Hydrolysis of 3-Benzoyl-2-thiohydantoin with Hydrochloric Acid. 2-Thiohydantoin,

$$\begin{array}{l} NH—CO \\ \;|\qquad\;\; | \\ CS\qquad | \\ \;|\qquad\;\; | \\ NH—CH_2 \end{array}$$

.—This hydantoin is formed quantitatively by hydrolysis of the above benzoyl derivative with strong hydrochloric acid. After evaporating to dryness on the steambath the thiohydantoin is then purified by crystallization from alcohol. The hydantoin melted with decomposition at 227° and was identical with the thiohydantoin which was prepared by hydrolysis of benzoylthiohydantoic acid.[1]

Condensation of Benzaldehyde with 3-Benzoyl-2-thiohydantoin. 4-Benzal-2-thiohydantoin,[1]

$$\begin{array}{l} NH—CO \\ \;|\qquad\;\; | \\ CS\qquad | \\ \;|\qquad\;\; | \\ NH—C : CHC_6H_5 \end{array}$$

.—Two grams of 3-benzoyl-2-thiohydantoin were condensed with a molecular proportion of benzaldehyde by digestion in glacial acetic acid in the presence of anhydrous sodium acetate. On cooling and pouring into water we obtained 1.6 grams of this benzalthiohydantoin. It melted at 258° and a mixture of this substance with some benzalthiohydantoin, which was prepared by hydrolysis of 1-benzoyl-4-benzalthiohydantoin, melted at the same temperature. Benzoic acid was identified in the acetic acid filtrate.

Analysis (Kjeldahl): Calculated for $C_{10}H_8ON_2S$: N, 1373; found, 13.27.

Action of Acetic Anhydride on 2-Thiohydantoin.—Five-tenths of a gram of 2-thiohydantoin was warmed with 10 cc. of acetic anhydride for exactly 30 minutes. The mixture was then cooled and poured into cold water when a clear solution was obtained. No acetylthiohydantoin separated. The aqueous solution was then evaporated to dryness and the residue obtained crystallized from hot alcohol. Pure thiohydantoin separated on cooling.

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STUDIES ON WATER DRINKING. VIII.[2] THE UTILIZATION OF INGESTED FAT UNDER THE INFLUENCE OF COPIOUS AND MODERATE WATER DRINKING WITH MEALS.

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Introduction.

Current Theories.—Notwithstanding the fact that many persons are

[1] Wheeler, Nicolet and Johnson, *Loc. cit.*

[2] Presented in abstract at the New Haven meeting of the American Society of Biological Chemists, December, 1910; Proceedings, Vol. II, p. xiv. This paper and the

accustomed to drinking considerable amounts of water with their meals, and with no apparent ill effect, the opinion has been and still is somewhat general, and the statement almost axiomatic, that the use of water with meals is injurious and harmful. The arguments advanced in proof of this statement are typical of that quasi-scientific reasoning which assumes, *a priori*, the truth of certain antecedents; the consequents must therefore logically be true.

A concrete statement of the views as generally held by many in the medical profession and, through them, by the general public, may be cited from Carrington:[1]

"We can lay down the definit and certain rule that it (water) should never be drunk at meals, and preferably not for at least one hour after the meal has been eaten. The effect of drinking water while eating is, first, to artificially moisten the food, thus hindering the normal and healthful flow of saliva and the other digestive juices; secondly, to dilute the various juices to an abnormal extent; and thirdly, to wash the food elements through the stomach and into the intestins before they have had time to become thoroughly liquefied and digested. The effects of this upon the welfare of the whole organism can only be described as direful."

It needs no argument to prove that such effects upon the organism would be direful, but the proof that such effects follow the drinking of water with meals is entirely wanting.[1] On the contrary, experiments have been made which show specifically that certain of these effects do not follow.

