OBSERVATIONS ON THE INFLUENCE OF LACTIC ACID 
FERMENTS UPON INTESTINAL PUTREFACTION IN A 
HEALTHY INDIVIDUAL. 

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Of all forms of bacterial invasion into the human body, there 
are few more difficult to reach and to combat than those of 
putrefactive bacteria in the intestinal tract. Since the more 
virulent of such organisms, colonizing in the intestines, have 
their development retarded or inhibited in an acid medium, an 
effort is being made by Metchnikoff and others to treat intestinal 
putrefaction by introducing into the stomach living cultures of 
lactic acid organisms, in the hope that these by continuing to 
grow in the intestine may there produce an acid reaction and so 
prevent putrefactive decomposition of the contents of the 
intestine. It has been proved by Metchnikoff¹ and Cohendy,² in the case of men, and by Herter and Kendall,³ in the case of 
monkeys, that certain of the lactic acid producing organisms, 
as B. bulgaricus retain their vitality while passing through the 
digestive tract and that when they are given by mouth, they 
can be grown in cultures from the feces. The effect upon 
intestinal putrefaction of such ingestion of lactic acid ferments 
can be discovered only by a large number of observations under 
normal and pathological conditions.

The following report is a contribution to such a body of evi-
dence. It is a study of the effect of the ingestion of certain 
lactic acid ferments upon intestinal putrefaction when they are 
given in combination with an ordinary mixed diet. The subject 
of the record was a healthy man, 43 years old, who throughout

¹ Metchnikoff: The Prolongation of Life.
the period of the experiment led a life of ordinary routine with regular hours of work, of exercise and of sleep. The observations extend over a continuous period of four months, during which time lactobacilline malt, bacillac, zoolak and plain milk were in turn taken in addition to an ordinary diet. The purpose of the experiment was to watch under these varying conditions, the development of putrefactive products in the intestines and the loss of nitrogen in the feces.

Throughout the period of experiment daily examinations of the urine and feces were made, occasionally two days specimens or urine being combined and examined together. The quantitative analyses of the urine and feces were made by Mr. Edward N. O'Brien. Throughout the most of the period the quantitative estimations were all done in duplicate, thus increasing the accuracy of the results. The following points were daily determined in the urine: the volume, color, reaction, specific gravity, the presence of indol and indolacetic acid, the reaction in the acid distillate with Millon's reagent, and the determination of the total nitrogen, the inorganic sulphates and the ethereal sulphates. In the feces, the total nitrogen, the percentage of water and the total amount of fresh and dried feces were determined. Also there were noted the color, consistency, reaction and Schmidt's test for hydrobilirubin. A portion of the feces was rendered acid with phosphoric acid and distilled. This distillate was tested with Millon's reagent and with the \( p \)-dimethylamino-benzaldehyde reagent. In Table I are given the average results of the quantitative determinations made during the different periods.

A series of observations had been made upon the same man during the summer of 1908. The record of his normal condition at that time was used for comparison with the observations noted in the present experiment. Including these former notes, the dietary periods of this series were the following:

1. **Methods.** The total nitrogen of the urine and of the feces was determined by the Kjeldahl method.

The inorganic and ethereal sulphates were determined by Folin's method (this *Journal* i, p. 131, 1906).

