THE INFLUENCE OF MILK FEEDING ON MORTALITY AND GROWTH, AND ON THE CHARACTER OF THE INTESTINAL FLORA.*

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I. THE INFLUENCE OF MILK FEEDING ON MORTALITY AND GROWTH. 1

The investigations which furnish the data presented in this paper extended over a period of about three years. The milk feeding experiments were the direct outcome of the researches that had been conducted for several years at the Storrs Agricultural Experiment Station on bacillary white diarrhea of chicks, and had as their chief object a study of the value of sour milk as a possible preventive and even curative agent in the elimination of bacillary white diarrhea. It was anticipated, in conformity with the views of Metchnikoff and his followers, that sour milk, in virtue of the acids that it contains, or as the immediate result of the acid-producing bacteria, might exert a beneficial influence in preventing or allaying the disease, if supplied soon enough.

The results of the first year were such as to leave little doubt as to the life-saving value of sour milk, in as far as bacillary white diarrhea was concerned. What appeared to be of far greater significance, however, was the marked influence of sour milk on the growth of the chicks and on the total death rate or mortality from all causes. In subsequent feeding experiments milk that was soured by Bacillus bulgaricus and sweet milk were employed, as well as ordinary sour

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milk. Throughout the different series of experiments the plan of investigation was essentially the same, and but for certain minor details was as follows:

The chicks, with few exceptions, were hatched in artificial incubators under as nearly uniform conditions as possible. When taken from the incubators the apparently sound chicks were divided uniformly into different lots, usually six. The number in each lot varied in the different experiments from fifteen to sixty. When sour milk was the only milk that was fed, as in the first series, the different lots or pens received the following treatment: Pen 1 was artificially infected with a bouillon culture of *Bacterium pullorum* when 24 to 36 hours old, and was fed sour milk; pen 2 was similarly infected, but received no milk; pen 3 was infected when 48 to 60 hours old and was fed sour milk; pen 4 was infected at the same age as pen 3, but was given no milk; pen 5 served as an uninfected control with sour milk, and pen 6 as an uninfected control without the milk.

In the numerous experiments which involved the use of two kinds of milk, as for example sweet and ordinary sour milk, the chicks were divided into equal lots, usually six. Pens 1, 2, and 3 were artificially infected with *Bacterium pullorum*, while pens 4, 5, and 6 were left uninfected. Pens 1 and 4 were fed the ordinary sour milk; pens 2 and 5 received the sweet or *bulgaricus* milk, according to the prearranged plan, and pens 3 and 6 went without milk. Thus double sets of controls were provided for.

Artificial infection with *Bacterium pullorum*, the organism which causes bacillary white diarrhea in chicks, was brought about by the use of a forty-eight hour bouillon culture which was administered with the aid of a medicine dropper. Three to six drops of the culture were placed in the beak in such a manner that the chick was compelled to swallow them. The milk was supplied in shallow galvanized pans having a capacity of about one pint. In order to prevent the chicks from wading in the pans coarse mesh wire was fastened over the tops. The pans were thoroughly cleaned at least

once a day, and scalded at definite intervals. In most of the experiments the milk was supplied twice a day.

Ordinary sour milk was usually obtained by adding a starter (sour milk) to fresh skimmed milk the day before it was to be used. The milk was kept at ordinary summer room temperature (25°-30° C.) either in pails or in milk bottles. *Bulgaricus* milk was prepared by sterilizing fresh skimmed milk, and after sufficient cooling, inoculating it with pure milk cultures of *Bacillus bulgaricus* which was procured in powder or tablet form from two reliable sources. The acidity was determined from day to day by titration. As a rule, the acidity was not permitted greatly to exceed 1.0 per cent. (in terms of lactic acid), since less of the milk was consumed when the acidity was high. The *bulgaricus* milk had the appearance of the product that is usually obtained with the use of *Bacillus bulgaricus*. Junket tablets were added in all but two instances to the sweet milk, in order to make it appear more attractive to the chicks. The conclusion was finally arrived at, however, that the junket tablets were unnecessary.

All the chicks were constantly supplied with an excess of feed. This consisted of the ordinary chick feed during the first week, after which a standard dry mash was fed. After the first year the feed going into each pen was weighed, and the amount left in the pans again weighed at the end of definite periods. The milk was also weighed, and complete records were kept of the milk and feed consumed per week.

The chicks were weighed at the beginning of the experiments, and once a week at as nearly the same hour as was possible. Daily mortality records were kept, and postmortem examinations were made of the chicks that died during the course of the experiments. Special emphasis was placed on the recovery of *Bacterium pullorum* from the internal organs and from the unabsorbed yolk.

In the entire investigation 5,118 chicks were employed. The majority of them were white Leghorns, though all the experiments of 1913 were conducted on white Plymouth Rocks. The small number of Rhode Island Red chicks that was used was of comparatively low vigor and was, therefore, less satisfactory than the others. The chicks were housed in small outdoor brooder houses which opened into large yards or runs, and in a large brooder house.
from which long runs extended from each pen. About the same
number of experiments was conducted under the two sets of hous-
ing conditions. The yards to which the chicks had access contained
sufficient grass to supply them with the necessary amount of green
food. The experiments were continued until the chicks were six
to seven weeks old, when they were removed to permanent quarters.

RESULTS OF THE MILK FEEDING EXPERIMENTS.

Since no records of the amounts of feed and of sour milk consumed were
kept in the investigations of the first year, the results are of far less importance
than those of the two following years. Furthermore, the lack of complete uni-
formity in the plans of the different experiments makes the averaging of results
somewhat difficult and unsatisfactory. It was most clearly shown throughout
the work, however, that the feeding of sour milk exerted a beneficial influence
on the growth and mortality of the chicks. The following tabulated results
(table I) of experiment F will serve as a good illustration.

<table>
<thead>
<tr>
<th>TABLE I.</th>
<th>Infected when 36 hrs. old.</th>
<th>Infected when 60 hrs. old.</th>
<th>Uninfected controls.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight per 10 chicks</td>
<td>5.45 lbs.</td>
<td>3.97 lbs.</td>
<td>4.75 lbs.</td>
</tr>
<tr>
<td>Mortality</td>
<td>3 or 8.1%</td>
<td>8 or 21.6%</td>
<td>2 or 5.4%</td>
</tr>
</tbody>
</table>

The total number of chicks used in the experiment was 222, or 37 in each pen.
In several instances the weight of the milk-fed chicks was, at the end of the
experiment, at least double that of the chicks that received no milk, whether
they were infected with *B. pullorum* or not. The sour milk chicks were stronger
and more vigorous than the others, particularly in the uninfected pens. This
was shown in different ways, as for example in the size and color of the combs,
the strength of the feet and legs, and in the various activities of the chicks.