1. The Effect of Water on the Digestive Juices. Saliva.—The degree of dryness of the food determins the amount of saliva poured out upon it, the drier the food the larger the amount of saliva that is secreted.[2] The kind of food introduced into the mouth determins also the physical properties of the saliva. It will be argued, therefore, that the taking of water with food prevents the normal secretion of saliva. In the experi-

two following were presented in abstract before the Second International Congress of Alimentary Hygiene and of the Rational Feeding of Man, Brussels, October, 1910; Proceedings, Vol. I, Section II, p. 30. They were also presented by Mr. Mattill to the Graduate School of the University of Illinois, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

[1] Although little experimental evidence substantiates the statement it will be granted at the start, that any circumstance that induces insufficient mastication of the food before swallowing is undesirable, the reason being that salivary digestion in the stomach is not to be overlooked and further, that the movements of the alimentary tract are insufficient to bring about the necessary fineness of division of the food particles. Therefore in all the discussion and experimental work that follows, water with meals means the taking of water when the mouth is empty; the food is masticated, as usual without the aid of water; water is never used to wash down the products of incomplete mastication.

ments that follow, however, since water is not mixed with the food while this is in the mouth, the effect of water on the secretion of saliva is only a residual one, that is, an effect due to the presence of whatever water may remain in the mouth after swallowing.

Gastric Juice.—The influence of water upon gastric secretion was investigated by Pavlov and Khizhin[3] and still earlier by Heidenhain[3a] and by Sanotskii[3b] and their findings have been confirmed by later investigators, especially by Foster and Lambert.[4] The first mentioned workers in experiments on dogs with Pavlov stomachs and divided vagi showed that water stimulates the flow of gastric juice if comparatively large amounts (400–500 cc.) are ingested, but that with small amounts (100–150 cc.) in half the cases observed, not the least trace of secretion could be found. "It is only a prolonged and widely spread contact of the water with the gastric mucous membrane, which gives a constant and positive result."[3] Since the vagi were divided, the effect of the water must have been that of a chemical excitant. The later investigators[4] in experiments on the influence of water when taken with food showed that water causes not only a more voluminous secretion but also a more acid secretion.

Pancreatic Juice and Bile.—Water also acts as an excitant of pancreatic juice.[5] When 150 cc. of water are introduced into the stomach of a dog the pancreas begins to secrete, or augments its flow, within a few minutes after the water has entered the stomach. Since this amount of water, according to Pavlov, is insufficient to excite a flow of gastric juice, the secretion of pancreatic juice is not secondary to a secretion of the other, but is a direct result of the presence of water in the stomach. In dogs with Pavlov pancreas fistulas Togami[6] has shown that in response to both chemical and psychical stimuli there is evident parallelism between the secretion of gastric juice and of pancreatic juice. Acids of all kinds act as powerful excitants of pancreatic secretion. The flooding of the small intestin with larger amounts of acid chyme means an increased production of pancreatic secretion and a consequent increased flow of pancreatic juice. The biliary secretion has also been shown to respond to pancreatic secretion and the digestive properties of the pancreatic juice are augmented in a very marked way by the bile. Hence the increased acidity of the gastric contents as a result of the stimulating action of water causes a much more active digestive juice to be poured out upon the chyme as it reaches the intestin. Furthermore, certain other experiments from this laboratory have shown increased pancreatic activity[6a] to follow water-drinking with meals, the index being the output of fecal amylase.[7, 7a]

Intestinal Juice.—The effect of water in the intestin has not been demonstrated as clearly as its effect in the middle portion of the alimentary canal. Under ordinary circumstances the intestinal juice is secreted only by those portions of the tube with which the food is in contact. Mechanical stimulation is effective in producing a secretion but it is shown that such secretion is comparatively poor in enzymes and contains only salt and water. When poured out upon food the intestinal juice is rich in enterokinase, but much more powerful stimulants even than food in this regard are the pancreatic enzymes; which one of them is active in this direction is not yet known.

2. The Effect of Dilution upon Enzyme Activity.—The reactions brought about by enzymes are like all other chemical reactions in that they are reversible. They do not proceed to completion unless the products of the reaction are removed as formed. In a concentrated solution the point at which the reaction comes to a standstill is reached sooner than in a dilute one, and in many instances the equilibrium of a reaction mixture may be disturbed by dilution; the reaction is forced toward completion if water is added. In the light of this fact the increased activity of gastric juice that has been observed under the influence of water may be due to the effect of dilution fully as much as to the increased acidity that accompanies it.

