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STUDIES ON WATER DRINKING: IX. THE DISTRIBUTION OF BACTERIAL AND OTHER FORMS OF FECAL NITROGEN AND THE UTILIZATION OF INGESTED PROTEIN UNDER THE INFLUENCE OF COPIOUS AND MODERATE WATER DRINKING WITH MEALS.

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Introduction.

By far the larger part of the organic material eliminated in the feces is of unknown nature and composition. A knowledge of the source of fecal material is thereby made the more difficult to obtain. Three sources are usually considered as contributing to the nitrogenous material excreted as feces: (1) food residues, (2) residues of the digestive juices and cellular material from the intestinal wall, (3) bacteria and their products. Each of these in turn has been emphasized as the principal contributing agent, but no attempt seems ever to have been made to determin the "nitrogen partition" in the feces.

That the food residues of an available diet form any considerable part of the excreted material has had to be denied since the early work of Voit on fasting feces. In feeding experiments on dogs this investigator[1] showed that the amount of nitrogen in the feces was not proportional to the amount of meat fed. No muscle fibers or protein could be detected in the feces. Voit[2] showed that the material produced in an isolated loop of the intestin of a dog was of a similar composition and contained the same amount of nitrogen as the feces of the normal intestin through which food was passing. Prausnitz,[3] in experiments on men, showed that the composition of the feces varied with the diet and gave a definition of normal feces as those resulting from the eating of any food that is completely digested and absorbed. His data also show that the amount of nitrogen in the feces is uninfluenced by the amount in the food, although Schierbeck[4] finds considerable variation in this respect. Rubner[5] found that in man the amount of feces and its nitrogen content are determined entirely by the cellulose content of the diet. In the same way he found that on a milk diet the resulting fecal mass was almost directly proportional to the quantity of milk ingested.

It is entirely probable that on a diet whose constituents are not entirely available the amount of feces is increased by the undigested cellulose, and the nitrogen content is increased by the larger amount of digestive juices secreted because of the larger volume of food and the accompanying increased peristalsis.

About one-third of the dry matter of human feces consists of bacteria, and at least one-half of the nitrogen of feces is bacterial in its origin.[6,7,8]

Little is known as to the conditions upon which the growth of the intestinal flora depends. In herbivora, whose food materials contain large amounts of cellulose, the presence of organisms that bring about the decomposition and utilization of this substance is an advantage. Even so, only 45 per cent. of the energy of such food is utilized. In the intestin of carnivora the existing micro-organisms are limited to unabsorbed protein and the residue of the digestive juices for their food supply. In man, living upon a diet that contains food material of both the available and the unavailable kind, a condition midway between might be expected, just as the relative length of the intestin lies between that of herbivora and carnivora.

A recent claim of Schottelius[11] is that the presence of bacteria in the intestin of vertebrates is a desirable condition. Among their functions are the following: to prepare the food for absorption, to stimulate peristalsis, to inhibit the growth of pathogenic bacteria, and to render the body immune to bacterial poisons and to pathogenic organisms in general. The investigations looking toward a determination of the possibility of normal life with a steril alimentary canal have yielded conflicting results;[9,10] the nature of the animal, of its food and its digestive mechanism are all important factors in deciding the question.

Since the supply of nutriment for the bacteria of the lower intestin must consist mainly of the nitrogenous residues of the digestive juices and of the unabsorbed foodstuffs that reach the large intestin, we should expect a decreased bacterial growth when this food supply is decreased. An increase in unabsorbed residues of digestive juices and foodstuffs should result in an increased bacterial growth. It would seem reasonable to suppose, therefore, that any influence leading to the incomplete digestion and absorption of food, especially of its protein portion, in the alimentary tract would result in increased elimination of nitrogen in the feces, or in increased bacterial growth in the lower intestin, or in both of these conditions.

It was thought that evidence on both of these points might be valuable in determining the probable effect of water ingestion with meals.

The only earlier experimental evidence as to the influence of water drinking with meals upon the utilization of food was obtained by Ruzicka[12] in an experiment upon himself. The conditions and routine of his experiment are referred to in the previous paper on the utilization of fat. During the first 2-day period of his experiment when no water was taken with the meals, 118.4 g. protein were ingested, 17.9 g. excreted; during the second 2-day period when water was taken with the meals, 125.9 g. protein were ingested, 16.5 g. excreted. Protein utilization was 84.9 per cent. in the first period and 86.9 per cent. in the second. Neither diet nor water ingestion was sufficiently uniform to allow any but the most general conclusions to be drawn.

More specific evidence comes from an investigation made in this laboratory by Fowler and Hawk.[18] Their subject was brought to nitrogen equilibrium on a constant and uniform diet and continuing this diet one liter of water was added to each meal for a time of five days; this was followed by a short final period in which the original conditions held. It appears that the average fecal output per day and the average dry matter per day in the feces were both much less during the water period than during either of the other periods, and that the average amounts during the final period were less than those of the preliminary period. More detailed examination of the feces was confined to the determinations of total and bacterial nitrogen on one stool in each of the three periods. These findings showed that both these forms of nitrogen were much reduced in amount during the period of copious water ingestion and that after water ceased to be used in unusual amounts these values did not immediately return to the values found for the preliminary period but were still lower than those during the final period. The authors concluded that these findings indicated a more economical utilization of the protein of the food. During the water period of five days the subject gained approximately two pounds in weight, and continued to gain for a number of months after the end of the experiment and the return to ordinary mixed diet. It could not be said that the water drinking had no effect, nor that it had an ill effect.

These conclusions as to the digestibility and availability of the foods during water-drinking were based upon analyses of but three stools, one in each of the three periods. The importance of the conclusion reached seemed to justify more extensive experiments along the same line, experiments in which each individual stool of the whole investigation should be subjected to similar examination. The present investigation was therefore planned on this basis.