The sour milk feeding exerted a decided influence on mortality from bacillary
white diarrhea as well as from all causes. In all of five complete experiments
the mortality of the infected chicks was lower in the pens that were supplied
with milk than in those which were not. In several instances the number of
deaths in the lots with no milk was at least twice as great as in the corre-
sponding pens that received milk, and in one case the ratio was approximately
3:1. Since the investigation of this first year involved the use of 1,044 chicks,
some importance should be given to these results. In two experiments that were
successfully carried through, and in which there were uninfected control lots,
it happened that not a single death occurred in the sour milk pens that were
not artificially infected with *B. pullorum*, while in the corresponding lots with
no milk the mortality was approximately 11 per cent.

The results of the first year are in perfect harmony with those of subsequent
investigations, in so far as the influence of milk feeding on growth and general
Leo F. Rettger.

mortality is concerned. The apparent discrepancies that exist in the data of 1912 and 1913, bearing on the influence of milk feeding on the mortality from white diarrhea, may be fully explained, as will be seen later.

As none but ordinary sour milk was employed in the above experiments, no satisfactory explanation presented itself as to how such significant results of a sour milk diet could be brought about. It seemed to be of considerable importance, therefore, to determine, if possible, to what factor or factors milk owed these properties. It was for this purpose largely that the extensive investigations of the following two years were carried on. More complete data were sought, also, on the value of milk feeding as such.

In the second series of investigations seven different experiments were carried on and successfully completed. In three of them ordinary sour milk was the only milk that was supplied, while in the remaining four both the naturally soured and bulgaricus milk were used. Altogether 1,824 chicks were employed. In the first three experiments full records were made of the total gain in weight per ten chicks, the total feed consumed, the gain in weight per lb. of feed, and the mortality. In three of the four experiments in which a comparative study was made of ordinary sour and of bulgaricus milk the following data were acquired: total gain per ten chicks, total feed consumed, total milk consumed, total solid matter fed including the milk, per cent. acid in milk, gain per ten chicks for each lb. of solid food, and mortality. As the fourth experiment was conducted on hen-hatched and hen-reared chicks, no food records could be kept.

Time and space do not permit of a complete survey of the results here; hence only brief summaries will be given.

**TABLE II.**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of chicks in each pen</th>
<th>Infected chicks</th>
<th>Uninfected chicks</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td>23</td>
<td>20</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>23</td>
<td>20</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>D</td>
<td></td>
<td>29</td>
<td>39</td>
</tr>
<tr>
<td>E</td>
<td></td>
<td>18</td>
<td>16</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>G</td>
<td></td>
<td>18</td>
<td>27</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>109, or 38%</td>
<td>187, or 42%</td>
</tr>
</tbody>
</table>

*For detailed statements of the results, including tables and curves, the reader is referred to Rettger, Kirkpatrick, and Jones, *loc. cit.*

*445 in each of the two infected groups, and 304 in each of the two uninfected groups.*
Influence of Milk Feeding on Mortality.

While the difference in the mortality of the two general infected groups (one supplied with sour milk and the other not) is but 4 per cent., the influence of sour milk feeding on mortality from all causes is encouraging (table II). Of the 304 uninfected chicks that were supplied with sour milk 37, or 12.2 per cent., died, as compared with a mortality of 36.5 per cent. in the corresponding group that received no milk. In other words, there were only one-third as many deaths in the former as in the latter. The mortality as a whole in the uninfected lots is considerably greater than it was during the previous year. This is undoubtedly due to a difference in the vitality of the chicks. In the investigations of 1912 strong white Leghorn stock was usually employed, while in the subsequent year white Plymouth Rock chicks which possessed no unusual vigor served as the subjects of investigation.

In the investigations of 1913 the chicks which were artificially infected with bouillon cultures of \textit{B. pullorum} were subjected to the infecting process only once; that is, aside from the possibilities of natural infection between chicks, the chicks received but one treatment with the organism, and that was at practically the same time that the sour milk was supplied.\textsuperscript{5}

\textbf{TABLE III.}

\textit{Combined Data Showing the Influence of Sour Milk Feeding on Growth.}

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected lots: 890</td>
<td>7.0 lbs.</td>
<td>4.3 lbs.</td>
<td>3.7 lbs., or 38.5%</td>
<td></td>
</tr>
<tr>
<td>Uninfected lots: 608</td>
<td>7.9 lbs.</td>
<td>4.6 lbs.</td>
<td>3.3 lbs., or 41.8%</td>
<td></td>
</tr>
</tbody>
</table>

The above summaries (table III) do not include any data on the feeding of \textit{bulgaricus} milk. They are based on work that was in a large measure merely a continuation of the investigation of the previous year. In the following condensed statements the results of the feeding of milk that was soured by \textit{B. bulgaricus} are included, and will therefore serve to show the relative merits of the two methods (table IV).

\textbf{TABLE IV.}

\textit{Gain Per Ten Chicks for Each Pound of Solid Matter, Including Milk Solids, Consumed.}

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected lots: 444</td>
<td>0.29 lb.</td>
<td>0.30 lb.</td>
<td>0.29 lb.</td>
<td></td>
</tr>
<tr>
<td>Uninfected lots: 444</td>
<td>0.32 lb.</td>
<td>0.30 lb.</td>
<td>0.26 lb.</td>
<td></td>
</tr>
<tr>
<td>Combined average: 888</td>
<td>0.305 lb.</td>
<td>0.30 lb.</td>
<td>0.28 lb.</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{5}The term sour milk is used throughout this paper to designate ordinary or naturally soured milk, in distinction from \textit{bulgaricus} milk.
Leo F. Rettger.

It is of interest to note that in the sour milk and the bulgaricus milk groups the gains per lb. of solid matter consumed are practically the same. In the lots that were not supplied with milk the average gain was noticeably less (approximately 8 per cent.). This difference, when taken by itself, is too small to be of much significance, but when considered along with other data should have some importance.

In order to make a comparative study of sour and of bulgaricus milk with special emphasis on acidity as an important factor, it was necessary to keep a full record of the acidity of both the sour and the bulgaricus product, and of the amounts fed. In table V the acidity is given in terms of lactic acid.