3. The Rapidity of the Passage of Food as Affected by Water.—That water begins to pass the pylorus soon after its ingestion has been shown by von Mering.[8] To a large dog with duodenal fistula 500 cc. of water were given through the mouth; within 25 minutes 495 cc. were collected through the fistula. It is probable that when water ingestion is accompanied by the taking of food the passage of water is somewhat delayed. In the experiments to be described it was shown that the equivalents of from one-half to three fourths of the amount of water ingested during a meal, if this amount was large, may be voided in the urine within 45–90 minutes thereafter. These facts would seem to give some ground for the contention that the food elements might be washed through the stomach and into the intestin before they were properly liquefied and digested.

It has been shown by Cohnheim[8a] however that when the fundus is filled with food material a specific mechanism comes into play after the introduction of liquid. Along the smaller curvature there is formed a trough which connects the antrum pylori with the cardiac opening, and this trough has been demonstrated anatomically by Kaufmann.[8b] In this trough water flows past the bolus of food lying in the stomach without as much as washing any of the exterior away. Even when digestion is at its height and when gastric juice is being secreted in large amounts, almost neutral water is often found leaving the stomach. Cohnheim further states that there is no dilution of the stomach contents by liquid food, and the accurate regulation of the pyloric sphincter is not disturbed whether water is taken with the meal or not.

From the considerations thus briefly reviewed the facts regarding the drinking of water with meals seem to be the following: (1) The ingestion of large amounts of water with meals not only does not hinder the normal flow of digestive juices, but acts as an excitant to their flow; (2) the digestive juices are not made less efficient by dilution; on the contrary, enzyme action is more complete the greater the dilution, within limits; (3) while the food elements might perhaps be washed through the stomach into the intestin more rapidly than is usual (contrary to Cohnheim's belief), yet over against this is the greater amount and efficiency of the digestive juices. The first two conclusions have been substantiated by experiment. The question as to the completeness of the digestion of the food and the degree to which it is utilized under the conditions of greater dilution and supposedly more rapid movement through the alimentary canal has had but little consideration.

The only experimental evidence upon the utilization of the fat of food as influenced by the amount of water taken with meals comes from an investigation by Ruzicka.[9] His conclusions were drawn from data obtained in two experimental periods of two days each, on a bread and meat diet, preceded, separated and followed by a day of milk diet. No attempt was made to have the fat intake uniform from day to day. During the first period water was taken at times and in amounts found desirable, except that none was taken during or immediately following a meal. In the second period approximately the same amount of water was ingested but it was taken during and immediately following the meals. The feces data include dry matter, nitrogen, fat, ash, and carbohydrate by difference. Simple ether extraction was employed in determining fat. The balance of utilization was 94.5 per cent. in the first period as against 95.1 per cent. in the second. The author draws the negative conclusion that a moderate water ingestion at meal time has no harmful influences on the utilization of the food. He emphasizes the adaptability of the organism and supposes that it is a matter of the rapid absorption of the superfluous water. More specific conclusions than these were hardly justified, since neither diet nor water ingestion was absolutely uniform as to time and amount, the water ingestion being particularly variable; it ranged from 300 to 522 cc. at meal time.

The Feces.—The nature and composition of human feces seems generally to be misunderstood. A recent statement is that the feces are chiefly the unabsorbed residues of intestinal excretions.[10] Another statement is to the effect that the feces consist chiefly of bacteria.[10a] A microscopical examination easily shows, however, that these claims are not true. The composition of feces as given by Schmidt and Strasburger[11] is as follows:

(1) Indigestible material in the food.

(2) Undigested material, which has for some reason escaped the action of the digestive juices.

(3) Residues of the digestive juices.

(4) Bacteria and the products of fermentation and putrefaction.

(5) Products of the epithelial wall, such as decayed cells, leucocytes, etc.

Fats are almost always found in feces, the amount being increased by an increase in the fats in the food. In addition to the food as a source of fat are the digestive juices and the cells of the alimentary epithelia which contain both fats and lipoids.

Many investigators believe that the percentage utilization of a given foodstuff in an available diet is a subject whose importance has been exaggerated. It is said that the percentage differences are so small as to be inconsiderable, particularly in view of the fact that only small quantities of a given substance are involved. Perhaps from the standpoint of the mere existence of the organism this may be true, but the question of continued efficiency is not a negligible one. It seemed probable, at least, that an examination of the feces with regard to their content of fat might give an indication as to the efficiency with which the fat of the food was digested under the influence of water ingestion with meals.

