protein. Cow's milk contains not quite twice as much lactose as protein and produces a putrefactive flora more commonly than does human milk. It has been repeatedly shown that when lactose is fed a fermentative type of flora results, which closely resembles that of breast-fed infants.

By the addition of lactose to cow's milk as mentioned above, such milk would be made to resemble more closely that of the human, thereby reducing the chances of successful invasion in children of putrefactive bacteria which tend to be inhibited in fermentative surroundings.

Aside from the possible therapeutic value of fermented milks there seems to be no question but that they are nutritious and refreshing and that their use should be encouraged among adults because of their food value.

## SUMMARY

The writer's experiments in feeding different diets to guinea pigs may be briefly summarized as follows:

(1) The feeding of fresh raw skim milk alters the fecal flora from a putrefactive to a fermentative type.

(2) The feeding of pasteurized milk, however, does not produce this change to as great an extent as does raw milk.

(3) Milk inoculated with a pure culture of _Bacillus bulgaricus_ results in a highly fermentative flora and shows _B. bulgaricus_ in large numbers during the period in which fed but immediately disappears when suitable carbohydrate food such as milk is withdrawn from the diet.

(4) The addition of 5% lactose to the milk fed, produces a flora very similar to that of breast-fed infants, _B. bifidus_ and _B. acidophilus_ predominating.

(5) Feeding pure cultures of _Bacillus bulgaricus_ in water produces no change.

(6) Feeding of Borden's Eagle Brand Condensed Milk, Horlick's Malted Milk and Mellin's Food all produce fermentative flora.

(7) *B. acidophilus* predominated in large numbers in the feces of the pigs fed milk containing *B. acidophilus* and it remained dominant as long as suitable food, such as milk, was furnished in the diet. However, upon discontinuing food suitable for the growth of *B. acidophilus*, it was supplanted by putrefactive organisms.

## CONCLUSIONS

The work presented in this paper has necessarily been limited but from the data obtained the following conclusions may be drawn:-

(1) The normal intestinal flora of the guinea pig, which is composed of a relatively high percentage of putrefactive organisms, may be changed temporarily to a more fermentative type of flora by the feeding of milk inoculated with lactic acid bacteria or by the addition of lactose to the diet. The writer also found that the feeding of Borden's Condensed Milk, Horlick's Malted Milk and Mellin's Food brought about a similar result.

(2) The length of time in which the fermentative flora, brought about by the feeding of milk inoculated with lactic acid bacteria, will persist after ingestion of the bacteria depends upon the type

of food consumed thereafter.

(3) Guinea pigs do not meet the requirements of an experimental animal for this kind of work.

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