**TABLE V.**

*Amounts of Acids in Milk Consumed.*

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Infected chicks</th>
<th>Uninfected chicks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fed sour milk</td>
<td>Fed bulgaricus milk</td>
</tr>
<tr>
<td>D</td>
<td>0.19 lb.</td>
<td>0.45 lb.</td>
</tr>
<tr>
<td>F</td>
<td>0.12 lb.</td>
<td>0.15 lb.</td>
</tr>
<tr>
<td>G</td>
<td>0.12 lb.</td>
<td>0.12 lb.</td>
</tr>
<tr>
<td>Total</td>
<td>0.43 lb.</td>
<td>0.72 lb.</td>
</tr>
</tbody>
</table>

The total amount of ordinary sour milk consumed was 115.6 lbs., and that of bulgaricus milk 105.5 lbs. In spite of this difference the total amount of acids in the bulgaricus milk (1.53 lbs.) was far in excess of what the sour milk contained (0.98 lb.). If the value of sour milk feeding is dependent upon the amounts of acids, or the degree of acidity, present, the results obtained with the bulgaricus milk should have been far better than those following the sour milk diet. That this was not the case is clearly shown in the following condensed results (table VI).

**TABLE VI.**

*Summary Showing the Comparative Influence of Sour Milk and of Bulgaricus Milk on Mortality.*

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of chicks</th>
<th>Mortality.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Infected chicks</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fed sour milk</td>
</tr>
<tr>
<td>D</td>
<td>50</td>
<td>7, or 14%</td>
</tr>
<tr>
<td>F</td>
<td>53</td>
<td>18, or 34%</td>
</tr>
<tr>
<td>G</td>
<td>45</td>
<td>25, or 55.5%</td>
</tr>
<tr>
<td>Total</td>
<td>148</td>
<td>50, or 33.7%</td>
</tr>
</tbody>
</table>

These figures require little comment. The total mortality of all chicks that received the naturally soured milk (both infected and uninfected) was 67, or 45.3 per cent, as compared with 70, or 47.3 per cent, for the bulgaricus chicks.
Influence of Milk Feeding on Mortality.

The small difference which is to the advantage of the sour milk feeding is, of course, within the limits of possible error in experiments of this kind, and should not be given much importance; nevertheless, the results clearly show that bulgaricus milk is of no greater value in reducing mortality than ordinary sour milk.

TABLE VII.

Figures Showing the Comparative Influence of Sour Milk and of Bulgaricus Milk on Growth (Experiments D, E, F, and G).

<table>
<thead>
<tr>
<th>Infected Chicks</th>
<th>Uninfected Chicks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed sour milk</td>
<td>Fed bulgaricus milk</td>
</tr>
<tr>
<td>6.13 lbs.</td>
<td>5.61 lbs.</td>
</tr>
<tr>
<td>7.50 lbs.</td>
<td>6.19 lbs.</td>
</tr>
</tbody>
</table>

Combined averages for infected and uninfected chicks:

Fed sour milk .......................................... 6.82 lbs.
Fed bulgaricus milk ..................................... 5.90 lbs.
Difference .......................................... 0.92 lb.

These figures (table VII) are for experiments D, E, F, and G. If we eliminate experiment E because hens were used as brooders and no records could be made of the amounts of feed and milk consumed, the averages are as follows (table VIII).

TABLE VIII.

Average Gain per Ten Chicks in Experiments D, F, and G.

<table>
<thead>
<tr>
<th>Infected Chicks</th>
<th>Uninfected Chicks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fed sour milk</td>
<td>Fed bulgaricus milk</td>
</tr>
<tr>
<td>5.48 lbs.</td>
<td>4.87 lbs.</td>
</tr>
<tr>
<td>7.1 lbs.</td>
<td>5.87 lbs.</td>
</tr>
</tbody>
</table>

Combined averages for infected and uninfected chicks:

Fed sour milk .......................................... 6.26 lbs.
Fed bulgaricus milk ..................................... 5.37 lbs.
Difference .......................................... 0.89 lb.

The pens that were supplied with the naturally soured milk (experiments D, F, and G) gained 0.89 of a lb. (or 14.2 per cent.) more per ten chicks than those which received the bulgaricus product. Since the former consumed more food (both dry feed and milk), but less acid, than the bulgaricus group, the greater gain in the sour milk pens must be attributed almost entirely to the food as such. The difference in gain per ten chicks closely corresponds with the difference in the total amount of solid food taken, namely 12.9 per cent., the sour milk chicks consuming 20.45 lbs. and the bulgaricus lots 17.81 lbs. Here again the differ-
ence is in favor of the naturally soured milk. Not only was there a larger absolute gain in weight in the sour milk chicks, but the gain per lb. of solid matter consumed was greater than in the bulgaricus lots. This difference has already been brought out in table II. The sour milk was the more appetizing, as 115.6 lbs. were consumed in experiments D, F, and G, while only 105.5 lbs. of the bulgaricus milk were utilized.

A COMPARATIVE STUDY OF THE INFLUENCE OF SWEET AND OF SOUR MILK ON MORTALITY AND GROWTH.

In the investigation of the past year (1914) eight complete experiments involving the use of 2,250 chicks were conducted. The plan of these experiments was practically the same as the preceding.

Each lot of newly hatched chicks was divided into six uniform groups. Pens 1, 2, and 3 were artificially infected with 48 hour bouillon cultures of B. pullorum, while pens 4, 5, and 6 were left untreated. Pens 1 and 4 were fed ordinary well soured milk; pens 2 and 5 sweet milk, and pens 3 and 6 no milk at all. Thus, pens 4, 5, and 6 served as controls for infected pens 1, 2, and 3; and pens 3 and 6 were controls with no milk for pens 1, 2, 4, and 5. In all but two of the experiments the sweet milk was curdled with rennet tablets. The milk was supplied at least twice each day, usually early in the morning and at noon. In every case of milk feeding the milk was supplied as soon as the chicks were removed from the incubators, approximately twenty-four hours after hatching. In six of the experiments the chicks were artificially infected at the time that they first received the milk, as well as on each of the following four days. In the other two experiments the first infection was postponed until three or four days after the earliest milk feeding. In every instance the bouillon culture was administered five times on five consecutive days. The delaying of infection with B. pullorum was for the purpose of giving the chicks all the advantages of early milk feeding that it might possess, especially in as far as increasing bodily vigor is concerned.

The results of these experiments appear in the tables under the following heads: numbers of chicks in each pen at the beginning of each week, weekly weights of the chicks, amounts of dry feed and of milk consumed per week as well as for the entire period, gains in weight per ten chicks for each pound of total solid matter, including milk consumed, and the mortality. The milk solids were estimated as 10 per cent. Table IX is a condensed statement of the data which bear on mortality.

6 Rettger, Kirkpatrick, and Card, loc. cit.
7 Rettger, Kirkpatrick, and Card, loc. cit.
Influence of Milk Feeding on Mortality.