Description.

General Plan.—The general plan of these experiments was to determin in a preliminary period the digestibility of fat in subjects living on a uniform diet. During a second period, with no change in diet, a given volume of water was to be added to that taken normally with each meal, and in a final period the conditions of the preliminary period should again obtain.

The subjects of the experiments were normal men, on the staff of assistants in the Department of Chemistry. The daily periods began and ended at 7 A.M., and the program was as follows: Body weights were taken at 7 A.M., after urinating and defecating. So regular the routine became that in only two or three instances throughout the eight to nine weeks of the experiments defecation did not come at this time. To insure accuracy, body weights were always taken without clothing. The morning meal was taken at 7.30, the noon meal at 12 or 12.15, and the evening meal at 5.30 or 5.45. The three meals were identical and consisted of graham crackers, butter, peanut butter, milk and water. Smaller quantities of water were taken at stated hours during the day. The men went about their duties as usual throughout the day and evening.

The urine was collected in 24-hour samples, the last portion being that passed before weighing in the morning. The urine was analyzed for total nitrogen, ammonia, urea, creatinine, creatine,[11a] total and ethereal sulfates, and indican.[11b]

The analysis of the feces was made on each individual stool. As passed it was weighed and thoroughly mixed until uniform throughout. The samples for analysis were then weighed out as quickly as possible to prevent loss by evaporation, which is very rapid. Charcoal was used as a "marker" to facilitate the separation of the feces of different periods; where the uniformity of the diet is not to be interfered with this method is the most satisfactory. One or two capsules (0.2 gram) of finely divided charcoal were taken before breakfast on the day beginning a new period. With but few exceptions the separations thus obtained were very distinct and entirely satisfactory.

The length of time between the taking of food and the appearance of the feces therefrom has been variously given. A recent statement is that particles fed to a man are not usually passed in his feces for two or three days.[10] The observations of the present experiments support the opinion as given by Strasburger[13] that normally this period is 24 hours. Throughout these experiments the charcoal given on one morning appeared in the last portions of the feces passed the next morning, in all but two cases, in both of which the separations were from an ordinary mixed diet, that is, at the beginning or at the end of the experiment.

Methods.—All analyses were made on fresh feces without previous drying, and were always made in duplicate unless the amount of material available was not sufficient. The analysis of the fresh stool[12] to our mind is the ideal method of feces examination. Certainly in view of Shimidzu's findings, mentioned below, we can place no dependence upon data obtained from the analysis of the dried sample.[13a] The analysis of each individual stool in the fresh condition of course demands the expenditure of much more time and energy than are necessitated in the analysis of composit samples of dried feces. However, the added accuracy and the greater value of the data obtained by means of the "fresh" procedure certainly warrant the extra effort.

The method selected for the determination of fat was that proposed and developed by Kumagawa and Suto[14] with the modifications added by Inaba.[15] This method, above all others, yields a product that can be considered to be more nearly pure fat than that yielded by any other methods of extraction.[1] The method as described by its authors is carried out upon air-dried materials but for our determinations no air-drying was employed. Shimidzu[15a] has shown that the drying of tissues on the

[1] This statement does not apply to the method of Folin and Wentworth (*J. Biol. Chem.*, **7**, 421–6 (1910)), with which we have had no experience. It appeared after the completion of this work.

water bath previous to the determination of their fat content by this method causes a loss of fat which may exceed 10 per cent. The loss is probably due to oxidation. It is probable, therefore, that in the determination of fat in feces by this method, the use of fresh material without previous drying yields most accurate results. The procedure involves the saponification of 5–10 grams of fresh feces by a 5 *N* sodium hydroxide solution for several hours; this is overneutralized with 20 per cent. hydrochloric acid, taking care to keep the mixture from becoming hot, and the acid liquid is extracted with ether. Any precipitate remaining is dissolved in hot normal sodium hydroxide, heated for about 15 minutes and extracted with ether; the acid aqueous solution that was first drained off is added and all the fat and fatty acid remaining go over into the ether portion. The combined ethers are evaporated, the residue purified by absolute ether and lastly by petroleum ether, and dried at 60° to constant weight. The fatty acids so obtained were crystallin and almost colorless; care in preventing overheating during the first neutralization and a sufficient drying of the last ether residue before taking up in petroleum ether are essential to obtaining them in pure form.