The indican was followed by means of Folin's method (*American Journal of Physiology*, xiii, p. 705).
<table>
<thead>
<tr>
<th>PERIOD</th>
<th>DIET AND LACTIC ACID FERMENT TAKEN</th>
<th>DAILY AVERAGE FOR 28 DAYS</th>
<th>2 &quot;</th>
<th>3 &quot;</th>
<th>FIRST 22 DAYS</th>
<th>SECOND 22 DAYS</th>
<th>7 DAYS</th>
<th>10 &quot;</th>
<th>PERCENTAGE OF WATER IN FRESH FECES</th>
<th>NITROGEN IN FRESH FECES</th>
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<tbody>
<tr>
<td>I</td>
<td>Mixed diet only</td>
<td>2.233 0.216 10.5 12.9 97.6 19.2 80.3 1.04 13.9</td>
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<tr>
<td>II</td>
<td>Mixed diet</td>
<td>2.187 0.179 12.2 12.1 65.8 16.7 74.6 1.16 13.2</td>
<td>2 &quot;</td>
<td></td>
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<tr>
<td>III</td>
<td>Mixed diet: Lactobacille tablets</td>
<td>2.721 0.292 9.0 14.8 132.3 25.9 80.4 1.73 16.6</td>
<td>3 &quot;</td>
<td></td>
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<tr>
<td>IV</td>
<td>Mixed diet: Lactobacille malt 50cc.</td>
<td>2.105 0.261 8.1 13.2 90.7 21.6 76.3 1.40 14.6</td>
<td></td>
<td>first 22 days</td>
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<tr>
<td></td>
<td>twice daily</td>
<td>2.072 0.254 8.2 12.6 96.0 21.8 76.2 1.41 14.0</td>
<td></td>
<td>second 22 days</td>
<td></td>
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<tr>
<td>V</td>
<td>Milk and biscuits only</td>
<td>2.448 0.150 16.3 14.7 124.3 25.4 79.7 0.89 15.5</td>
<td>7 days</td>
<td></td>
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<tr>
<td>VI</td>
<td>Mixed diet, breakfast and dinner.</td>
<td>2.153 0.251 8.7 12.9 115.0 28.3 75.4 1.28 14.1</td>
<td>Plain milk and biscuits, lunch</td>
<td>10 &quot;</td>
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<tr>
<td>VII</td>
<td>Mixed diet, breakfast and dinner.</td>
<td>2.456 0.263 9.3 14.2 109.0 25.2 66.8 1.30 15.5</td>
<td>Bacillae and biscuits, lunch</td>
<td>10 &quot;</td>
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<tr>
<td>VIII</td>
<td>Mixed diet, breakfast and dinner.</td>
<td>2.424 0.267 9.0 14.2 128.6 29.4 67.7 1.41 15.6</td>
<td>Plain milk and biscuits, lunch</td>
<td>10 &quot;</td>
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<td>IX</td>
<td>Mixed diet, breakfast and dinner.</td>
<td>2.375 0.187 12.8 13.9 120.7 27.4 77.3 1.40 15.3</td>
<td>Zoolak and biscuits, lunch</td>
<td>10 &quot;</td>
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<tr>
<td>X</td>
<td>Mixed diet only</td>
<td>2.409 0.180 13.4 14.3 122.7 23.7 80.0 1.53 16.0</td>
<td>15 &quot;</td>
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<tr>
<td>XI</td>
<td>Mixed diet, breakfast, lunch and dinner.</td>
<td>2.516 0.239 10.5 14.0 129.2 27.9 78.4 1.63 15.7</td>
<td>Zoolak, one liter daily</td>
<td>7 &quot;</td>
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Lactic Acid Ferments and Putrefaction

I. Ordinary mixed diet: 28 days, May 29 to June 26, 1908.
II. Ordinary mixed diet: 2 days, December 8 and 9.
III. One lactobacilline tablet after each meal. Ordinary mixed diet: 3 days only, 1 December 10 to 12.
IV. Lactobacilline malt, 50 cc. twice daily before meals. Ordinary mixed diet: 44 days, December 13 to January 25.
V. Plain milk and biscuits only: 7 days, January 26 to February 1.
VI. One liter of plain milk and 90 grams of biscuits only for lunch. Ordinary mixed diet for breakfast and dinner: 10 days, February 3 to 12.
VII. Bacillac, one liter and biscuits, 90 grams for lunch. Ordinary mixed diet for breakfast and dinner: 10 days, February 13 to 22.
VIII. Plain milk, one liter, biscuits, 90 grams for lunch. Ordinary mixed diet for breakfast and dinner: 10 days, February 23 to March 4.
IX. Zoolak, one liter and biscuits, 90 grams for lunch. Ordinary mixed diet for breakfast and dinner: 10 days, March 4 to 15.
X. Ordinary mixed diet: 15 days, March 15 to 29.
XI. Zoolak, one liter daily in addition to ordinary mixed diet three times daily: 7 days, March 30 to April 6.