### TABLE IX.

Influence of Sweet and of Sour Milk Feeding on Mortality.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>No. of chicks</th>
<th>Mortality</th>
<th>Infected chicks</th>
<th>Uninfected chicks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fed sour milk</td>
<td>Fed sweet milk</td>
</tr>
<tr>
<td>A</td>
<td>31</td>
<td>11</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>B</td>
<td>38</td>
<td>17</td>
<td>22</td>
<td>31</td>
</tr>
<tr>
<td>C</td>
<td>38</td>
<td>20</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>D</td>
<td>39</td>
<td>2</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>E</td>
<td>38</td>
<td>20</td>
<td>22</td>
<td>30</td>
</tr>
<tr>
<td>F</td>
<td>60</td>
<td>49</td>
<td>48</td>
<td>60</td>
</tr>
<tr>
<td>G</td>
<td>59</td>
<td>33</td>
<td>22</td>
<td>49</td>
</tr>
<tr>
<td>H</td>
<td>50</td>
<td>66</td>
<td>66</td>
<td>50</td>
</tr>
</tbody>
</table>

Total mortality, by pens 151, 145, 201, 65, 61, 114, or 40% or 39% or 54% or 17% or 16% or 30%.

Total mortality for the three infected groups 497, or 44.2 per cent.

Total mortality for the three uninfected groups 240, or 21.3 per cent.

The results obtained in experiments A and B were not as satisfactory and well defined as in the others. The chicks were hatched in March, and hence were kept indoors for the greater part of the time that they were under observation, the weather being cold. Considerable leg weakness developed, which was confined largely to the pens receiving milk. The sour milk pens in A were most seriously affected. More milk was consumed by these chicks than in the sweet milk lots. Furthermore, the conditions were more favorable for leg weakness at the time experiment A was in progress than later. It appeared most evident that the milk-fed chicks were growing too fast, under the conditions of close confinement, for the legs to support their weight.

The mortality in experiment F was unusually high in all the pens, especially in the uninfected lots. These figures alone constitute a large part of the total mortality. They are explained by the fact that the chicks used in this experiment were of low vitality, which was shown in various ways, aside from the high death rate. However, the proportion that the mortality figures of the milk-fed chicks bear to those which were not supplied with milk is about the same as the average for the eight experiments.

According to the above data there is a slight difference in the value of sweet and of sour milk feeding. Among the infected chicks the mortality was 2.5 per cent. greater in the sour milk than in the sweet milk pens, while among the uninfected the difference amounted to 5.9 per cent.

The value of milk feeding as such is clearly demonstrated in the
table. Not only was there a great reduction in the mortality of the uninfected lots that received milk (either sweet or sour), which amounted to almost 100 per cent., but there was a marked difference in the death rate of the milk-fed infected chicks, as compared with those which remained without milk, the difference amounting to approximately 30 per cent. In other words, there were almost twice as many deaths in the uninfected lots which were denied the milk than in the corresponding sweet or sour milk pens, and almost one-third more deaths in the infected groups that did not receive the milk than in the corresponding milk-fed lots. These results are in harmony with those of the first series of experiments (1912). Sufficient evidence is at hand, therefore, to show that the feeding of milk, either sweet or sour, exerts a most decided influence in lowering the general death rate of young chicks, and if fed soon enough greatly reduces the mortality from bacillary white diarrhea.

That the feeding of sour milk in the second series of investigations did not materially affect the death rate from bacillary white diarrhea may be explained readily by the fact that the milk was not supplied soon enough before the chicks were subjected to the artificial infection, and hence failed to increase the vigor and resistance of the chicks before the bacteria in question established themselves and the disease ran its natural course.

**TABLE X.**

*Influence of Sweet and of Sour Milk Feeding on Growth.*

*Average Total Gain in Weight per Ten Chicks.*

<table>
<thead>
<tr>
<th>Infected chicks</th>
<th>Uninfected chicks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fed sour milk.</td>
</tr>
<tr>
<td></td>
<td>Fed sweet milk.</td>
</tr>
<tr>
<td></td>
<td>No milk.</td>
</tr>
<tr>
<td></td>
<td>Fed sour milk.</td>
</tr>
<tr>
<td></td>
<td>Fed sweet milk.</td>
</tr>
<tr>
<td></td>
<td>No milk.</td>
</tr>
<tr>
<td>4.62 lbs.</td>
<td>4.17 lbs.</td>
</tr>
<tr>
<td>2.65 lbs.</td>
<td>5.07 lbs.</td>
</tr>
<tr>
<td></td>
<td>4.72 lbs.</td>
</tr>
<tr>
<td></td>
<td>3.22 lbs.</td>
</tr>
</tbody>
</table>

Combined averages, all chicks:

- Fed sour milk ........................................ 4.84 lbs.
- Fed sweet milk ....................................... 4.44 lbs.
- Fed no milk ......................................... 2.88 lbs.
Influence of Milk Feeding on Mortality.

TABLE X.—Concluded.

Gain in Weight per Ten Chicks for Each Pound of Total Solid Matter Consumed as Food.

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of chicks employed</th>
<th>Fed sour milk</th>
<th>Fed sweet milk</th>
<th>No milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected</td>
<td>1,125</td>
<td>0.25 lb.</td>
<td>0.24 lb.</td>
<td>0.19 lb.</td>
</tr>
<tr>
<td>Uninfected</td>
<td>1,125</td>
<td>0.27 lb.</td>
<td>0.26 lb.</td>
<td>0.22 lb.</td>
</tr>
<tr>
<td>Combined (infected and uninfected)</td>
<td>2,250</td>
<td>0.26 lb.</td>
<td>0.25 lb.</td>
<td>0.205 lb.</td>
</tr>
</tbody>
</table>

In every instance (table X) the feeding of sweet and of sour milk was followed by a marked increase in the weights of the chicks beyond that which took place in the chicks which received no milk, the difference often amounting to as much as 80 per cent., and in two cases to more than 100 per cent. The differences vary in a large measure in direct proportion to the amounts of total solid matter consumed. Nevertheless, a review of the original tables will show that, aside from its appetite-stimulating properties, the milk served to bring about a more complete utilization of the food.

The combined data on the 2,250 chicks show that those which received the sour milk gained 0.26 of a pound per ten chicks for each pound of total solids consumed; that those which were fed sweet milk made a gain of 0.25 of a pound; and those that were without milk gained only 0.20 of a pound. In other words, the milk-fed chicks gained 25 per cent. and 30 per cent. more in weight per pound of solid matter used than the chicks which received no milk. The gains per pound of solid food in the sour milk chicks were 5 per cent. greater than in the sweet milk lots. As this difference is so small as to lie within the limits of possible error, it should not be given too much importance. Furthermore, it offsets the slight difference in mortality that favored the sweet milk feeding (table IX).