Description.

General Plan.—The general plan and routine of the experiment has been described in the preceding paper.

Methods.—All analyses were made on fresh feces without previous drying. The samples usually weighed out with the approximate weights were as follows:

(*a*) Two 2-gram samples for total nitrogen.
(*b*) Two 2-gram samples for bacterial nitrogen.
(*c*) Two 2-gram samples for acid-alcohol extract.

Total Nitrogen.—During the earlier experiments dried feces were used for the total nitrogen determinations, with unsatisfactory results due to loss of nitrogen during the drying. That there is such a loss the data clearly show; this has been observed before.[14,14a] In the later experiments the determination of total nitrogen was made upon the fresh material with satisfactory results.

Bacterial Nitrogen.—The method for bacterial nitrogen is described in another paper from this laboratory.*

Residue Nitrogen.—As explained more in detail under the determination of bacterial nitrogen, residue nitrogen is that which comes from the well-washed sediments in the sedimentation for bacterial nitrogen. It represents undigested and insoluble nitrogen that occurs in the larger particles of the feces.

Extractive Nitrogen.—The sample for acid-alcohol extract was rubbed up in a small Erlenmeyer flask with a known volume of 95 per cent. ethyl alcohol made 0.2 per cent. acid with hydrochloric acid. The flask was stoppered and was allowed to stand at room temperature for a week, being shaken up at least once each day. Nitrogen was then determined on an aliquot portion (one-half) of the alcohol originally added. This represents such nitrogenous end-products as are below the proteose stage, and the soluble nitrogen of the digestive juices and of the pigments. Almost invariably this amount is less than that obtained by a similar extractive method on the bacterial sample as described under bacterial nitrogen. This may be due to the greater fineness of division that is secured in the case of the latter and perhaps also to the solvent action of the 0.2 per cent. hydrochloric acid used in making the bacterial suspensions.

All determinations of nitrogen of whatever form were made by the Kjeldahl method. Instead of metallic mercury, copper sulfate was used as catalyst in the digestion.

Experiments on Copious Water Drinking with Meals.

As mentioned before, subjects H and W were put on the same diet; the amounts had to be altered before nitrogen equilibrium was reached. The quantity and composition as finally given were as follows:

	Amount (per meal).	Nitrogen.
Graham crackers	150 grams	2.087 grams
Peanut butter	20 "	0.882 "
Butter	25 "	0.020 "
Milk	450 (cc.)	2.360 "
	Total	5.349
Protein		33.44 grams
Protein per day		100.32

On this diet a condition of nitrogen equilibrium was attained approx-

* Mattill and Hawk, *J. Exp. Med.*, **14**, 433 (1911).

imately at the end of the third day. The exact nitrogen balance may be seen from the following:

Subject H.	Nitrogen in feces	2.153
	Nitrogen in urine	14.036
	Nitrogen in excreta	16.189
	Nitrogen in food	16.046
		—0.143
Subject W.	Nitrogen in feces	2.385
	Nitrogen in urine	14.534
	Nitrogen in excreta	16.919
	Nitrogen in food	16.047
		—0.873

TABLE I.—SUBJECT W.

		Nitrogen distribution.						Percentage of total fecal nitrogen found in.		
	Number of stool.	Fecal nitrogen (det.).	Fecal nitrogen (calc.).	Bacterial + soluble nitrogen.	Bacterial nitrogen.	0.2 per cent. HCl-soluble nitrogen.	Acid-alc.-soluble nitrogen.	Bacterial.	HCl-soluble.	Acid-alc.-soluble.
Preliminary period. 3 days.	1	1.738	1.911	1.737			0.445			23.3
	2	2.333	2.536	2.305			0.599			23.6
	3	0.913	0.961	0.874			0.193			20.1
	4	1.649	1.747	1.588			0.586			33.5
	Total	6.633	7.155	6.504			1.823			
	Average	2.211	2.385	2.168	1.279 (calc.)	0.889 (calc.)	0.608			25.5
Water period. 5 days.	5		(1.387)	1.296			0.280			15.2
	6	0.301	0.287	0.268			0.085			28.2
	7	1.337	1.433	1.339			0.416			29.0
	8	1.499	1.593	1.489			0.402			25.2
	9	1.653	1.771	1.655			0.458			25.9
	10	0.554	0.640	0.598			0.175			27.3
	Total		7.111	6.645			2.401			
	Average		(1.422)	1.329	0.784 (calc.)	0.545 (calc.)	0.480			33.4
Final period. 3 days.	11	1.410	1.459	1.364	0.817	0.547	0.456	56.0	37.5	31.2
	12	1.805	1.896	1.772	0.970	0.802	0.571	51.2	42.3	30.1
	13	0.448	(0.431)	0.403	0.294	0.109	0.122	65.6	24.3	27.2
	14	1.329	1.499	1.401	0.866	0.535	0.373	57.8	35.7	24.9
	Total	4.992	5.286	4.940	2.947	1.993	1.522			
	Average	1.664	1.762	1.647	0.982 0.972 (calc.)	0.664 0.675 (calc.)	0.507	55.7	37.7	28.8

