[FROM THE LABORATORIES OF PHYSIOLOGICAL CHEMISTRY OF THE UNIVERSITY OF ILLINOIS AND OF JEFFERSON MEDICAL COLLEGE.]

FASTING STUDIES. XIII. THE OUTPUT OF FECAL BACTERIA AS INFLUENCED BY FASTING AND BY LOW AND HIGH PROTEIN INTAKE.

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So far as we are aware, the influence which fasting exerts upon the output of fecal bacteria has not been studied. Schmidt and Strasburger[1] state that during fasting the output of dry bacteria is one and one-third gram per day. No data are submitted. The amount of fecal bacteria excreted per day during a fast should, of course, decrease because of the withdrawal of food and the consequent absence of the products of the digestion of such food from the contents of the intestine. However, one would not expect a complete cessation of bacterial growth, inasmuch as albuminous secretions continue to be poured into the lumen of the intestine even during a fasting interval.[2]

The course of intestinal putrefaction as measured by the urinary indican excretion has been followed in a few cases. One of the first experiments of this sort was that made by Müller[3] upon the professional faster Cetti. He found no indican in the urine after the third day of fasting. The procedures for the detection of indican were not fully developed at that time. One would naturally expect to find indican in the urine of a fasting individual because of the fact mentioned above, that secretions and juices containing protein material are poured into the intestine even during periods of inanition. Baumstark and Mohr[4] also report the absence of indican in the urine of a fasting dog after the elimination of the "hunger feces." In our experience, fasting feces continue to be passed throughout a fasting interval of over 100 days.[5] Experiments made in our laboratory have also shown a pronounced decrease in the indican output of a 76 kilogram man during a seven-day period of inanition.[6]

Description.

Data as to subject, diet and experimental periods are given in a previous paper.[7] A description of methods is also given in another connection.[8]

[1] "Die Fäces des Menschen," p. 286.
[2] Mosenthal, *Proc. Soc. Exp. Biol. Med.*, **8**, 40 (1910).
[3] *Berl. klin. Woch.*, **24**, 433 (1887); also *Virchow's Arch.*, p. 133, supplement (1893).
[4] *Z. exp. Path. Ther.*, **3**, 687 (1906).
[5] Howe, Mattill and Hawk, *J. Biol. Chem.*, **11**, 103 (1912).
[6] Sherwin and Hawk, *J. Biol. Chem.*, **11**, 169 (1912).
[7] Wilson and Hawk, THIS JOURNAL, **36**, 137 (1914).
[8] Blatherwick and Hawk, *Biochemical Bulletin*, **3**, No. 9, 1913.

Discussion of Results.

During the preliminary period of four days (21.86 grams of nitrogen per day), the average daily excretion of fecal nitrogen was 2.815 grams (see Table I). Of this amount 1.571 grams or 55.82% was bacterial nitrogen. The actual excretion of bacteria was 14.34 grams per day, constituting 34.03% of the dry feces.

TABLE I.

Subject E—Fast.

Stool No.	Weight of stool. Grams.	% dry matter.	Wt. dry matter. Grams.	Fecal N. Grams.	Bacterial N. Grams.	Bact. dry matter. Grams.	% dry bact. in dry feces. Grams.	Bact. N in fecal N %.
Preliminary Period (4 Days).								
1	97.0	22.23	21.56	1.412	0.752	6.861	31.82	53.26
2	166.0	22.65	37.60	2.368	1.351	12.327	32.78	57.05
3	184.5	23.34	42.94	2.891	1.513	13.805	32.15	52.33
4	215.0	22.31	47.96	3.081	1.747	15.940	33.24	56.70
5	35.5	26.55	9.43	0.614	0.379	3.458	36.69	61.73
6	31.0	29.12	9.03	0.893	0.543	4.954	54.88	60.81
Total	729.0	...	168.52	11.259	6.285	57.345	...	...
Average	182.3	23.12	42.13	2.815	1.571	14.336	34.03	55.82
Fasting Period (7 Days).								
1	81.0	11.34	9.185	1.504	0.352	3.212	34.97	23.40
2	38.5	19.47	7.495	0.682	0.354	3.230	43.10	51.91
Total	119.5	...	16.68	2.186	0.706	6.442	...	...
Average	17.1	13.99	2.38	0.312	0.101	0.920	38.62	32.29
Low Protein (4 Days).								
1	44.0	18.28	8.043	0.633	0.358	3.266	40.61	56.55
2	19.0	8.98	1.706	0.143	0.075	0.684	40.09	46.05
Total	63.0	...	9.75	0.776	0.433	3.950	...	...
Average	15.75	15.48	2.44	0.194	0.108	0.988	40.51	55.80
High Protein (5 Days).								
1	192.5	21.61	41.59	2.779	1.564	14.270	34.31	56.28
2	230.0	14.76	33.94	2.210	0.944	8.613	25.38	42.71
3	86.5	19.95	17.25	1.127	0.589	5.374	31.15	52.26
4	135.0	22.60	30.51	2.014	1.114	10.164	33.31	55.31
5	180.5	22.79	41.13	2.659	1.549	14.133	34.36	58.25
6	48.5	25.44	12.34	0.820	0.490	4.471	36.23	59.76
Total	873.0	...	176.76	11.609	6.250	57.025	...	...
Average	174.6	20.25	35.35	2.322	1.25	11.405	32.26	53.84