The results of the entire investigation of milk feeding, which extended over a period of almost three years, fully justify the conclusion that milk is an important factor of diet in as far as mortality and growth are concerned. Whether milk is fed as sweet, ordinary sour, or Bacillus bulgaricus milk, the effect is essentially the same. Hence, milk as such possesses one or more ingredients which are of unique significance. No experiments have been undertaken, in connection

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* Rettger, Kirkpatrick, and Card, loc. cit.
Leo F. Rettger.

with the general problem of milk feeding, to determine what these physiologically active substances are. Some reference to the work of Osborne and Mendel will be of special interest here.

These investigators have shown that butter fat, when fed to rats which failed to complete their growth on a diet consisting of pure proteins, starch, protein-free milk, and commercial lard, enabled the rats to regain their lost growth. They have also acquired an abundance of evidence to indicate that lactalbumin is of particular value in fostering growth. In other words, lactalbumin furnished the necessary factors in their experiments which are required for normal growth. Since lactalbumin is rich in both tryptophane and lysine, it is but natural to assume that this milk protein owes its unique dietary properties to these two substances that it contains in its molecule. Osborne and Mendel have apparently demonstrated that tryptophane is necessary for maintenance, but wholly inadequate to produce growth, and that lysine is indispensable for growth.

II. THE INFLUENCE OF MILK FEEDING ON THE CHARACTER OF THE INTESTINAL FLORA.

That the value of the feeding of milk does not lie in acids that may be present in the milk, nor in acid-producing bacteria which as a rule constitute a large part of the organisms occurring in milk, has been clearly demonstrated in the foregoing investigations. These results are not in harmony with the ideas of many, who hold that sour milk, or the acid product which is prepared with the aid of the so called "Bulgar" or "Bulgarian tablets," is of great dietary importance because of the presence of these acid-producing bacteria, or of their acid products.

After experimenting upon himself, Leva arrived at the conclusion that the ingestion of Metchnikoff's lactobacilline brought about an acclimatization of B. bulgaricus in the intestine. This organism was found to be present in the feces on and after the fifth day following the use of the tablets. He also observed that during the investigation the amounts of aromatic oxyacids, hippuric acid, and phenol in the urine were decreased.

In an extensive investigation in which thirty different hospital patients were employed as subjects, besides the author himself, Cohendy was led to conclude that the use of milk which is soured by B. bulgaricus causes a marked transformation of the intestinal flora, which is made apparent by the deodorization

9 Osborne, Mendel, Ferry, and Wakeman, loc. cit., 1913, xv, 311; 1913-14, xvi, 423; 1914, xvii, 401.
10 Osborne, Mendel, Ferry, and Wakeman, loc. cit., 1914, xvii, 325.
12 Cohendy, M., Compt. rend. Soc. de biol., 1906, lviii, 602.
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of the feces and by a decrease in the amounts of conjugate sulphates in the urine. He ascribed the change to the lactic acid bacillus, without due regard to the milk itself.

Belonovsky employed white mice. The diet consisted of sterilized wheat grain to which cultures of \( B. \text{bulgaricus} \) had been added. Twelve days after the beginning of the experiment a marked change was noted in the character of the intestinal flora. There was a large increase in the number of Gram-positive organisms, and a corresponding decrease in gas-producers. Similar results, although less pronounced, were obtained with mice that were fed sterilized wheat and killed cultures of \( B. \text{bulgaricus} \). The author further states that in young nursing mice the intestinal flora presented practically the same microscopic appearance as in the older mice which received the wheat and \( \text{bulgaricus} \) cultures. Direct microscopic examination of the feces of the mice that received the \( \text{bulgaricus} \) bacillus cultures failed to reveal a predominance of the \( \text{bulgaricus} \) type over other organisms. In fact, it is to be inferred that bacilli of this description were very few. The writer believes that the action of \( B. \text{bulgaricus} \) is not due entirely to the bacilli, or to the lactic acid, but to other products as well.

The opinions cited above are essentially the same as those which Metchnikoff announced and reiterated from time to time, and which have apparently gained a firm hold in the minds of many scientists as well as laymen. It is claimed that the wonderful accomplishments of \( B. \text{bulgaricus} \) are closely linked with its power to destroy or eliminate harmful bacteria from the intestinal tract, particularly those having putrefactive properties. Hence, by its ability to prevent autointoxication, it serves as an important therapeutic agent, even to the extent of preventing premature old age.

In the investigations upon which this part of the paper is based at least seventy-five white rats were employed. A moderate number of experiments was conducted also on chicks of different ages and on adult fowls. The work was the natural outcome of the earlier investigation of Rettger and Horton on the intestinal flora of white rats kept on experimental and ordinary mixed diets.

Special emphasis has been given to organisms which belong to the type of \( B. \text{bifidus} \) (Tissier) and of \( B. \text{acidophilus} \) (Moro), though other types of intestinal organisms were not overlooked or neglected when they were present in appreciable numbers. \( B. \text{acidophilus} \) assumed much prominence in the earlier feeding experiments which were carried on in connection with the elaborate investigations of Osborne and Mendel on the influence of pure pro-

tein diets on growth. When the rats were transferred from the ordinary mixed to the pure protein diets a marked transformation in the character of the intestinal flora invariably took place. The flora became more simplified, very few types of bacteria being found after an interval of two or three days following the change of food. Organisms belonging to the acidophilus group always became numerous, and at times were so prominent as to exclude all other types except Bacillus bifidus. This change in the intestinal flora was undoubtedly independent of the pure proteins, but, as later experiments have indicated, bore a definite relation to the protein-free milk which formed a part of the pure protein diet.

The experiments recorded here will be presented under three different heads; namely, the influence of milk feeding, the influence of carbohydrate feeding, and the influence of the ingestion of Bacillus bulgaricus, on the intestinal flora. The results of only a limited number of experiments which are typical will be given in detail.

The methods employed were essentially the same in the different series of experiments. The rats were kept in cages which were constructed of rather coarse wire net and supported at some distance over a tray which was covered daily with clean paper, so that the feces could be collected with as little chance of contamination as possible. Samples of feces were collected at regular intervals, sometimes daily. From 0.2 to 0.4 of a gram of the samples was vigorously shaken in test-tubes containing eight to ten cubic centimeters of sterile water and a little broken glass until a uniform suspension was obtained. From these suspensions agar plates and Veillon tubes were prepared in the desired dilutions. Smears were made also on slides and stained by the Gram method.

For plate-pouring different kinds of agar medium were used; namely, plain agar, dextrose agar (1 per cent. dextrose), acid dextrose agar (1 per cent. dextrose, 0.5 per cent. acetic acid), and neutral whey agar (neutral whey 1 liter, peptone 10 grams, agar 15 grams, and Liebig’s meat extract 3 grams). The dextrose agar was used at practically all times, especially in the Veillon tubes.