Discussion of Data from Subject W, Table I.—As mentioned before the determinations of fecal nitrogen in this experiment were unsatisfactory because of the loss of volatil nitrogenous compounds in drying. That nitrogen was lost is very evident from the values of bacterial + soluble nitrogen which are in almost all cases larger than the corresponding total nitrogen. The so-called bacterial + soluble nitrogen comes from the determination of bacterial nitrogen and its significance will be clear by referring to the description of the method as given in a recent article.[1] If the acid suspension after removing the last sediment of non-bacterial substance is not treated with alcohol, but is directly transferred to Kjeldahl flasks, the nitrogen so determined is not only bacterial but includes in addition all nitrogen that is soluble in 0.2 per cent. hydrochloric acid or that has become so during the time of manipulation. This datum is spoken of as bacterial + soluble nitrogen. In later experiments it was shown that the ratio of total nitrogen to bacterial + soluble was fairly constant at 1.10 in the preliminary period and 1.07 in the following periods. Applying this factor to the values under bacterial + soluble nitrogen the values under fecal nitrogen (calc.) are obtained. Although these are not values obtained by analysis, they are more correct than those actually obtained for the reasons given. Taking either of these values, however, the average daily amount of total nitrogen excreted during the water period is only two-thirds of the average daily amount excreted during the preliminary period, and about four-fifths the average daily amount of the final period. The average daily amount in the final period is only slightly higher than that of the water period, and only three-fourths of what it was in the preliminary, showing that the good effect of the water is not immediately lost.

The question as to the kind of fecal nitrogen that was decreased in amount cannot be answered on the basis of analytical data, since the bacterial and acid-soluble nitrogen were not separated during the early part of the experiment. From later experiments in which this separation was made, a factor was calculated and found to be very uniform for different subjects throughout the various periods. On this basis 59 per cent. of the combined bacterial + soluble nitrogen is nitrogen belonging to bacterial substance. That the factor as applied does not fall far short of representing actual conditions may be gathered from the close agreement between the calculated values and those obtained by actual analysis of the stools of the final period. Applying this factor to the values for combined bacterial + soluble nitrogen the nitrogen of bacterial substance in the preliminary period was 1.279 grams per day, in the water period 0.784 gram per day, and in the final period 0.972 gram per day. These values indicate that bacterial nitrogen was de-

[1] Mattill and Hawk, *J. Exp. Med.*, 14, 433 (1911).

creased under the influence of copious water drinking and furtnermore, in common with the results found for total fecal nitrogen, this condition was not transitory but more or less permanent. The same statement may be made regarding the nitrogen soluble in 0.2 per cent. hydrochloric acid. The acid-alcohol-soluble nitrogen averaged 25.5 per cent. of the total fecal nitrogen during the preliminary peiiod, 33.4 per cent. during the water period, and 28.8 per cent. during the final. This may mean that the digestion during the water period resulted in nitrogenous end products which are more soluble. This increased percentage of acid-alcohol-soluble nitrogen in the feces during the water period does not indicate decreased absorption, for the *absolute* amount of this form of nitrogen in the feces is *decreased* from 0.608 gram during the preliminary period to 0.480 gram in the water period and rizes only slightly above this value, 0.507 gram, during the final period, showing that absorption of the soluble end products is more complete under the influence of water. More probably, however, this form of nitrogen represents the residual portion of digestive and intestinal juices which are known to increase in amount under the influence of water ingestion, especially the gastric and pancreatic secretions and the bile. If this is so, it is a very important fact, for even though during copious water ingestion, the flow of these secretions is stimulated, and as a result of increased peristalsis the amount of cast-off cellular material in the intestin is increased, the amount of fecal nitrogen instead of being increased, as, indeed, it must be fiom these sources, is, on the contrary, actually *decreased.* It follows from this that the digestibility of protein material during a period of copious water-drinking was increased even beyond what the data indicate, since part of the excreted nitrogen is known to come from the larger amounts of digestive juices secreted under the stimulating influence of water.

Discussion of Data from Subject H, Table II.—The values for total fecal nitrogen, either those determined directly on dry feces or those calculated directly from the bacterial + soluble nitrogen, show that the average daily excretion of nitrogen was 1.833 g. (detd.) during the preliminary period, 1.442 g. during the water period, and 1.636 g. during the final period. It was thus much less during the water period than during either of the others, and the average daily amount after the water was less than that before it.

As with subject W, the kinds of fecal nitrogen that suffered a decrease cannot be stated on the basis of analysis. The results on applying to the value for bacterial + soluble nitrogen the factor 0.59, which was obtained from later experiments, as has been explained, show that the average bacterial nitrogen per day was decreased from 1.155 in the preliminary to 0.875 in the water period, rizing to 1.044 in the final. The average daily output as determined for the final period is 1.128, showing that the factor used is accurate. The same proportionate differences are to be noticed in the values for soluble nitrogen. It is evident that both bacterial and soluble nitrogen in the feces underwent a marked decrease during the period of copious water drinking.

TABLE II.—SUBJECT H.

		Nitrogen distribution.						Percentage of total fecal nitrogen found in		
	Number of stool.	Fecal nitrogen (detd.).	Fecal nitrogen (calc.	Bacterial + soluble nitrogen.	Bacterial nitrogen.	0.2 per cent. HCl soluble nitrogen.	Acid-alc.-soluble nitrogen.	Bacterial.	HCl-soluble.	Acid-alc.-soluble.
Prelim. period. 3 days.	1........	0.636	0.743	0.675			0.208			28.0
	2........	1.214	1.427	1.297			0.342			24.0
	3........	2.206	2.654	2.413			0.756			28.5
	4........	1.443	1.635	1.486			0.653			39.9
	Total.........	5.499	6.459	5.871			1.959			
	Average......	1.833	2.153	1.957	1.155 (calc.)	0.802 (calc.)	0.653			30.3
Water period. 5 days.	5........	0.514	0.537	0.502			0.280			52.1
	6........	1.113	1.249	1.167			0.447			35.8
	7........	0.695	0.761	0.711			0.238			31.3
	8........	3.325	3.602	3.366			1.134			31.5
	9........	1.563	1.788	1.671			0.655			36.6
	Total.........	7.210	7.937	7.417			2.754			
	Average......	1.442	1.587	1.483	0.875 (calc.)	0.608 (calc.)	0.551			34.7
Final period. 3 days.	10........	1.229	1.415	1.322	0.819	0.503	0.503	57.9	35.6	35.6
	11........	0.442	0.477	0.446	0.302	0.144	0.119	63.3	30.2	24.9
	12........	1.510	1.763	1.648	1.082	0.566	0.563	61.4	32.1	31.9
	13........	1.726	2.028	1.895	1.180	0.715	0.671	58.2	35.3	33.1
	Total........	4.907	5.683	5.311	3.383	1.928	1.856			
	Average.....	1.636	1.894	1.770	1.128 1.044 (calc.)	0.643 0.726 (calc.)	0.619	59.6	34.0	32.7