The feces of the fasting period were quite readily separated from those of the other periods because of the different character of the stools. Neither carmine nor charcoal was utilized in making the separation. The feces passed during the fasting interval (2 stools) were more fluid in character and darker in color than the feces resulting from the cracker-milk diet of the feeding periods. The fasting feces when examined macroscopically apparently consisted principally of mucous and residues of secretions. Microscopical examination demonstrated large numbers of bacteria.

The bacterial suspension, however, had a much different appearance than did that of the stools passed during feeding. After adding the alcohol the supernatant liquid assumed a reddish green tinge in contrast to the customary brown color seen in such suspensions. When the bacterial substance had settled it also possessed a greenish tinge. Bile may have been a contributing factor in this color variation.

The weight of fecal nitrogen excreted per day during the fast was 0.312 gram, of which 0.101 gram or 32.29% was of bacterial origin. The actual output of dry bacteria per day was but 0.92 gram, a most marked decrease from the daily value of 14.34 grams observed during the preliminary feeding interval.

Following the fast, the subject was fed a low-protein (5.23 grams of nitrogen per day) diet for a period of four days. Two stools were collected during this interval. The amount of fecal nitrogen excreted daily was 0.194 gram, of which 0.108 or 55.80% was of bacterial origin. The average daily excretion of dry bacteria was 0.988 gram or 40.51% of the total fecal (dry) output.

The original high-protein diet of the preliminary period was now resumed. The average weight of fecal nitrogen passed per day during this five-day period was 2.322 grams. Of this total excretion 55.84% or 1.250 grams was bacterial nitrogen. The actual output of bacterial substance was 11.405 grams or 32.26% of the dry feces.

When the four parts of the experiment are compared, the variations in the bacterial data are very striking. The amount of bacterial dry substance excreted per day, for example, was 14.336 grams during the preliminary period, 0.92 gram for the fast, 0.988 gram for the period of low-protein ingestion and 11.405 grams for the final high-protein period. Similar variations were observed in the bacterial nitrogen values. The very marked decrease in bacterial values during the fasting interval was expected because of the absence of food digestion products in the lower intestine. That the bacterial output for the low protein period was about the same as that for the fasting interval is significant. That there was no greater bacterial development when 5.23 grams of nitrogen were daily introduced into the body than when *no nitrogen* was introduced may be taken as evidence of very thorough utilization of the food nitrogen, thus leaving no extra pabulum for the microörganisms. The fact that the fecal nitrogen values, both total and bacterial, were not so great during the final period as during the preliminary period, although an identical diet was ingested in each instance, may also be taken as evidence of a better utilization of the protein constituents of the diet following the fast. Undoubtedly the need of nitrogen to make good the tissue destroyed during the fast played an important part in producing the better food utilization.

It will be noted that the percentage of dry bacteria in dry feces was increased during the fasting and low-protein periods. This was to be expected, since the ingestion of large amounts of protein (21.86 grams of nitrogen) would increase the amount of undigested and indigestible residues, while the increase of bacteria would not be relatively as great.

It is interesting to note that the feces passed during fasting contain smaller numbers of bacteria per day than during a period of normal feeding, but that the fasting feces contain a larger relative proportion of bacteria than do feces passed during such an interval of feeding.

Bacteria versus Indican.—In the appended table (II) will be found a comparison of the data for the excretion of fecal bacteria and urinary indican by the subject of the present investigation. The indican values are taken from data reported by Sherwin and Hawk.[1]

TABLE II.—RELATIONSHIP OF FECAL BACTERIAL NITROGEN TO URINARY INDICAN OUTPUT.

	Experimental period.			
Constituent determined.	Preliminary.	Fasting.	Low-protein. Excretion per day.	High-protein.
Indican (mgs.)	48.9	32.9	74.1	79.6
Bacterial nitrogen (grams)	1.571	0.101	0.108	1.250

It will be noted that the indican values are very high for the low-protein period following the fast. This phenomenon is difficult of explanation. If this high indican value is to be interpreted as being due to increased putrefaction, brought about by less complete absorption of protein from the intestine, it would be logical to expect that the total nitrogen of the feces would be proportionately increased. However, our fecal nitrogen data (Table I) show no such increase. The daily output of fecal nitrogen for the preliminary period was 2.815 grams, whereas the daily output for the period of low-protein ingestion subsequent to the fast was but 0.194 gram. In other words, the ratio between the nitrogen of the diets was as 4 : 1 while the ratio between the resultant fecal nitrogen values was as 14.5 : 1. It is apparent, therefore, that instead of being relatively increased, the nitrogen in the feces has undergone a very pronounced *relative decrease*. This finding of itself, considered apart from all associated factors, might be interpreted as indicative of a much more satisfactory absorption during the period of low-protein ingestion, subsequent to the fast.