It is evident that by this method the unsaponifiable substances are determined along with the fatty acid and the authors[14] give a satisfactory procedure by which these may be determined. It was shown by Inaba[15] that the unsaponifiable substances in the feces amount to about 10 per cent. of the total fatty acids determined and that a separation of these substances is of importance, if most accurate results are desired. Inasmuch as a uniform diet was fed in these experiments, any difference in the fat content of the feces from one period to another was probably subject to no correction on this account.

Experiments on Copious Water Drinking with Meals.

General Description.—The first experiments on Subjects H and W may now be considered in detail. Subject H was a tall well-proportioned man weighing 70.22 kilograms at the beginning of the experiment. He had been on a diet of comparatively simple variety and as he was not fond of milk and drank neither tea nor coffee, water comprized the chief liquid portion of his diet. Subject W was of smaller stature and weighed 63.2 kilograms at the beginning of the experiment. He was accustomed to the diet as offered by a club table of the better grade and usually drank water sparingly. He regularly smoked a cigar after the evening meal and did so throughout the experiment. Both subjects were put upon the same uniform diet of graham crackers, butter, peanut butter and milk. It contained 180 grams of fat per day distributed for each food and meal as follows:

	Amount.	Fat.
Oatmeal crackers......	150 grams	11.7 grams
Peanut butter.........	20	9.2
Butter................	25	21.1
Milk..................	450 (cc.)	18.0
Water.................	100 cc.	....
		Total, 60.0

In addition, 200 cc. of water were taken at 10 A.M., at 3 P.M., and again in the evening or just before retiring, making a total of 900 cc. of water per day during the three-day preliminary period.[1] On the morning of the fourth day before breakfast charcoal was taken and during the five days following one liter of water was added to the menu of each meal, making 1100 cc. per meal and a total of 3900 cc. per day. On this diet both subjects record a feeling of fulness that sometimes became temporarily slightly uncomfortable. It was necessary to urinate frequently especially during the first few hours after the meal; for a short time after eating there was a desire to remain quiet and inactive, as is the case after any full meal; within three-quarters of an hour or an hour, approximately half the water taken at the meal was voided. Both subjects record that the feeling of fulness and lassitude noted immediately after meals became less marked after the second day of the water period. Both felt perfectly well at all times and had normal appetites. After the fourth day H records that he did not notice the feeling of fulness which followed the high water ingestion of the first few days of the water period.

The period of copious water ingestion lasted five days. On the morning of the sixth day charcoal capsules were taken before breakfast and during that and the two following days the diet of the preliminary period was resumed. The experiment ended with the taking of charcoal on the morning of the fourth day of this period.

Discussion of Results.—The data upon the excretion of fat in the feces during these three periods are given in Tables I and II.

Subject H, Table I.—The data show that the average daily excretion of fat during the preliminary period was 8.37 grams, during the water period 7.16 grams and 9.22 grams during the final period. The digestion and absorption of fat were seemingly more complete during the water period than during the preliminary period and upon the withdrawal of water the excretion of fat rose to an amount that was higher than before the period of water ingestion. A slight gain in weight was recorded,

[1] The water supply (see Fowler and Hawk, *J. Exp. Med.*, 12, 390 (1910)) of this community is from deep wells and for use in these experiments it was softened by the addition of five liters of saturated lime water to thirty liters of the tap water. After standing several hours or a day the precipitate was filtered off. This water had an agreeable taste; its alkalinity was 70 to phenolphthalein, 180 to methyl orange, and its hardness determined by soap solution was 92 parts per million.

70.29 kilograms on the morning of the first day of water and 70.88 kilograms on the morning of the first day after the water. This gain of 600 grams was not lost for at least three months thereafter.

TABLE I.—SUBJECT H.

Preliminary period. 3 days.		Water period. 5 days.		Final period. 3 days.	
Number of stool.[1]	Fat.	Number of stool.[1]	Fat.	Number of stool.[1]	Fat.
1	3.23	5	2.16	10	6.06
2	5.16	6	5.65	11	2.14
3	9.73	7	3.58	12	8.79
4	7.00	8	16.59	13	10.68
Total	25.12	9	7.80	Total	27.67
Average	8.37	Total	35.78	Average	9.22
		Average	7.16		

TABLE II.—SUBJECT W.