The percentage of acid-alcohol-soluble nitrogen rose from an average of 30.3 in the preliminary to 34.7 during the water period and fell to 32.7 in the final, while the actual amount fell from 0.653 in the preliminary to 0.551 in the water and rose to 0.619 gram per day in the final period. The actual amount of this form of nitrogen was considerably decreased under the influence of water drinking. The suggestion may be made again that the increased percentage output was probably due to the increased volume of digestive juices, the secretion of which was stimulated by the ingestion of water.

Summary.

The findings on both subjects in this experiment show a decreased elimination of all forms of fecal nitrogen during the period in which water (1000 cc. additional) was taken with each meal. No ill effects could be seen and the beneficial effect of water was not temporary but was prolonged beyond the time during which water was taken.

Experiments on Moderate Water Drinking with Meals.

In the experiments upon protein utilization under the influence of a water ingestion of 500 cc. with each meal, the same general methods were employed. Total nitrogen determinations were made on the fresh moist material and the loss in volatil nitrogen compounds due to drying was thus avoided. A more accurate and trustworthy separation of the bacterial + soluble nitrogen was made by an efficient centrifugation of the final alcohol suspension from which the clear liquid had been pipetted off. The nitrogen of the precipitated material could more truly be called bacterial nitrogen, that of the liquid, acid-soluble nitrogen.

On the basis of the experience gained in this and similar investigations we cannot agree with certain statements made recently by Mendel and Fine[1] as to the determination of the total nitrogen content of feces. They say: "The error incident to this procedure (drying), however, did not appear to us to warrant serious attention, at least until certain details of metabolism operations, such, *e. g.*, as the accurate division of feces belonging to successive periods, reaching a higher stage of perfection." If we examin Tables I and II of the present paper it will be observed that *the values obtained by us for the combined bacterial and soluble nitrogen of fresh feces are in nearly every instance larger than the total nitrogen values obtained from the analysis of the same feces after drying.* We are firmly convinced that the ideal method of feces analysis embraces the examination of the individual stools in the *fresh* condition. This procedure, of course, entails much more labor than the less accurate practice of utilizing the dried feces, but we believe that the added accuracy richly compensates the investigator. In certain connections the individual fresh stools may be preserved for several days and an analysis made of a composit sample of the moist feces.[14b]

Throughout most of this experiment the values for bacterial nitrogen and for nitrogen in the alcohol extract of bacteria, that is the acid-soluble nitrogen, were determined along with a determination of the bacterial + soluble nitrogen, that is, the same suspension without alcohol treatment. The agreement between the last-named and the sum of the first two is very satisfactory; in almost all cases they would pass as duplicates. The fact that the alcohol used was not previously freed from pos-

[1] Mendel and Fine, *J. Biol. Chem.*, **10**, 309 (1911).

sible traces of nitrogen may account for the uniformly higher values given by the sum of the separate alcohol-soluble and bacterial determinations.

It was found from these data that the bacterial nitrogen was 59 per cent. of the combined bacterial + soluble nitrogen and this ratio was used in the preceding experiment. The ratio of total fecal nitrogen to bacterial-soluble nitrogen used in the first experiment was obtained from the values for these forms in this experiment. In both Tables III and IV this was approximately 1.10 in the earlier periods and 1.07 in the later periods.

Acid-alcohol-soluble nitrogen was determined as before, and the determination of residue nitrogen was made throughout this experiment.

The diet of subject W, who had served before, was slightly reduced from what it had been in the preceding experiment. The amounts and composition were as follows:

	Amount (per meal).	Nitrogen.
Graham crackers	125 grams	1.776 grams
Peanut butter	20 "	0.868 "
Butter	25 "	0.015 "
Milk	400 (cc.)	1.917 "
		4.577
Protein		28.61
Daily protein		85.83

The diet of subject E consisted of

Graham crackers	150 grams	2.120 grams
Peanut butter	20 "	0.868 "
Butter	25 "	0.015 "
Milk	400 (cc.)	1.917 "
		4.920 "
Protein		30.75
Daily protein		92.25

For water ingestion see preceding paper.

Discussion of Data from Subject W, Table III.—Because of the difficulty experienced in obtaining nitrogen equilibrium the preliminary period of W was divided by taking charcoal on the eighth day, but with no change in diet. On the 13th day W's nitrogen balance was as follows:

Nitrogen in feces	1.360
Nitrogen in urine	12.361
Nitrogen in excreta	13.721
Nitrogen in food	13.731
	+0.010

The influence of a restricted amount of water and the latent period after which its effects appeared, as explained in the previous paper, are to be noted in the protein utilization as they were in the fat utilization. The average daily fecal nitrogen excretion during the first part of the preliminary period rizes from 1.275 to 1.360 in the second part, bacterial + soluble nitrogen from 1.142 to 1.233, acid-alcohol-soluble from 0.284 to 0.320, and residue nitrogen from 0.148 to 0.154. A comparison of the nitrogen data of this preliminary period with the nitrogen data of the preliminary period of the first experiment shows the average daily total fecal nitrogen output to be 2.385 in the first as against 1.275 in the second; bacterial + soluble nitrogen 2.168 in the first as against 1.142 in the second; acid-alcohol-soluble 0.608 as against 0.284. The average percentage utilization of protein in the first experiment was 86.3 per cent. as against 90.7 per cent. in the second. These data showing so pronounced an improvement in the digestion and utilization of protein are on an individual living on the same kind of diet, but separated by a period of three months in which water drinking with ordinary meals was practised.

