A METHOD FOR THE QUANTITATIVE DETERMINATION OF FECAL BACTERIA.*

BY H. A. MATTILL AND P. B. HAWK.

(From the Laboratory of Physiological Chemistry of the University of Illinois, Urbana.)

The composition of feces, both of animals and of man, during fasting and on many different diets, has been determined frequently, but despite the many investigations there are widely different statements as to the nature and probable source of the substances found. The apparent uniformity in kind and quantity of fecal material resulting from different diets is a striking fact and has been explained in several different ways: that the feces are chiefly the unabsorbed residues of intestinal excretions (1) which might naturally vary but little, or that the feces consist chiefly of the bodies of dead and living bacteria (2), whose amount might also be fairly constant. Probably neither of these views is correct. It was shown by Strasburger (3) that ordinarily about one third of the dry substance of the feces consists of microorganisms, and that from one fourth to one half of the fecal nitrogen is bacterial in its origin.

The importance of the bacterial flora in the intestinal tract has been emphasized in a recent investigation by MacNeal, Latzer, and Kerr (4), and in investigations, reported almost simultaneously with this, by Sato (5) and by Berger and Tsuchiya (6). The first mentioned investigators examined 266 stools of twelve men, on a normal mixed diet, during a prolonged metabolism experiment. The results include microscopic differential counts by several methods, gravimetric determinations of bacterial substance and determinations of the ratio of bacterial nitrogen to total fecal nitrogen. By MacNeal's modification of Strasburger's method of fractional sedimentation, these investigators found the average daily output of dry bacteria to be 5.34 gm., the average daily bacterial dry substance to be 26.9 per cent. of the fecal dry substance, and the average daily bacterial nitrogen 46.3 per cent. of the total fecal nitrogen. They

* Presented in abstract at the New Haven meeting of the American Society of Biological Chemists, December, 1910. Received for publication, June 29, 1911.
conclude that direct quantitative determinations of fecal bacteria furnish evi-
dence of the extent and nature of the bacterial growth in the intestine, and that
this seems to be a delicate index of intestinal conditions.

In the opinion of Sato (5), an examination of the feces is as important as
that of the stomach contents, perhaps even more so. He examined thirty con-
secutive stools (mixed diet) by the method of Strasburger and found an average
daily excretion of dry bacteria equal to 8.54 gm. and an average of 24.39 per
cent. of dry bacteria in dry feces.

By the same method, the results of Berger and Tsuchiya (6) show very much
smaller values for these quantities; an average daily excretion of 3.023 gm. dry
bacteria, which form 12.6 per cent. of the dry feces. Tobaya (7) found a value of
11.22 per cent. for this ratio. The last mentioned workers consider the lower
results to be more accurate than those of Sato, Strasburger, and others, because
these investigators employed hand centrifuges, which are less rapid in their
action and less easily maintained under uniform control. None of these last
mentioned investigations include data on the nitrogen content of bacteria.

There are several methods for the quantitative determination of bacterial
substance gravimetrically, or rather several modifications of Strasburger’s
method of fractional sedimentation (2). According to the technique originally
used by this investigator, 2 c.c. of a fecal suspension are subjected to two serial
centrifugalizations to remove the non-bacterial solid material. The method of
Steele (8) involves a single centrifugalization of 5 c.c. of a fecal suspension,
followed by filtration through muslin to remove the remaining non-bacterial
solid matter. The method of MacNeal (4) begins with a 2 to 3 gm. sample of
fresh feces, which is brought into suspension in 0.2 per cent. hydrochloric acid in
a centrifuge tube and subjected to three serial centrifugalizations, or four if
necessary.

PURPOSE AND CONDITIONS OF THE EXPERIMENTS.

In the belief that the quantity of intestinal bacteria would furnish
evidence as to the comparative utilization, particularly of the pro-
tein material of the food, under different conditions, it was deemed
advisable to determine the nitrogen content of the bacterial portion
of each individual stool obtained throughout several metabolism
experiments. The object of these experiments was to discover the
influence of varying amounts of water taken with the meals upon
the digestion and absorption of food. Since the diet of the sub-
jects of these experiments was absolutely uniform from meal to
meal, any variation in the bacterial content of the feces could not
be attributed to the effect of different diets.

The subjects of the experiments and their diet and daily routine
have been more fully described elsewhere.1 The daily periods began
and ended at 7 A. M. after urinating and defecating. The three

meals were taken as regularly as possible each day at 7:30, 12:15, and 5:30; the meals were identical and consisted of graham crackers, butter, peanut butter, milk, and water, a diet containing but small amounts of cellulose.