There were no symptoms produced by the varying diets excepting that twice during the period when lactobacilline malt was taken the eating of an apple was followed by a severe attack of colic with a slight diarrhoea.

The variations in the amounts of indol and phenol and their derivatives excreted from the body in the urine and feces are used as an approximate index of the degree of intestinal putrefaction since indol and phenol are not formed in the body in appreciable quantities excepting through the activity of putrefactive bacteria and barring some especial focus elsewhere, only in the intestines. The amount of indican and phenol and allied substances excreted cannot however be taken as an absolute index of that formed in the intestine as there may be some degree of putrefaction in the food brought before it is ingested and there may be putrefactive changes in the food in the stomach if there is an absence of hydrochloric acid in the gastric contents.

1 The period of three days when lactobacilline tablets were given is included in the record as it formed a part of the period of experiment but no conclusions are drawn from the observations at that time because the period was of such short duration.
Ethereal Sulphates. Daily estimations of the ethereal sulphates in the urine were made because the indol, phenol, skatol and allied putrefactive products which are absorbed from the intestines become linked with sulphuric acid in the body and are excreted as ethereal (or combined) sulphates. In health the amounts of the ethereal sulphates vary regularly with the amount of proteid ingested and bear an approximately constant ratio to the nitrogen excreted in the urine. (The ratio of the amount of ethereal sulphates excreted in health, measured as sulphuric acid, to the total nitrogen of the urine is as 1.4 to 1.5 : 100 "coefficient of Amann").

The accompanying charts show the relative amounts of nitrogen and ethereal sulphates (estimated as H₂SO₄) excreted daily in the urine. Chart I gives the absolute daily amounts throughout the experiment. Chart II gives the average daily amounts for the different periods, thus showing more clearly the variations due to the different diets. The ordinates for the nitrogen curves and the sulphuric acid curves bear the ratio of 100 to 1.5 so that in health the two curves should approximately coincide.

Apart from the plain milk period the intestinal putrefaction, as measured by the ratio of the ethereal sulphates to the nitrogen in the urine was least when an ordinary mixed diet was given with no additional fermented milk or malt. This is seen in the period of one month in June 1908 (Period I), in the period of two days only just before the beginning of the experiment (Period II) and in the period of sixteen days which came almost at the end of the experiment (Period X).

In the period when zoolak and biscuits only were taken for lunch with a mixed diet for breakfast and dinner (Period IX) the ethereal sulphates became less than normal but in the last period when a liter of zoolak was taken daily in addition to an ordinary diet for lunch as well as breakfast and dinner (Period XI) the intestinal putrefaction again increased over that of the period before when no fermented milk was taken. In both these zoolak periods the ratio of ethereal sulphates was less than during the time when other forms of the products of lactic acid fermentation were given. The intestinal putrefaction as measured by the ratio of the ethereal sulphates was highest dur-
Chart 1. Showing daily amounts of ethereal sulphates as $\text{H}_2\text{SO}_4$ (solid line) and of nitrogen (broken line) excreted in the urine.

The scales of ordinates are so arranged that the two curves should normally coincide.
CHART II. Showing average daily amounts of ethereal sulphates as H₂SO₄ (solid line) and of nitrogen (broken line) excreted in the urine during the different periods.

The scales of ordinates are so arranged that the two curves should normally coincide.
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During the period when lactobacilline malt was given (Period IV) but it was nearly as high during the periods when bacillac was given (Period VII) and when a liter of plain milk with biscuits only was taken for lunch with an ordinary mixed diet for breakfast and dinner (Periods VI and VIII).

The week when plain milk and biscuits only were taken forms a break between the lactobacilline malt period and that which followed, showing that the influence of the lactobacilline malt was not carried over to the later periods. The ethereal sulphates are always markedly reduced when a patient is put on a milk diet, as is shown in the charts (Period V).

Indican. The examination made the first day of the experiment when a general mixed diet was taken showed a trace of indican in the urine. The next day on the same diet there was none.