With the fourteenth day 500 cc. of water were added to the diet of of each meal and this was continued for ten days. A five-day period followed in which the original conditions prevailed.

By referring to Table III it will be seen at once that the nitrogen of the various periods presents no striking differences.

The values for total, bacterial and other forms show fluctuations which are too small to admit of conclusions. The largest proportionate variation is seen in the residue nitrogen. This, as was explained, was obtained from the solid material that was sedimented in the procedure for bacterial nitrogen. Its percentage of the total nitrogen, 11.3, in the preliminary period, fell to 9.5 in the water period, and still lower, to 8.2 in the final period. If these small differences are significant, they point to a condition of better digestion.

Discussion of Data from Subject E, Table IV.—On the diet given, the nitrogen balance of Subject E at the end of the sixth day was as follows:

Nitrogen in feces	1.926
Nitrogen in urine	13.320
Nitrogen in excreta	15.246
Nitrogen in food	14.761
	—0.485

An examination of the data in Table IV again reveals no striking differences in the nitrogen values from one period to another. The variations in average daily amounts of nitrogen in its various forms are, as in the case of W, too small to be significant, with the possible exception of the

TABLE III.—SUBJECT W.

	Number of stool.	Nitrogen partition. Total fecal nitrogen.	Bacterial + soluble nitrogen.	Bacterial soluble separate.	Bacterial nitrogen.	0.2 per cent. HCl soluble nitrogen.	Acid-alc.-soluble nitrogen.	Residue nitrogen.	Percentage of total fecal nitrogen found in Bacterial + soluble nitrogen.	Bacterial.	0.2 per cent. HCl soluble.	Acid-alc.-soluble.	Residue.
Prel. per. I. 7 days.	1.......	2.152	1.906				0.460	0.323	88.6			21.4	15.0
	2.......	0.618	0.556				0.143	0.076	90.0			23.2	12.2
	3.......	0.878	0.789				0.213	0.100	89.9			24.3	11.4
	4.......	1.070	0.990				0.234	0.109	92.5			21.9	10.2
	5.......	3.075	2.760				0.684	0.269	89.8			22.3	8.8
	6.......	1.131	0.993				0.255	0.159	87.8			22.6	14.0
Total..........		8.924	7.994				1.990	1.035					
Average........		1.275	1.142				0.284	0.148	89.6			22.3	11.6
Prel. per. II. 6 days.	7.......	0.845	0.790	0.816	0.483	0.336	0.190	0.071	93.6	57.2	39.5	22.5	8.4
	8.......	1.265	1.208	1.199	0.693	0.506	0.277	0.127	95.5	54.8	40.0	21.9	10.0
	9.......	1.491	1.324	1.388	0.791	0.597	0.362	0.132	88.8	53.0	40.1	24.3	8.9
	10.......	2.213	2.008	2.057	1.107	0.949	0.544	0.271	90.7	50.0	42.9	24.6	12.3
	11.......	0.673	0.574	0.549	0.287	0.262	0.161	0.109	85.3	42.6	39.0	23.9	16.1
	12.......	1.254	1.136	1.084	0.590	0.494	0.298	0.169	90.6	47.0	39.4	23.8	13.5
	13.....	0.418	0.355	0.358	0.194	0.163	0.088	0.044	85.1	46.6	39.1	21.8	10.5
Total..........		8.157	7.396	7.450	4.144	3.306	1.920	0.922					
Average........		1.360	1.233	1.242	0.691	0.551	0.320	0.154	90.7	50.8	40.5	23.5	11.3

Water period 10 days. 14	0.388	0.352	0.368	0.200	0.168	0.103	0.028	90.6	51.4	43.3	26.5	7.2
15	2.044	1.959	1.867	0.984	0.884	0.530	0.207	95.8	48.1	43.2	25.9	10.1
16	1.029	0.990	0.986	0.563	0.424	0.249	0.083	96.3	54.7	41.2	24.2	8.0
17	2.044	1.929	1.929	1.087	0.841	0.521	lost	94.4	53.2	41.2	25.5	....
18	0.899	0.790	0.831	0.440	0.390	0.215	0.123	87.8	49.0	43.4	24.0	14.0
19	1.616	1.510	1.495	0.873	0.621	0.404	0.163	93.4	54.0	38.4	25.0	10.1
20	0.377	0.337	0.345	0.198	0.147	0.087	0.042	89.3	52.5	39.1	23.0	11.1
21	2.355	2.249	2.254	1.298	0.957	0.583	0.195	95.5	55.1	40.6	24.8	8.3
22	1.672	1.533	1.549	0.881	0.668	0.409	0.172	91.7	52.7	40.0	24.5	10.3
23	1.918	1.751	1.813	0.996	0.817	0.498	0.211	91.3	51.9	42.6	26.0	11.0
Total	14.343	13.400	13.437	7.520	5.917	3.599	1.224					
Average	1.434	1.340	1.344	0.752	0.592	0.360	0.136	93.4	52.4	41.3	25.1	9.5
Final period 5 days. 24	0.775	0.730	0.724	0.416	0.307	0.184	0.062	94.2	53.7	39.6	23.8	8.0
25	1.497	1.383	1.391	0.823	0.568	0.355	0.099	92.4	55.0	38.0	23.7	6.6
26	0.758	0.694	(0.694)	0.461	0.233	0.195	0.079	91.6	60.9	30.8	25.7	10.5
27	1.845	1.738	1.955	1.160	0.795	0.488	0.163	94.2	62.9	43.1	26.5	8.8
28	1.238	1.153	1.201	0.758	0.444	0.292	0.100	93.1	61.2	35.8	23.6	8.1
29	0.875	0.830	0.872	0.539	0.333	0.207	0.070	94.8	61.6	38.1	23.6	8.0
Total	6.988	6.528	6.837	4.157	2.680	1.721	0.573					
Average	1.398	1.306	1.368	0.832	0.536	0.344	0.115	93.4	59.5	38.4	24.6	8.2

Table IV.—Subject E.