Each stool was passed into a weighed porcelain dish which was then weighed again. The consistency of the stool determined the ease with which a uniform mixture could be obtained. Bone spatulas proved to be very satisfactory for breaking up and crushing the larger masses. This was done as quickly as possible to prevent loss by evaporation, which is very rapid. In obtaining the samples for analysis, the use of glass-stoppered weighing bottles and small porcelain spatulas was found most satisfactory. Weighing by difference was more accurate as well as more rapid than by the direct method.

BACTERIAL NITROGEN. THE SEPARATION OF BACTERIA FROM FECES.

The method which was adopted for this procedure and determination is a simplification of that of MacNeal. About two grams of feces are accurately weighed and placed in a fifty cubic centimeter centrifuge tube. To the feces in the tube a few drops of 0.2 per cent. hydrochloric acid are added, and the material is mixed to a smooth paste by means of a glass rod. Further amounts of the acid are added with continued crushing and stirring until the material is thoroughly suspended. The tube is then whirled in the centrifuge at high speed for one half to one minute. The suspension is found sedimented into more or less definite layers, the uppermost of which is fairly free from the larger particles. The upper and more liquid portion of the suspension is now drawn off by means of a pipette and transferred to a beaker. The sediment remaining in the tube is again rubbed up with the glass rod with the addition of further amounts of dilute acid, and again centrifugalized for one half to one minute. The supernatant liquid is pipetted off and added to the first, the same pipette being used for

2 A 25 cubic centimeter pipette is the most satisfactory size; to facilitate observation, the delivery tube is bent near the bulb to an angle of about 120 degrees.
Quantitative Determination of Fecal Bacteria.

the one determination throughout. A third portion of the dilute acid is then added to the sediment, which is again mixed by stirring and again centrifugalized. All the washings are added to the first one, and during the process care is taken to wash the material from the walls and mouth of the centrifuge tube down into it. Finally, when the sediment is sufficiently free from bacteria, the various remaining particles are visibly clean, and the supernatant liquid after centrifugalization remains almost clear. This is removed to the beaker in which are now practically all the bacteria present in the original portion of feces, together with some solid matter not yet separated. In the centrifuge tubes there is a considerable amount of bacteria-free solid matter. This sediment and the others following are all combined in a Kjeldahl flask and their nitrogen content is determined. It has been called the residue nitrogen, and it represents undigested and insoluble nitrogen.

The suspension is now transferred to the same centrifuge tube, centrifugalized for a minute, and the supernatant liquid transferred to a clean beaker by means of the same pipette. The tube is then refilled from the first beaker and thus all the suspension centrifugalized a second time. The beaker is finally carefully washed with the aid of a rubber-tipped glass rod, the second sediment in the centrifuge tube is washed free of bacteria by means of this wash water and by successive portions of the dilute acid, and the supernatant liquid after centrifugalization is added to the contents of the second beaker. The second clean sediment is added to the first. The bacterial suspension now in the second beaker is again centrifugalized in the same way and a third portion of bacteria-free sediment is separated. Frequently a fourth serial centrifugalization is performed,—always if the third sediment is of appreciable quantity. At all stages of the separation, small portions of the dilute hydrochloric acid are used, so that the final suspension shall not be too voluminous. Ordinarily it amounts to 125 to 200 cubic centimeters. At the same time, the final amount of fluid should not be too small, as shown by Ehrenpfordt (9), because the vis-

A convenient support for the pipettes is a wire spring on a glass base, such as is used on a desk for pen-holders. The delivery tube, just where it is bent, is inserted between the wires, and any liquid not delivered collects in the bend of the tube.
To the final bacterial suspension an equal volume of alcohol is added and the beaker set aside to concentrate. A water bath at 50° to 60° C. is very satisfactory. After two or three days, when the liquid is concentrated to about fifty cubic centimeters, the beaker is removed and about 200 cubic centimeters of alcohol are added. The beaker is covered and allowed to stand at room temperature for twenty-four hours. At the end of this time the bacterial substance is generally settled, so that most of the clear supernatant liquid, of dark brown color, can be directly siphoned off without loss of solid matter. The remainder is then transferred to centrifuge tubes, centrifugalized, and the remaining clear liquid pipetted off. The sediment consists of the bodies of the bacteria, and is transferred to a Kjeldahl flask for nitrogen determination. This is the bacterial nitrogen. Where a determination of bacterial dry substance is desired, the sediment of bacteria is extracted by absolute alcohol and ether in succession, transferred to a weighed porcelain crucible, and dried at 102° C. to constant weight. This dried sample is then used in the nitrogen determination. Our procedure differs from that of MacNeal in that the bacterial dry matter is not determined. A saving of about seven days’ time and of considerable labor is accomplished by this omission.