Throughout the various periods there was but little difference in the amount of indican found, usually there was none, often a trace only, seldom more than a trace. During the week when milk and biscuits only were taken, the indican was somewhat increased. Also when a liter of plain milk was taken at noon with a mixed diet for breakfast and dinner the amount of indican was more than during the other periods, at one time giving a marked reaction (75 in a color scale in which Fehling’s Solution equals 100).

Indolacetic Acid. The urine throughout the experiment showed only traces of indolacetic acid excepting during the period when lactobacilline malt was given. At that time the reaction became more marked.

Phenols. While indol and the phenols are both regularly formed by the putrefactive processes in the intestine, there is no definite parallelism between the amounts of the phenol and indican found in the urine. This is due to variation, always occurring in the amounts formed, the amounts absorbed from the intestine and the amount of indol oxidized in the body.

The phenol and allied substances reacting with Millon’s reagent in the urinary distillate were found only in small amounts throughout the period of experiment although occasionally there was a marked reaction for these bodies. The average amount of phenol was greatest during the period when lactobacilline malt.
was taken and in the period when a general mixed diet alone was taken. It was least in the period when zoolak and biscuits only were taken for lunch and the amount was exceptionally low when plain milk and when bacillac with biscuits only were taken for lunch.

Indol and Skatol in the Feces. The acid distillate from the feces was tested daily with the $p$-dimethylamino-benzaldehyde reagent. This serves as a delicate test for skatol and indol, giving a rosy or red color with indol, and a violaceous or purple color with skatol. Other substances react with this reagent but in the acid distillate from the feces these products of putrefaction, indol and skatol, seem to be dominant in giving the reaction. The average intensity of this reaction was least in the two periods when zoolak was given. It was nearly as low in the periods when an ordinary mixed diet was given and in one of the periods when plain milk and biscuits were given for lunch in addition to an ordinary mixed diet for breakfast and dinner. It was most marked during the periods when bacillac, lactobacilline malt and lactobacilline tablets were given.

Phenols in the Feces. The phenols are so readily absorbed from the intestine that only small amounts are ordinarily found in the feces. Throughout this series of observations, the acid distillate from the feces was tested with Millon's reagent but the amount of phenols present was always too small to be detected by the method used.

Reaction of the Feces. The reaction of the feces was alkaline throughout the period of experiment, excepting for three days. Twice during the period when the subject was on an ordinary mixed diet, the feces became acid and one day during the last period, when zoolak was added to a full mixed diet, the reaction was slightly acid. These changes to an acid reaction were associated with a tendency to diarrhœa. That the reaction of the feces was alkaline during the periods when the fermented milk and malt were taken, showed that all the acid produced by the lactic acid organisms had been neutralized or absorbed from the intestines before the feces were voided. This result is in contrast to the observations of Grekoff, who found in patients suf-

Suffering from intestinal affections, that when the cases resulted favorably the fecal matter gave an acid reaction after the fourth or fifth day of treatment with lactic acid preparations.

*Schmidt's Reaction in the Feces.* When a concentrated solution of mercuric chloride is thoroughly mixed with the feces a marked red color with yellowish fluorescence may develop. This has been regarded as a test for hydrobilirubin. It has been found more marked in cases of intestinal putrefaction and has been thought to indicate an increase in reduction processes in the intestine due to the excessive action of putrefactive bacteria. Throughout the period of experiment daily tests were made for Schmidt's reaction in the feces. This reaction was negative or very slight during the lactobacilline malt period and it was slight throughout the most of the period when bacillac was given. The reaction was marked throughout all periods when plain milk was given, either with biscuits only or in addition to a mixed diet. There was a moderate reaction during the periods when an ordinary diet of zoalak added to an ordinary diet were given.

*Lactic Acid Bacilli in the Feces.* At the close of the lactobacilline malt period and again a week after the lactobacilline malt was discontinued, the coarse lactic acid bacilli in the lactobacilline malt (*B. bulgaricus?*) were grown in cultures from the feces by Dr. A. I. Kendall.