Another surprising observation in this connection is the fact that the actual average daily output of bacteria during the low-protein feeding period was only about $^1/_{14}$ as great as during the preliminary interval. That increased putrefaction should come coincidently with a decrease in the bacteria value to approximately $^1/_{14}$ of the original, is certainly a surprising observation. What factors may have been instrumental in bring-

[1] Sherwin and Hawk, *J. Biol. Chem.*, 11, 169 (1912).

ing about such a condition of affairs? In the first place it is entirely possible that the intestinal flora before the fast, although highly developed as was indicated by an average daily output approximating 14 grams, was nevertheless made up in large part of non-indole-forming bacteria. They may, in turn, have been relatively inactive in so far as the actual formation of indole was concerned. Under such conditions then, we would have a low indole output from an intestinal flora which was copious in quantity. A condition such as this could, of course, be brought about by proper manipulation of the diet.[1] In the experiments from this laboratory under consideration, however, the conditions were such as to lead one to expect a pronounced intestinal putrefaction at the very point where the low indican value was obtained, *i. e.*, on the high-protein diet. We could further cite, from the data thus far obtained from experimentation upon subject E in this and other connections, facts which seem to indicate that there is, of necessity, no uniform relationship between the urinary indican excretion and the fecal bacteria output under a similar dietary régime.

During the fast it is possible that certain indole-forming organisms formed a progressively increasing proportion of the intestinal flora due to their greater resistance to the rigors of the fasting régime. Therefore, at the end of the fast, although there was an actual reduction in the mass of the flora, these indole-formers may have made up the major portion. Unhampered by the presence and activities of other types of microorganisms these active indole-organisms were able to bring about a very rapid formation of indole and the stimulated absorptive mechanism caused this indole to be very completely absorbed and rapidly put through the subsequent detoxicating process which yielded the urinary indican. These factors may have played an important part in the peculiar relationship which we note between fecal bacteria and urinary indican output.

Conclusions.

By means of a seven-day fast the daily excretion of fecal bacterial nitrogen by a 76 kilogram man (E) was reduced from 1.571 grams to 0.101 gram, whereas the actual weight of the excreted bacterial substance was reduced from 14.336 grams to 0.920 gram per day. The percentage of the fecal nitrogen which was present as bacterial nitrogen was decreased from 55.82% to 32.29% as a result of the fast. The percentage of dry bacteria in dry feces was slightly increased.

The output of bacterial nitrogen and the output of bacterial substance were approximately the same on a low-protein diet as during fasting. With the ingestion of a high-protein diet these values underwent an immediate and pronounced increase.

[1] Kendall, *J. Med. Res.*, **29**, 411 (1911).

The percentage of the fecal nitrogen which was composed of bacterial nitrogen was about the same in the periods of low- and high-protein ingestion.

There was no definite relationship between the excretion of fecal bacteria and that of urinary indican.

The ingestion of 5.23 grams of nitrogen *after* the fast was followed by an excretion of fecal bacteria which was only $^1/_{14}$ as great as when four times that amount of nitrogen was ingested *before* the fast.

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STUDIES ON WATER DRINKING. XVI. THE INFLUENCE OF DISTILLED WATER DRINKING WITH MEALS UPON FAT AND CARBOHYDRATE UTILIZATION.

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So far as we are aware, the only study of the utilization of fat and carbohydrate, as influenced by the ingestion of large volumes of water at meal time, is that reported by Mattill and Hawk.[1] In the tests cited in that report the subjects were young men and the water employed was a freshly prepared *softened* water. A somewhat better utilization was secured when large volumes of water were taken with meals. The variations, however, were not very marked except in the case of the fat utilization by one subject. In this case, the percentage utilization was increased from 94.3% to 96.5% as a result of high water intake.

The statement is frequently made that distilled water ingestion, because of the absence of electrolytes in such water, has a harmful influence upon the animal body. In this connection, Findlay[2] says that the ingestion of distilled water is followed by the swelling of the surface layers of the gastric epithelium and that "salts also pass out, and the cells may die and be cast off. This may lead to catarrh of the stomach." Harlow[3] and Koeppe[4] also believe that catarrh of the stomach may follow the ingestion of distilled water (iced). Nocht[5] and Winkler[6] report experiments which failed to demonstrate that any bad effect followed the drinking of distilled water. Spitta[7] believes that we have, as yet, no definite

[1] *J. Am. Chem. Soc.*, **33**, 1978 (1911).

[2] "Physical Chemistry and its Applications in Medical and Biological Science," London, **1905**.

[3] Quoted by Oehler.

[4] *Deut. med. Wochschr.*, **1898**, 624.

[5] *Hyg. Rundschau.*, **1892**, 273.

[6] *Z. physikal. diät. Ther.*, **8**, 671 (1905).

[7] "Rubner's Handbuch Hygiene," Leipzig, **1911**, 28.