		Nitrogen distribution.							Percentage of total fecal nitrogen found in				
		Total fecal nitrogen.	Bacterial + soluble nitrogen.	Bacterial soluble separate.	Bacterial nitrogen.	0.2 per cent. HCl soluble nitrogen.	Acid-alc.-soluble nitrogen.	Residue nitrogen.	Bacterial + soluble.	Bacterial.	0.2 per cent. HCl soluble.	Acid-alc.-soluble.	Residue.
Prel. period. 7 days.	1......	1.141	0.945				0.236	0.159	82.8			20.7	13.9
	2......	0.461	0.457				0.106	0.056	99.1			23.0	12.1
	3......	2.420	2.251				0.530	0.235	93.0			21.9	9.7
	4......	2.867	2.591				0.679	0.312	90.4			23.7	10.9
	5......	1.219	1.104				0.262	0.130	90.6			21.5	10.7
	6......	2.846	2.587				0.654	0.328	90.9			23.0	11.9
	7......	1.744	1.581				0.366	0.206	90.6			21.0	11.8
	8......	0.784	0.731				0.176	0.072	93.3			22.5	9.2
Total.........		13.481	12.247				3.010	1.497					
Average.......		1.926	1.750				0.430	0.214	90.9			22.3	11.1

Water period. 10 days.	9	1.048	0.923	0.921	0.536	0.386	0.233	0.138	88.0	51.1	36.8	22.2	13.2
	10	1.837	1.713	1.723	1.085	0.638	0.380	0.194	93.2	59.1	34.7	20.7	10.6
	11	1.158	1.087	1.091	0.685	0.406	0.241	0.072	93.9	59.2	35.1	20.8	6.3
	12	2.577	2.398	2.405	1.372	1.033	0.571	0.214	93.0	53.3	40.1	22.2	8.3
	13	3.133	2.678	2.700	1.443	1.257	0.713	0.376	85.5	46.1	40.1	22.8	12.0
	14	1.896	1.706	1.767	1.019	0.748	0.419	0.192	90.0	53.8	39.5	22.1	10.1
	15	2.389	2.059	2.165	1.083	1.081	0.589	0.242	86.2	45.4	45.3	24.7	10.1
	16	1.144	1.015	1.032	0.523	0.509	0.264	0.140	88.7	45.7	44.5	23.1	12.3
	17	0.811	0.806	0.787	0.425	0.362	0.207	0.102	99.4	52.4	44.7	25.5	12.6
	18	2.567	2.377	2.353	1.343	1.010	0.630	0.232	92.6	52.3	39.3	24.5	9.0
	19	0.818	0.790	0.808	0.445	0.363	0.205	0.075	96.5	54.4	44.4	25.1	9.2
Total		19.378	17.551	17.752	9.958	7.794	4.452	1.978					
Average		1.938	1.755	1.775	0.996	0.779	0.445	0.198	90.6	51.4	40.2	23.0	10.2
Final period. 4 days.	20	0.943	0.829	0.828	0.425	0.403	0.213	0.146	87.9	45.1	42.7	22.5	15.4
	21	1.855	1.675	1.703	1.015	0.688	0.429	0.196	90.3	54.7	37.1	23.1	10.6
	22	2.045	1.861	1.864	1.068	0.796	0.446	0.199	91.0	52.2	38.9	21.8	9.8
	23	2.251	2.086	2.158	1.243	0.915	0.556	0.230	92.7	55.2	40.6	24.7	10.2
	24	0.538	0.493	0.502	0.195	0.308	0.126	0.059	91.7	36.2	57.2	23.4	10.9
Total		7.631	6.944	7.055	3.946	3.109	1.769	0.830					
Average		1.908	1.736	1.764	0.987	0.777	0.442	0.207	91.0	51.7	40.7	23.2	10.9

values for the residue nitrogen. The percentage of the total nitrogen found in this form during the preliminary period, 11.1, fell to 10.2 in the water period and rose to 10.9 in the final. Similar variations, and in the same direction, were noted with W. Attention may be called again to the satisfactory agreement of the values for bacterial + soluble nitrogen with the values of the sum of these two determined separately, which thus furnishes a valuable check on the determinations.

The protein data on Subjects W and E during this experiment on moderate water drinking with meals do not justify the drawing of any but negative conclusions; they do not show that the use of water was attended by any undesirable results. The data obtained on the utilization of carbohydrate and fat during this period, which are presented in the following and preceding papers, show that where analytical methods are sufficiently exact to give significant results the effect of moderate water drinking is in the same direction as that of copious water drinking, though much less marked.

Copious Water Ingestion by an Individual Accustomed to Taking Considerable Water with Meals.