The alcohol which is siphoned off, the last portions of which are separated from the bacteria by centrifugalization, contains such nitrogen as has been extracted from the bacteria and other fecal material, and, in addition, all fecal nitrogen which is soluble in 0.2 per cent. hydrochloric acid or which has become so during the time of manipulation. This comprises all unabsorbed soluble digestion products, the soluble nitrogen of the digestive juices and bile pigments, and the nitrogenous extractives of the bacteria. The alcohol precipitate may contain, in addition to bacterial substance, protein material which is not bacterial in its origin, such as mucin, and the application of this method to pathological stools of such a nature that the amount of this substance is increased, would involve a correction on this account. This is true for all the methods in which a precipitation by alcohol is employed.
investigation upon the accuracy of Strasburger's method, Ehrenpfordt (9) attempted, by various means, to eliminate the soluble protein precipitable by alcohol, but he was not successful. He suggests that the final large dilution of an originally small sample would practically eliminate this source of error.

When the extraction by alcohol was not performed and nitrogen was determined directly on the supernatant fluid after the last sedimentation, the amount of nitrogen was considerably higher. It was, in fact, but little below the total fecal nitrogen and bore a constant ratio to it. This shows that the alcohol extraction does remove nitrogenous substances,—an action which MacNeal, Latzer, and Kerr (10) have thought to be not impossible, although they say the amount of nitrogen in dry bacterial substance is ten to fifteen per cent. greater when calculated upon extracted residue. Since the method herein described does not include a determination of dry bacterial substance, it is not possible to show from the data whether this is true here. Most frequently, in investigations upon bacterial proteins, the substance employed is the bacterial residue after extraction with alcohol and ether.

The same investigators (10) found the nitrogen content of the dry bacterial residues to vary from ten to twelve per cent. with an average of 10.96 per cent., a value which they verified on pure cultures, and which agrees with the values found by other investigators, among them Cramer (11) and Leach (12). In an examination of pure cultures prepared on a large scale, Nicolle and Alilaire (13) found the nitrogen content of *Bacillus coli* to be 10.32 per cent. In view of the susceptibility of microorganisms and their prompt adaptation to their surroundings, results obtained under other than natural conditions have little value in this connection. A value of 10.97 per cent. for the average nitrogen content of dried extracted fecal bacteria was obtained by Fowler and Hawk (14), who used the method of MacNeal for the determination. Inasmuch as it has been shown by various investigators that such bacteria as are present in the feces contain on the average about 11 per cent. of nitrogen, the values for bacterial nitrogen as determined by our method may conveniently serve as a basis for the calculation of the actual output of bacterial substance.
For the following tables, which include data on total fecal nitrogen and on bacterial nitrogen by our method, we have calculated the bacterial dry matter of each stool on the basis of a nitrogen content of 10.96 per cent. Also for purposes of comparison, the amount of dry bacterial substance in fecal dry substance has been calculated.

**DISCUSSION OF RESULTS.**

Of the sixty-six stools completely examined in this way, the data on twenty-three are given as representative. The averages are on the basis of the fecal excretion per day throughout the entire period.