In reviewing the record of this case, one has to recognize the narrow limits of the experiment. The observations were made upon only one person and he was in health. During each period an ordinary mixed diet was taken together with the fermented milk or malt. The observations with regard to each form of lactic acid ferment were continued for only ten days in succession with the exception of lactobacilline malt when the record covered forty-four successive days. The variations from the normal, as would be expected, were only slight. No generalizations can be made from a single case. Individual idiosyncrasies of digestion are so marked that the saying is constantly being proved that what is one man's meat is another man's poison. The results as they stand are interesting, however, especially as showing that in this case the subject's condition, as indicated by the ratio of ethereal sulphates excreted, was best when he was taking an ordinary mixed diet of his own choosing, with no form of lactic acid
ferment or of milk food excepting the small amount of milk added to the tea and coffee. The excretion of phenol in the urine was slightly greater during the period when a mixed diet alone was taken but this was the only respect in which the subject's condition was not fully as favorable or even more favorable than during the other periods.

During the period when zoolak and biscuits only were taken for lunch, there were days when the ratio of the ethereal sulphates was very low, lower than at any other time excepting when biscuits and milk were the only foods taken. This is best shown in Chart I. The amount of phenol excreted in the urine was also the lowest during the period when zoolak and biscuits only were taken for lunch. But in the last period when a liter of zoolak was taken in addition to three meals daily of a mixed diet the subject's condition again became less satisfactory than when no fermented milk was added to the diet.

Lactobacilline malt was given continuously for so long a period (44 days) that its effect can be more definitely stated than that of the milk preparations. The period was divided into two parts to determine whether the condition varied in the second half from the first half of the time. The averages for these two halves of the period vary but slightly in any respect as can be seen by the table of averages of the periods (Table I). The subject's condition was definitely less satisfactory during the lactobacilline malt period than at any other time. During this period the ratio of ethereal sulphates was greater, the excretion of phenol was greater, the reaction for indol and skatol in the acid distillate from the feces was more marked than during any other period. Also, the percentage of nitrogen lost in the feces during the second half of the period was greater than at any other time excepting the last period when zoolak was taken in addition to a full diet. Twice during this period there occurred attacks of severe colic when apples were eaten, the combination of the lactobacilline malt and the fruit apparently causing the colic. There was more constipation during this period. Throughout the other periods the bowels were fairly regular. The amount of fresh feces and of dried feces was least during the lactobacilline malt period. This was evidently due to the fact that the lactobacilline malt lessened the appetite for other food as the nitrogen in
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the urine was also low at this time, showing that there was a lessening and not an increase in the amount of nitrogenous food absorbed from the intestine. Schmidt’s reaction in the feces was negative throughout the lactobacilline malt period. If, as is supposed, this indicates diminished reduction processes in the intestines due to a lessening of the activity of putrefactive bacteria, the patient’s condition in this regard was better than at any time during the experiment.

The periods in which a liter of plain milk with biscuits was taken for lunch with a mixed diet for breakfast and dinner showed a definite increase in intestinal putrefaction over the periods when a mixed diet with only the small amount of milk added to the tea and coffee was taken three times in a day. When milk and meat were both taken the ethereal sulphates were increased (Charts I and II, Periods VI and VIII), the indican was increased, Schmidt’s reaction in the feces was more marked; but the reaction for phenol in the urine was slight during these periods.

Emphasis should be laid upon the fact that the observations in this paper have no bearing upon the use of fermented milks alone or with a diet free from meat. The giving of protein in the form of milk rather than of meat regularly reduces the amount of intestinal putrefaction. The fermented milks give a variety to a milk diet, they are found by many to be more appetizing and refreshing than plain milk and in certain forms of gastric and intestinal indigestion they are better borne than plain milk. The object of this investigation as before stated was only to determine the effect of fermented milks in controlling intestinal putrefaction when a general mixed diet including meat was taken. In this case it was found that they exerted no specially favorable influence in this way but in so far as the subject’s condition varied it was better when the lactic acid ferments were not added to the general diet.

In conclusion I am glad to acknowledge my indebtedness to Mr. E. N. O’Brien for his cooperation in performing a large part of the analytical work connected with this study.
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