Following the preceding experiment, a period of six days formed the preliminary period for this experiment, the subject of which was E. The diet was the same as before and at the beginning of the 5-day water period the nitrogen balance was as follows:

Nitrogen in feces	1.957
Nitrogen in urine	12.775
Nitrogen in excreta	14.732
Nitrogen in food	14.761
	+0.029

Discussion of Data on Subject E, Table V.—Although both carbohydrate and fat data (discussed in other places) show differences that signify an increased utilization of these foodstuffs during the ingestion of one and one-third liters of water additional with each meal, a comparison of the data on the excretion of nitrogen in its various forms during the three periods of this experiment allows no positive conclusions to be drawn. The differences are too small to be significant. A negative conclusion, however, is entirely justifiable, when it is seen that the absorption of over four liters of water during the day, and most of this taken during the meals, had no untoward effect upon the digestion and absorption of the food. The probable reason for the fact that no change in the direction of better digestion could be noticed is that Subject E habitually took considerable amounts of water with his meals, and the experimental conditions were thus little different from the usual régime.

Table V.—Subject E.

	Nitrogen distribution.							Percentages of total fecal nitrogen found in				
	Total fecal nitrogen.	Bacterial + soluble nitrogen.	Bacterial soluble separate.	Bacterial nitrogen.	0.2 per cent. HCl soluble nitrogen.	Acid-alc.-soluble nitrogen.	Residue nitrogen.	Bacterial + soluble.	Bacterial.	0.2 per cent. HCl soluble.	Acid-alc.-soluble.	Residue.
Prel. period, 6 days. 1.......	0.572	0.517	0.521	0.307	0.214	0.131	0.067	90.4	53.7	37.4	22.9	11.7
2.......	1.116	1.051	1.094	0.692	0.402	0.273	0.073	94.2	62.1	36.0	24.5	6.5
3.......	3.036	2.821	2.898	1.715	1.183	0.706	0.233	92.9	56.5	39.0	23.2	7.7
4.......	1.940	1.813	1.836	1.088	0.748	0.451	0.176	93.5	56.1	38.6	23.3	9.1
5.......	2.104	2.003	2.066	1.205	0.861	0.489	0.174	95.2	57.3	40.9	23.2	8.3
6.......	2.383	2.175	2.225	1.264	0.961	0.521	0.252	91.3	53.1	40.3	21.9	10.6
7.......	0.591	0.553	0.570	0.364	0.206	(0.080)	0.042	93.7	61.5	34.9	(13.5)	7.1
Total.........	11.741	10.933	11.210	6.635	4.575	2.650	1.016					
Average.......	1.957	1.822	1.868	1.106	0.763	0.457	0.169	93.1	56.5	39.0	(23.4)	8.7
Water period, 5 days. 8.......	1.119	1.026	1.069	0.640	0.424	0.250	0.115	91.7	57.1	37.9	22.4	10.3
9.......	0.627	0.590	0.606	0.365	0.241	0.143	0.050	94.1	58.2	38.5	22.8	8.0
10.......	3.633	3.429	3.512	2.040	1.472	0.756	0.292	94.4	56.2	40.5	20.8	8.0
11.......	1.216	1.103	1.130	0.659	0.471	0.285	0.103	90.7	54.2	38.8	23.4	8.5
12.......	2.250	2.095	2.178	1.081	1.097	0.570	0.225	93.1	48.1	48.7	25.3	10.0
13.......	0.994	0.885	0.916	0.528	0.389	0.213	0.077	93.8	55.8	41.2	22.6	8.2
Total.........	9.788	9.128	9.411	5.312	4.094	2.216	0.861					
Average.......	1.958	1.826	1.882	1.062	0.819	0.443	0.172	93.3	54.3	41.8	22.6	8.8
Final period, 3 days. 14.......	1.790	1.658	1.712	1.042	0.670	0.389	0.189	92.6	58.2	37.4	21.7	10.6
15.......	1.336	1.241	1.283	0.767	0.516	0.304	0.111	92.9	57.4	38.6	22.8	8.3
16.......	2.800	2.561	2.664	1.555	1.109	0.607	0.250	91.5	55.5	39.6	21.7	8.9
17.......	0.340	0.303	0.300	0.159	0.141	0.092	0.040	89.3	47.0	41.4	27.0	11.8
Total.........	6.265	5.763	5.958	3.523	2.435	1.392	0.590					
Average.......	2.088	1.921	1.986	1.174	0.812	0.464	0.197	92.0	56.2	38.9	22.2	9.4

General Discussion.

The general conclusion from all these findings is that during water ingestion with meals there is a better digestion and a more complete utilization of the protein food and that this effect is much less marked with a small water ingestion than with a large one. It is also more or less permanent, with the result that in an individual accustomed to taking considerable water with meals the effects of decreasing or increasing the volume ingested are not immediately obvious.

There is one objection to the conclusion that this is caused by water drinking. It has frequently been observed in experiments on men that the prolonged administration of a given diet causes the enzyme content of the digestive juices so to change as to be best adapted to digesting the food. It might be argued, therefore, that although the food was as well digested during the latter part of these experiments as in the beginning, this was a result of adaptation which counteracted the undesirable effects of water drinking. A comparison of the data of the final periods with those of the water periods is sufficient to show that the withdrawal of water was accompanied by a pronounced change in favor of the water drinking, or by no appreciable change in digestibility and utilization.

Any supposed effect of adaptation is also counterbalanced by the effect of loss of appetite due to the monotony of the diet. London and Pevsner[15] found that in dogs the stomach contents were more rapidly passed on to the duodenum when the factor of appetite was present than when it was absent. They conclude that the larger amount of secretion, that is, the appetite juice, was the cause. If there is any increased efficiency in "appetite" juice over the ordinary secretion in man then the digestive power of all the juices is at least not increased by the factor of appetite after partaking of over one hundred meals that were absolutely uniform in the kind and quantity of food they contained.