These experiments were conducted largely by Thomas G. Hull, and appear in part in another paper (Hull, T. G., and Rettger, L. F., Centralbl. f. Bakteriol., iet Abt., Orig., 1914, lxxv, 219).
Influence of Milk Feeding on Mortality.

All the rats were fed bread and green vegetables as the basic diet. In the different experiments the special agents to be studied were added to the basic diet. In several instances the bread was ground in a meat grinder. Numerous examinations of the feces of white rats that had been subsisting upon bread and green vegetable food for at least three or four days had shown that the intestinal flora were of the usual mixed type, the absence, or but a small number, of *Bacillus acidophilus* and *Bacillus bifidus* being particularly noticeable. Hence, such a diet was of much value in bringing the flora to a standard or uniform basis as a definite starting or end point in the different experiments.

**MILK FEEDING.**

In none of the experiments was the milk supplied until examination of the feces demonstrated that *Bacillus acidophilus* and *Bacillus bifidus* were absent from the feces, or that they were comparatively rare. Furthermore, control rats which received only the bread and vegetable diet were employed along with the others. The milk, which was usually whole milk, was poured over the dried bread crumbs or in the water dish in place of the water. The following experiments are given here as examples.

*Experiment A.*—Rats 1, 2, and 3 received 20 to 30 c.c. of milk three times a week for four weeks, in addition to the basic diet, while rats 4, 5, and 6 were given the bread and vegetable food only. During the entire period *B. acidophilus* was practically absent from the feces of the control rats, whereas in the milk-fed rats it was abundant after the first few days and continued so until the milk feeding was discontinued, after which the *acidophilus* group again disappeared.

*Experiment B.*—Rats 2 and 3 received, in addition to the bread and vegetable diet, 10 c.c. of milk daily for ten days. After the first three days *B. acidophilus* appeared, and numerous colonies of this organism were obtained on the agar plates. It was also very abundant in smears prepared from the feces, although various other types were present in small numbers.

*Experiment C.*—Rat 23 was supplied with 50 c.c. of milk daily for forty days. Within two to three days *B. acidophilus* became very numerous, which was shown by the large numbers of colonies on the agar plates, and by the small number of other types, excepting *B. bifidus*, in the microscopic mounts. Besides *B. acidophilus*, the Gram stained smears contained numerous rods which were smaller than the others, and which showed evidence of branching (*B. bifidus*). In the Veillon tubes *B. acidophilus* was quite abundant throughout the length of the tubes in the form of the typical shaggy colonies, while the smooth, disc-like colonies of *B. bifidus* appeared in sharp contrast in the deeper portions of the
tubes. As the experiment progressed *B. bifidus* increased in numbers, and in a large measure supplanted *B. acidophilus*.

At the termination of the forty days' period but little milk was taken by the rat, and although milk was supplied for the next ten days very little was consumed. As a consequence, *B. bifidus* disappeared from the intestine, and the number of *B. acidophilus* was greatly reduced, while other types again became more common.

Postmortem examinations were made of rats 2 and 3 after they had been on the bread, vegetable, and milk diet for ten days. Cultural and microscopic tests of the gastro-intestinal tract revealed the presence of *B. acidophilus* in large numbers, and in almost pure form, in the small intestine. It was also found to be abundant in the large intestine, but here it was mixed with various other organisms.

Numerous other experiments on milk feeding, both with white rats and with chicks of different ages, have given similar results. Sufficient evidence is at hand, therefore, to show that profound alterations in the character of the intestinal flora, at least of the white rat and the common domestic fowl, are brought about by the addition of milk to a diet which has established a flora in which the *acidophilus* and *bifidus* types of bacteria are few or absent. In this transformation the usual mixed flora are, in a large measure at least, supplanted by ones rich in *Bacillus acidophilus* or *Bacillus bifidus*, or both of these organisms. A limited number of experiments on man have given results similar to the above. The complete transformation of types is, however, not accomplished so readily as in the rat or in fowls.

Since *Bacillus acidophilus* and *Bacillus bifidus* are both carbohydrate-consuming organisms, and have but slight proteolytic properties, it seemed but natural to look for a possible explanation of the above results in the lactose content of the milk. For this purpose numerous feeding experiments were conducted on white rats with seven different carbohydrates.

### CARBOHYDRATE FEEDING.

The following carbohydrates were used: lactose, sucrose, maltose, dextrose, levulose, galactose, and dextrine. At first each carbohydrate was fed in the form of a concentrated aqueous solution, which was poured over the bread crumbs; later three to four grams of the carbohydrate in question were placed on the bread and moistened...
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with a small amount of water. The lactose feeding experiments were carried on for periods varying from one to seven weeks.

**Experiment A.**—Rat 13 was fed lactose for fifteen days. On the third day *B. acidophilus* was observed in appreciable numbers in the smears and agar plates prepared from the feces, after which it practically disappeared, and *B. bifidus* was found to be present in almost pure form in the smears.

**Experiment B.**—Rat 12 received lactose for thirty days, but little change was observed in the intestinal flora, neither *B. acidophilus* nor *B. bifidus* being present in appreciable numbers in the slides, plates, or Veillon tubes. No explanation can be given for this irregularity in the results, except that the rat was peculiarly resistant to bacterial changes in the intestine, which as a rule are readily induced by change of diet.

**Experiment C.**—Rats 16 and 17 obtained milk sugar for eleven days. *B. bifidus* became quite prominent on the second day and continued to the end of the period. *B. acidophilus* also was present in small numbers.

**Experiment D.**—Rat 35 was given lactose for thirteen days. *B. acidophilus* became very abundant at the outset, but soon diminished in numbers, while *B. bifidus* increased to such an extent as to exclude almost all other forms.

**Experiment E.**—Rat 26 received lactose for fifty days. For a short time the intestinal flora consisted largely of *B. bifidus* and, to a lesser extent, of *B. acidophilus*. Later *B. bifidus* was practically the only organism present.

**Experiment F.**—Rats 21 and 22 were fed milk sugar for sixteen days; Nos. 14, 15, and 16 for twenty days; Nos. 17 and 19 for eight days; Nos. 23, 27, and 28 for seven days, and No. 9 for ten days. In every instance *B. bifidus* became very abundant soon after the addition of the lactose to the diet. In general, the following changes took place in the intestine. By the second or third day *B. acidophilus* and *B. bifidus* began to make their appearance. After having attained a certain maximum *B. acidophilus* gradually decreased in number, and was practically absent at the close of the period, with very few exceptions. *B. bifidus* increased very fast, however, and in every case it became the predominant form.