**TABLE I.**

*Bacterial Nitrogen. Subject W.*

<table>
<thead>
<tr>
<th>Number of stool</th>
<th>Weight of stool in gm.</th>
<th>Per cent. dry matter</th>
<th>Amount dry matter in gm.</th>
<th>Fecal nitrogen in gm.</th>
<th>Bacterial dry substance (calculated) in gm.</th>
<th>Per cent. dry bacteria in fecal nitrogen</th>
<th>Per cent. bacterial nitrogen in fecal nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>62.8</td>
<td>25.61</td>
<td>16.1</td>
<td>0.845</td>
<td>0.483</td>
<td>4.494</td>
<td>27.49</td>
</tr>
<tr>
<td>8</td>
<td>98.8</td>
<td>26.72</td>
<td>26.4</td>
<td>1.265</td>
<td>0.693</td>
<td>6.319</td>
<td>60.39</td>
</tr>
<tr>
<td>11</td>
<td>41.8</td>
<td>31.23</td>
<td>13.1</td>
<td>0.673</td>
<td>0.287</td>
<td>2.616</td>
<td>20.05</td>
</tr>
<tr>
<td>12</td>
<td>104.9</td>
<td>24.28</td>
<td>25.5</td>
<td>1.254</td>
<td>0.590</td>
<td>5.378</td>
<td>21.11</td>
</tr>
<tr>
<td>15</td>
<td>147.5</td>
<td>27.52</td>
<td>40.6</td>
<td>2.044</td>
<td>0.984</td>
<td>8.977</td>
<td>22.12</td>
</tr>
<tr>
<td>16</td>
<td>75.4</td>
<td>26.20</td>
<td>19.8</td>
<td>1.029</td>
<td>0.563</td>
<td>5.131</td>
<td>25.97</td>
</tr>
<tr>
<td>17</td>
<td>144.8</td>
<td>27.45</td>
<td>39.8</td>
<td>2.044</td>
<td>1.087</td>
<td>9.973</td>
<td>24.96</td>
</tr>
<tr>
<td>21</td>
<td>166.0</td>
<td>26.32</td>
<td>44.8</td>
<td>2.355</td>
<td>1.298</td>
<td>11.84</td>
<td>26.43</td>
</tr>
<tr>
<td>27</td>
<td>142.7</td>
<td>25.05</td>
<td>35.8</td>
<td>1.845</td>
<td>1.156</td>
<td>10.58</td>
<td>29.57</td>
</tr>
<tr>
<td>28</td>
<td>81.4</td>
<td>27.06</td>
<td>22.8</td>
<td>1.238</td>
<td>0.758</td>
<td>6.914</td>
<td>30.33</td>
</tr>
<tr>
<td>31</td>
<td>136.0</td>
<td>25.5</td>
<td>34.6</td>
<td>1.895</td>
<td>1.072</td>
<td>9.775</td>
<td>28.24</td>
</tr>
</tbody>
</table>

Average daily excretion of dry bacteria

6.07  26.0  54.1

In the data of table I, subject W, the calculated percentage of dry bacteria in dry feces varies from 20.05 per cent. to 30.33 per cent., with an average value of 26.0 per cent. The percentage of fecal nitrogen found existing as bacterial nitrogen varies from 42.6 per cent. to 62.9 per cent., with an average of 54.1 per cent.

The data of table II, subject E, show the calculated percentages of dry bacteria in dry feces to vary from 24.45 per cent. to 35.29 per cent., with an average of 29.9 per cent. The percentage of fecal nitrogen existing as bacterial nitrogen varies from 45.4 per cent. to 61.5 per cent., the average being 53.7 per cent.

A closer examination of the tables reveals no relation between the percentage of dry matter and the percentage of dry bacteria in dry...
Quantitative Determination of Fecal Bacteria.

TABLE II.

Bacterial Nitrogen. Subject E.