Evidence has been adduced in another place to show that the action of water is not such as to cause undigested food particles to be washed through the pylorus prematurely, and thereby place a more than proportionate burden upon the lower digestive tract. That a premature opening of the pylorus, resulting in a shortening of gastric digestion, is uneconomical, has been shown by Cohnheim.[16] The importance of the stomach in protein digestion is not clear despite extensive experimentation bestowed upon the subject.

London and Polovzova[17, 18] found that with but few exceptions proteins are not absorbed in the stomach, but that with few exceptions most proteins are made soluble in the stomach to about 78 per cent., the ratio between proteoses, peptones and residual substances being 59.3, 32.9, 7.8. The soluble products of gastric digestion are quickly attacked by the intestinal juices. Examination of stomach contents[19] reveals the fact that peptones, peptides and amino acids may be absorbed; while it is shown that the enzymes of the stomach have the ability completely to hydrolyze proteins to these end-products, yet it is also shown that the length of time which pepsin requires to bring this about is far in excess of the time in which protein remains in the stomach.

The importance of the stomach in protein assimilation has been emphasized recently by Carrel, Meyer and Levene,[20] who showed that after removal of the larger part of the small intestin, although the absorption of ingested protein is diminished, the rate of assimilation and retention of the absorbed protein follows the same course as in normal animals. They conclude that the stomach and not the intestin is the most important organ for protein assimilation. London and Dmitriev[21] showed that the removal of the small intestin in a dog results in the death of the animal in about five weeks. Ordinarily, if as much as seven-eighths of the small intestin is removed, carbohydrate and especially protein assimilation rapidly return to normal but not so with the assimilation of fat. Somewhat similar results have been obtained by Underhill.[22] After resection of 80 per cent. of the small intestin Axhausen[23] found that the absorption of protein as well as of fat was very much lower than normal. Results obtained after experimental removal of various portions of the alimentary tract are always subjected to this correction that the different organs may change their function and character in the direction of compensation. Thus, after gastrectomy in dogs, Carrel, Meyer and Levene[24] observed a high nitrogen retention which disappeared in ten to twelve weeks after the operation. The explanation suggested is that the pancreatic and intestinal secretions that are minimal immediately after gastrectomy return to normal at a later period and protein is again fully digested before absorption. They also note a hypertrophy of the upper end of the duodenum after gastrectomy.

Since the presence of water along with food in the stomach is hardly of long enough duration to affect either the food or the mucous membrane, the changes for the better digestion and utilization of the protein material that have been observed must take place principally in the intestin. Some experimental work has been done on the absorption of proteins by living membranes. Zunz[25] in experiments on dogs upon protein digestion and absorption in the stomach and small intestin *in situ* has shown that the osmotic pressure of the solutions of protein introduced scarcely changes in the stomach when this is tied off, but in the small intestin it tends toward that of the blood and usually becomes lower than this. Surface tension is lower than that of the blood in both the stomach and intestin. With low proteose content the surface tension decreases in both regions. In the intestin, Zunz concludes, the digestive processes tend to bring the concentration, osmotic pressure and surface tension of the contents to the optimum for absorption. The organism itself seems to strive to secure a dilution of the products of digestion such that they can be most readily and completely absorbed.

The phenomena of absorption still lack a unifying physical explanation; in fact, each investigation seems to disclose new and unknown factors. Filtration, osmosis, the selective action of membranes and the nature and behavior of colloids are some of the important factors, upon an understanding of which the solution of the many problems depends.

Conclusions.

Without attempting to suggest any further explanations than those given at the end of the preceding paper, it may be said that the ingestion of large amounts (1000 cc.) of water with meals caused the protein constituents of the food to be more completely utilized, as shown by a decrease in all forms of nitrogen in the feces, including bacterial, 0.2 per cent. hydrochloric-acid-soluble, acid-alcoholic extractive, and residue nitrogen.

When 500 cc. of water were taken with meals no significant changes in protein utilization could be observed, as there were in fat and carbohydrate; the protein data do, however, admit of the negative conclusion, that absolutely no undesirable effects were to be observed as a result of the ingestion of 500 cc. of water with the meals. Even when over four liters of water were taken daily, with the meals, there was no indication of untoward effects as a result.

As before, the beneficial results of water ingestion with meals were not transitory, but were more or less permanent, extending beyond the time of the experimental period.

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STUDIES ON WATER DRINKING: X. FECAL OUTPUT AND ITS CARBOHYDRATE CONTENT UNDER THE INFLUENCE OF COPIOUS AND MODERATE WATER DRINKING WITH MEALS.

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Introduction.

It has been said that the amount of feces, as well as its nitrogen content, depends entirely upon the cellulose content of the food materials, the first being the result of the inability of the organism to digest cellulose, the second being due to the increased desquamation of intestinal epithelium as a result of heightened peristalsis and to an accompanying increase in the amount of digestive fluids secreted.

Aside from possible traces of the less common complex carbohydrates, the only carbohydrates ever present in normal feces under ordinary conditions are cellulose and starch. It has been shown by Lusk[1] that the decomposition of cellulose does not result in the formation of glucose, and its nutritive value is probably in the fatty acids formed from it. In a study of the digestibility of carbohydrate, therefore, a consideration of the possible digestion of cellulose is unnecessary.

The source of starch in the feces is ingested vegetable food, the cellulose envelopes of which, as a result of insufficient disintegration, have not become accessible to the action of the digestive juices. The manner of preparing the food has much to do with the extent of this disintegration; the efficiency of the mastication also plays a part, and the activity of the digestive juices and the extent of the churning to which the food is subjected in the intestin also have an influence. All other conditions remaining the same, the amount of carbohydrate found in the feces should furnish some indication as to the digestibility of carbohydrate in the organism, as well as to the extent of cellular disintegration by which it has become available. In the series of observations reported in the preceding papers on the utilization of protein and fat under the conditions of water drinking with meals attention was also paid to the comparative