Several lactose rats were killed for the purpose of making bacteriological examinations of the digestive tract. In rat 9 no colonies of *B. acidophilus* or *B. bifidus* were obtained from the stomach. A small number of the *acidophilus* type was found in the duodenum, while throughout the remainder of the intestine *B. bifidus* was quite abundant. In rat 26 (fed lactose for fifty days) *B. bifidus* was present in large numbers in the stomach, the small intestine below the duodenum, and in the large intestine. *B. acidophilus* was found also, but to a limited extent. Rat 15, which required an unusual length of time for *B. bifidus* to establish itself, contained *B. acidophilus* in the stomach and throughout the small and large intestine, while *B. bifidus* was plentiful in the ilium and in the large intestine.

The foregoing experiments demonstrate that both milk and lactose, when fed in sufficient quantities, have a pronounced influence on
the character of the intestinal flora, each bringing about a profound change in which the ordinary mixed flora give way to ones that are more simplified and which are made up largely of *Bacillus acido-philus* or *Bacillus bifidus*, or both. That milk owes this property to the lactose which it contains can hardly be doubted. Since almost half of the total solids of milk is milk sugar, namely, about 5 per cent., it is not surprising that milk when fed even in small amounts has such a marked influence on the flora of the intestine.

A large number of feeding experiments was conducted in which sucrose, maltose, dextrose, levulose, galactose, and dextrine were used in their turn. Very little or no change could be observed in the intestinal flora of any of the rats. In order to determine whether the negative results were due to an inhibiting influence of the different carbohydrates, a limited number of feeding experiments was carried out in the following manner. Rats which had been receiving lactose, and which exhibited the characteristic flora of milk- or lactose-fed rats, were given, besides the usual amount of milk sugar (three to four grams daily), an equal amount of the carbohydrate in question. No decrease could be observed in the numbers of organisms of the *acidophilus* and *bifidus* types, nor was there any other apparent change. The failure, therefore, of the intestinal bacteria to be affected by any of these carbohydrates, except lactose, can not be ascribed to any inhibitory action which they or their products may exert.

Thus far one significant fact has been demonstrated repeatedly; namely, that diet may be a very important factor in determining the character of the intestinal flora.

The above results are in accord with those of Sittler,\(^7\) who found that when lactose is fed to children that subsist on cow's milk the typical *bifidus* flora of breast-fed infants are present. Similar results were obtained with so called "malt soup." On the other hand, cane sugar did not react in this way. The limited number of experiments which was carried on with dextrose seemed to indicate that it played the same part as lactose. This claim could not be substantiated by us.

Quite recently Distaso and Schiller\(^8\) claim to have shown that the feeding of milk, lactose, or dextrine to white rats which have been on a diet of bread and meat brings about a change in the intestinal flora from the usual mixed to


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the *bifidus* type. No mention in their brief publication is made of *B. acidophilus*. Our results on the feeding of dextrine are not in harmony with those just cited. The difference may be due to the dextrine employed. Further work to determine the influence of dextrine, and starches as well, is under way.

It is surprising that so little attention has been given in past investigations to the occurrence of *B. acidophilus* (Moro), and to the conditions that influence its numbers in the intestine. In our work on the intestinal flora of the albino rat and of the common domestic fowl, organisms of the *acidophilus* type have been met with almost constantly. In the feces of man and of guinea pigs we have found them frequently also, and at times in considerable numbers. While *B. bifidus* (Tissier) was in most instances associated with *B. acidophilus*, the latter was, as a rule, much more prominent. Exceptions to this rule occurred in some of the lactose feeding experiments.

THE INFLUENCE OF THE INGESTION OF *BACILLUS BULGARICUS* ON THE CHARACTER OF THE INTESTINAL FLORA.

The feeding of *Bacillus bulgaricus* was accomplished in two ways. In some instances the rats received milk cultures of the organism. These cultures were prepared by inoculating sterilized skimmed milk with the organism and incubating for twenty-four hours at 37°C. By the other method large surfaces of dextrose agar were inoculated with *Bacillus bulgaricus*. After an incubation period of twenty-four hours the surface growth was washed off with sterile water. The water suspensions were fed as such in small dishes, or they were poured over the bread crumbs. In all the *bulgaricus* feeding the regular basic diet of bread and vegetables was employed. In some of the experiments sterile milk was fed to one or two of the rats, instead of the inoculated milk or the bacterial suspensions, also the washings of sterile agar.

It was difficult at the outset to distinguish between *Bacillus bulgaricus* and *Bacillus acidophilus*, and especially the colonies on dextrose agar. Certain marks of distinction were noted, however, but the identification was never regarded as satisfactory without the milk acidification test. Since *Bacillus bulgaricus* rapidly produces enough acid to coagulate the milk, and a recently isolated *acidophilus* strain does so only after more prolonged incubation, this test was of considerable importance.

Special precautions had to be taken to minimize the chances of

contamination of the feces by *Bulgaricus* bacilli that were supplied with the food. This was accomplished with no little effort.

**Experiment A.**—Six rats were employed. Rats 1 and 4 received the basic diet of bread and vegetables together with a watery suspension of *B. bulgaricus*; rats 2 and 5 were given the washings of sterile agar, in addition to the regular diet, while Nos. 3 and 6 received the bread and vegetable only. The experiment continued for four weeks, with daily feeding. *B. bulgaricus* was observed but twice in the feces of the rats that were abundantly supplied with it. Furthermore, no differences could be detected between the flora of rats 1 and 4 and those of the four rats which were not supplied with the organism in question. In all the rats the typical mixed flora prevailed.

In postmortem examinations conducted on rats 1 and 4 eighteen hours after the last feeding of *B. bulgaricus*, an organism resembling the bulgaricus bacillus was found in very small numbers in the stomach and duodenum, while from the remainder of the intestine not a single colony of this organism could be obtained.

**Experiment B.**—Rat 21 received the stock diet together with a washed culture (water suspension) of *B. bulgaricus*; No. 22 was given the regular diet plus 50 c.c. of milk that was fermented by *B. bulgaricus*, and rat 23 the usual diet plus 50 c.c. of sterilized milk. From the feces of rat 21 single colonies resembling those of *B. bulgaricus* were obtained three times; from No. 22 individual colonies of the *bulgaricus* type were obtained but four times, while in the plates from rat 23 no colonies of *B. bulgaricus* were detected, though colonies of *B. acidophilus* were quite abundant. This experiment continued over a period of four weeks.