<table>
<thead>
<tr>
<th>Number of stool</th>
<th>Weight of stool in gm.</th>
<th>Per cent. dry matter</th>
<th>Amount dry matter in gm.</th>
<th>Fecal nitrogen in gm.</th>
<th>Bacterial nitrogen in gm.</th>
<th>Bacterial dry substance (calculated) in gm.</th>
<th>Per cent. dry bacteria in dry feces</th>
<th>Per cent. bacterial nitrogen in fecal nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>124.6</td>
<td>22.30</td>
<td>27.8</td>
<td>1.744</td>
<td>0.987</td>
<td>9.005</td>
<td>32.36</td>
<td>66.6</td>
</tr>
<tr>
<td>11</td>
<td>63.8</td>
<td>20.10</td>
<td>18.6</td>
<td>1.158</td>
<td>0.685</td>
<td>6.250</td>
<td>33.01</td>
<td>59.2</td>
</tr>
<tr>
<td>12</td>
<td>159.0</td>
<td>24.30</td>
<td>41.1</td>
<td>2.577</td>
<td>1.372</td>
<td>13.52</td>
<td>20.45</td>
<td>53.3</td>
</tr>
<tr>
<td>14</td>
<td>135.3</td>
<td>22.06</td>
<td>31.1</td>
<td>1.896</td>
<td>1.019</td>
<td>9.200</td>
<td>29.87</td>
<td>53.3</td>
</tr>
<tr>
<td>15</td>
<td>192.4</td>
<td>19.58</td>
<td>37.7</td>
<td>2.389</td>
<td>1.083</td>
<td>9.880</td>
<td>26.21</td>
<td>45.4</td>
</tr>
<tr>
<td>16</td>
<td>79.2</td>
<td>24.58</td>
<td>19.5</td>
<td>1.144</td>
<td>0.523</td>
<td>4.750</td>
<td>24.45</td>
<td>45.7</td>
</tr>
<tr>
<td>22</td>
<td>147.7</td>
<td>21.19</td>
<td>24.3</td>
<td>2.045</td>
<td>1.068</td>
<td>6.742</td>
<td>28.40</td>
<td>52.2</td>
</tr>
<tr>
<td>b1</td>
<td>35.2</td>
<td>28.88</td>
<td>10.2</td>
<td>0.572</td>
<td>0.397</td>
<td>2.709</td>
<td>27.45</td>
<td>53.7</td>
</tr>
<tr>
<td>b3</td>
<td>202.2</td>
<td>24.05</td>
<td>19.89</td>
<td>3.036</td>
<td>1.715</td>
<td>15.64</td>
<td>31.41</td>
<td>56.5</td>
</tr>
<tr>
<td>b 8</td>
<td>18.6</td>
<td>27.14</td>
<td>24.9</td>
<td>0.591</td>
<td>0.264</td>
<td>2.317</td>
<td>35.29</td>
<td>61.5</td>
</tr>
<tr>
<td>b12</td>
<td>25.8</td>
<td>14.51</td>
<td>38.2</td>
<td>2.250</td>
<td>1.082</td>
<td>9.860</td>
<td>25.81</td>
<td>48.1</td>
</tr>
<tr>
<td>b17</td>
<td>50.6</td>
<td>11.06</td>
<td>5.6</td>
<td>0.340</td>
<td>0.159</td>
<td>1.454</td>
<td>25.96</td>
<td>47.0</td>
</tr>
</tbody>
</table>

Average daily excretion of dry bacteria ................................. 9.57 29.9 53.7
Average daily excretion of dry bacteria, subject W (table I) .............. 6.97 26.0 54.1
Average daily excretion of dry bacteria, subjects W and E .................. 8.27 27.95 53.9

feces, but such a relation is highly improbable, since it has been shown that bacteria do not possess a typical water content (15), (16), (17). With but a few exceptions, however, the percentage of dry bacteria in dry feces varies in the same way as the percentage of bacterial nitrogen in fecal nitrogen. This means that on a uniform diet the bacterial nitrogen of the feces varies, in general, in the same way as the amount of dry matter in the feces. The same statement can also be made regarding the fecal nitrogen in its relation to total dry matter. It is thus shown to be unnecessary to obtain the dry bacteria as a preliminary step in the determination of their nitrogen content.

Combining the data from both tables, the bacterial dry substance constitutes 20.05 to 35.29 per cent. of the fecal dry substance, with an average of 27.95 per cent. MacNeal, Latzer, and Kerr (18) found the variation to be from 14.03 to 42.53 per cent., with an average value of 26.0 per cent. dry bacterial substance in dry feces, a value about 1 per cent. lower than our own. The combined data show a variation of 42.6 to 62.9 per cent. for the portion of bacterial fecal nitrogen, the average value being 53.9 per cent. The investigators just mentioned (19) found the variations in this quan-
tity to be from 23.3 to 66.8 per cent., with an average of 46.3 per cent. While our values show smaller variations, they are uniformly higher, the average being 7.6 per cent. more than in the investigations of MacNeal, Latzer, and Kerr.

Since in the determination of dry bacterial substance, a factor obtained by other investigators was used, our results upon the daily output of dry bacterial substance have not the value of original determinations, although from the uniform results of several investigators upon the nitrogen content of dry bacteria there seems to be a reasonable basis for calculation. Among the determinations of the daily excretion of dry bacteria are the following (previously mentioned):

<table>
<thead>
<tr>
<th>Grams</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Strasburger</td>
<td>8.0</td>
</tr>
<tr>
<td>Sato</td>
<td>8.54</td>
</tr>
<tr>
<td>Berger and Tsuchiya</td>
<td>3.023</td>
</tr>
<tr>
<td>MacNeal, Latzer, and Kerr</td>
<td>5.34</td>
</tr>
<tr>
<td>Mattill and Hawk</td>
<td>8.27</td>
</tr>
</tbody>
</table>

The data on the percentage of dry bacteria in dry feces include the following:

<table>
<thead>
<tr>
<th>Per cent.</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Strasburger</td>
<td>24.3</td>
</tr>
<tr>
<td>Schittenhelm and Tollens</td>
<td>42.0</td>
</tr>
<tr>
<td>Lissauer*</td>
<td>8.67</td>
</tr>
<tr>
<td>Tobaya</td>
<td>11.22</td>
</tr>
<tr>
<td>Sato</td>
<td>24.39</td>
</tr>
<tr>
<td>Berger and Tsuchiya</td>
<td>12.6</td>
</tr>
<tr>
<td>MacNeal, Latzer, and Kerr</td>
<td>26.9</td>
</tr>
<tr>
<td>Mattill and Hawk</td>
<td>27.95</td>
</tr>
</tbody>
</table>

These widely different values have all been obtained by Strasburger's method, or by modifications thereof. It is claimed by Sato (5), as well as by Ehrenpfordt (9), that in normal individuals passing normal stools, diet has no appreciable effect on the quantity of bacteria excreted. All the variations shown above are caused, according to Ehrenpfordt (9), by differences in the centrifugalization procedure. A two minute centrifugalization causes non-bacterial matter to be left in suspension, and gives high results

*Quoted by Ehrenpfordt, loc. cit.
Quantitative Determination of Fecal Bacteria.

(Strasburger, Sato), while a ten minute centrifugation by a precipitation of considerable bacteria along with the non-bacterial matter causes low results (Tobaya, and Berger and Tsuchiya). Ehrenpfordt claims that an absolute separation by centrifugation is not possible, but that the more accurate values probably lie between those given and are more nearly obtained by an intermediate centrifugation time, i.e., about five minutes.

It should be emphasized in this connection that the technique of serial centrifugation, in which a two minute period is used, will, if properly conducted, obviate to a large degree the difficulty mentioned by Ehrenpfordt. Furthermore, the fact that different observers do not agree does not invalidate the gravimetric method as compared with the counting methods, and a given procedure in the hands of one observer yields entirely comparable results.

Notwithstanding the negative conclusions regarding the effect of diet, a possible reason for our uniformly higher results in the percentage of bacterial nitrogen in fecal nitrogen is suggested by the findings of Herter and Kendall (20). These investigators found in experiments on kittens and monkeys that frequent alternations in the chemical nature of the diet were beneficial, since they interfered with the establishment of any one type of bacteria in the intestine and thereby diminished intestinal putrefaction. The subjects of our experiments were on an absolutely uniform diet both as to kind and quantity of food.

In addition to a possible uniform difference in the procedure of sedimentation, it is to be recalled that Strasburger's original method, as well as most of its modifications, includes alcohol and ether extraction, while our method involves only an alcohol extraction. Any ether-soluble nitrogenous substances, such as lecithin and other lipoids, would not be extracted as completely by our method as by the other methods, and without any values as to the weight of our dried bacteria it can not be stated whether the nitrogen values on the basis of dry bacteria are greater or less when these are extracted merely with alcohol, than when both alcohol and ether are used.

Since bacterial nitrogen is desired, it seems more reasonable to avoid as much as possible the removal by solvents of such nitrog-
H. A. Mattill and P. B. Hawk.

Endogenous substances as are normal constituents of bacteria. On this basis the higher values obtained by our method are probably nearer the true value for "bacterial" nitrogen than where the organisms are subjected to ether extraction.

This method will also be applied to the determination of bacteria in the feces of herbivora, where the diet, although not so completely available contains a large amount of cellulose.

SUMMARY.

From results of which the foregoing data form a part, it appears that the amount of bacterial nitrogen in the feces is a valuable index to intestinal conditions, and the method herein described is a simple and satisfactory one for making this determination. It involves three serial centrifugalizations of a two gram sample of the fresh feces brought into suspension in 0.2 per cent. hydrochloric acid. The bacterial suspension finally obtained is concentrated and extracted by alcohol, and nitrogen is determined in the precipitated material. The complete data on a given stool can be obtained in about five days, and one operator can take care of three or four stools in duplicate in one day.

On an absolutely uniform diet of simple and easily digested food during a period of three to four weeks, the average amount of bacterial nitrogen in two subjects was found to be 53.9 per cent. of the total fecal nitrogen, and this percentage, though higher than that obtained by workers heretofore, is probably more nearly a true value for bacterial nitrogen, because no ether extraction was employed.

The average daily amount of dry bacteria, calculated on the basis of the nitrogen values, is 8.27 grams.

BIBLIOGRAPHY.

Quantitative Determination of Fecal Bacteria.