**Experiment C.**—Rat 13 received bread and lettuce plus the water suspension of *B. bulgaricus*; No. 32 was given the same diet plus 50 c.c. of sterile milk and the water suspension; and rat 23 the same basic diet plus 50 c.c. of sterile milk. *B. bulgaricus* could at no time be found in the feces of the rats (13 and 32) that were fed the living bacilli; nor was there any apparent difference between the intestinal flora of rats 32 and 23. This experiment lasted ten days.

In postmortem examination one of the three rats that were given *B. bulgaricus* in water suspension failed to show the presence of this organism in any portion of the digestive tract; in the second a very small number was recovered from the jejunum and ilium only. The third rat had been receiving lactose for some time. *B. bulgaricus* could not be recovered from it, while *B. bifidus* was found in large numbers.

In all the rats that received milk, whether it was sterile or inoculated with *B. bulgaricus*, *B. acidophilus* and *B. bifidus* were at all times more or less prominent, as in the previous milk-feeding experiments. On the other hand, in spite of the large numbers of *bulgaricus* bacilli that were fed to some of the rats, very few could be recovered from the feces, and at no time did this organism establish itself in the intestine, even after the daily ingestion of the bacilli for periods of from ten days to four weeks.

These observations are in accord with those of Luerssen and Kühn, 21 who failed to establish *B. bulgaricus* in the intestine of man by the continued use of

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yoghurt, and with the views of Distaso and Schiller, who claim to have shown quite recently that the ingestion of large numbers of this organism by rats which are receiving milk or lactose does not result in its acclimatization in the intestine, and that \textit{B. bifidus} is the predominating type of bacteria.

Oehler\textsuperscript{23} conducted some feeding experiments with yoghurt on mice and monkeys, and stated that the \textit{bulgaricus} bacillus could be demonstrated with ease in the feces during the yoghurt feeding period. Within two to three days after the yoghurt feeding was discontinued, \textit{B. bulgaricus} again disappeared from the intestine. It should be borne in mind that large quantities of milk as such were supplied in the form of the yoghurt, and hence the conditions for the development of \textit{B. acidophilus} were most favorable. Some doubt may be felt, therefore, as to whether Oehler's \textit{B. bulgaricus} may not have been the Moro bacillus. It is conceivable, however, that any species of organism may be recovered in limited numbers from the feces if ingested in extremely large numbers.

Herter and Kendall\textsuperscript{24} found that in a monkey which was killed three to four hours after it had been fed 500 c.c. of milk that had been well soured by bacillac very few \textit{bulgaricus} bacilli were present in the large intestine. In an earlier experiment on this monkey they had failed to detect \textit{B. bulgaricus} in the feces after feeding the sour milk (bacillac) daily for three days. These same authors state that the intestinal flora of cats and monkeys were rapidly altered when a diet of meat or eggs was followed by one of milk and dextrose. The most important change was the substitution of an acidophilic, non-proteolyzing type of flora for one which was strongly proteolytic.

Even \textit{B. coli}, especially a foreign strain, appears to be unable to establish itself in the intestine as the result of the feeding of this organism. This was clearly demonstrated by Rettger and Horton\textsuperscript{25} who supplied white rats which were on diets containing pure protein as the only available nitrogenous food with vast numbers of colon bacilli. In one of the experiments a very small increase of \textit{B. coli} took place in the intestine; while in another there was no perceptible increase, although \textit{B. coli} was fed at frequent intervals for a period of almost four weeks.

Similar results were obtained by Seiffert\textsuperscript{26} who showed that a strain of \textit{B. coli} isolated from another species failed to inure itself to the conditions which obtain in the human intestine after being taken into the system \textit{per os}. A strain which had been isolated from the same person, however, rapidly multiplied and was found in abundance in the intestine. Raubitschek\textsuperscript{27} also claims to have demonstrated that foreign organisms are unable to establish themselves in the intestine, except after at least partial immunization of the host to the particular organisms.

Kulka\textsuperscript{28} conducted a series of experiments on man and rabbits. His results appear to be very decisive, demonstrating that \textit{B. metchnikovi} and \textit{B. prodigiosus},

\begin{footnotes}
\item 22 Distaso and Schiller, \textit{loc. cit.}
\item 23 Oehler, R., \textit{Centralbl. f. Bakteriol., 2te Abt.,} 1911, xxx, 149.
\item 25 Rettger and Horton, \textit{loc. cit.}
\item 26 Seiffert, G., \textit{Deutsch. med. Wehrschr.,} 1911, xxxvii, 1064.
\item 28 Kulka, W., \textit{Arch. f. Hyg.,} 1914, lxxxii, 337.
\end{footnotes}
when introduced either by mouth or by subcutaneous, intravenous, or intraperitoneal injection, do not appear in the feces. Mitchell and Bloomer obtained similar results in the common domestic fowl with the typhoid bacillus.

GENERAL CONCLUSIONS.

Throughout the investigations upon which a large part of this paper is based the favorable influence of milk feeding on mortality and growth was most apparent. Mortality from all causes was frequently reduced to at least one-half of what obtained among the chicks that received no milk, while the milk-fed chicks in some experiments gained twice as much in weight as those that were without this article of diet. Although the influence of milk feeding was less pronounced on the mortality of chicks that were artificially infected with Bacterium pullorum, quite an appreciable difference in mortality was always noted if the milk was fed at least one or two days before the first administration of the bouillon cultures of the organism in question.

Practically the same results were obtained, whether sweet or sour milk was fed, and no differences 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 exist in the milk as such, rather than in any milk acids or milk bacteria that may be present.

Milk and lactose diet exert a very important influence on the character of the intestinal bacteria, especially in white rats and in the common domestic fowl. Within a few days after the ingestion of milk or lactose a transformation of the flora takes place in which the usual mixed bacterial flora give way to ones that are more simplified, and in which Bacillus acidophilus and Bacillus bifidus are, as a rule, prominent. It is to be assumed that milk has this influence in virtue of the large amount of lactose which it contains. Other carbohydrates, besides milk sugar, failed to bring about such a transformation.

The ingestion of foreign bacteria, even in large numbers, does not of itself bring about an elimination or displacement of the common intestinal microorganisms. Vastly more important is the influence of diet, especially milk and lactose. The feeding of Bulgara

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tablets or other preparations which contain as the supposedly active agent the bacillus of Metchnikoff and Mazé, without due regard to the use of milk, can, therefore, be of little, if indeed of any, value. The beneficial effects which it is claimed have been derived from the use of yoghurt, and other oriental sour milk products have in all probability been due to the milk as such, rather than to the bacteria which they contained. This view is strongly supported by the extensive milk feeding experiments on chicks which are recorded in this paper, and also by the results which show the influence of milk and of lactose feeding on the intestinal flora of white rats and of the common domestic fowl.