Preliminary period. 3 days.		Water period. 5 days.		Final period. 3 days.	
Number of stool.	Fat.	Number of stool.	Fat.	Number of stool.	Fat.
1	9.22	5	5.80	11	5.56
2	10.60	6	1.31	12	7.76
3	3.85	7	5.98	13	1.84
4	7.00	8	6.41	14	6.34
Total	30.67	9	7.01	Total	21.50
Average	10.22	10	2.64	Average	7.17
		Total	29.15		
		Average	5.83		

Subject W, Table II.—During the preliminary period there was an average daily excretion of 10.22 grams of fat in the feces. During the water period this was reduced to an average of 5.83 grams per day and in the final period it rose to 7.17 grams per day, an amount only slightly above that of the water period. From these data it would appear that during the period of copious water drinking the fats of the food were more completely digested and absorbed than either before or after this period and that this effect of the water drinking was not temporary but more or less permanent. In the case of W also a slight gain in weight accompanied the experiment. On the morning of the first day of water his weight was 63.46 kilograms; at the end of this period it was 64.16 kilograms. This gain of 700 grams might be attributed to retained water, except for the fact that it was not lost subsequently. After the lapse of three months, during which time the subject was on an ordinary mixed diet, his weight was identically the same as at the end of the water period of this experiment. While great significance can not be attached to so small a change in weight, even granted that it is not due to water, it must nevertheless be borne in mind that the diet throughout the experiment was absolutely uniform with the exception of the water ingestion.

[1] Weights of all stools are included in the third paper of the series.

It seemed reasonable to assume that the decreased excretion of fat during the water period was due to more complete utilization as a result of the large volumes of water ingested, and several explanations could be suggested. Of first importance was the direct stimulating effect of water upon the digestive juices. In his first experiments on dogs Pavlov[3] found that a large amount of water (500 cc.) caused a flow of gastric juice, while a small amount (150 cc.) in half the cases observed had not the least effect. He states that the important factor is a prolonged and widely spread contact of water with the gastric mucous membrane. This contact can hardly be called prolonged because of the rapid passage of water through the pylorus; this very circumstance, however, might make a large volume of water effective as against a small volume in that the former did secure a more widely spread contact than the latter, and perhaps also for a slightly longer period of time. To obtain further information on this point it was considered worth while to make another experiment upon the effect of a smaller amount of water taken with meals, but whose use should extend over a longer period of time.

Experiments on Moderate Water Drinking with Meals.

Description, Methods, Etc.—The plan of the experiment was exactly the same as that of the previous one. Two subjects were maintained on a uniform diet of small water content for several days. Then during a period of ten days in which the same diet was continued, 500 cc. of water in addition to the usual amount were taken with each meal. In the final period the conditions of the preliminary period were again in force. The daily routine was the same as in the preceding experiment. Charcoal was used to separate the feces of the different periods and the analyses were made on each individual stool in a fresh condition.

After an interval of about three months W, of the preceding experiment, again served as subject. In the meantime he had been at the same table as before, had had much the same kind of food, and in general the same dietary habits with the exception that he had formed the habit of taking more water with his meals than before the first experiment. His weight at the beginning of this experiment was 64.18 kilograms, almost exactly the same as at the end of the first experiment.

Subject E was of the average build and weighed 73.6 kilograms. His habits of eating were irregular. During the previous year he had for a time lived on one substantial lunch-counter meal a day,[1] later on two, and during the months preceding the experiment on three at a regular table. He was accustomed, ordinarily, to taking considerable amounts of water with his meals.

The food of each meal, and its fat content were as shown:

[1] Howe, Mattill and Hawk, THIS JOURNAL, 33, 570 (1911).

(In grams.)	Amount.	Fat.
Oatmeal crackers	150	12.9
Peanut butter	20	9.2
Butter	25	21.1
Milk	400 (cc.)	16.0
Water	100 cc.	
	Total,	59.2

The diet of W was slightly reduced from what it had been before and was as follows:

(In grams.)	Amount.	Fat.
Oatmeal crackers	125	10.8
Peanut butter	20	9.2
Butter	25	21.1
Milk	400 (cc.)	16.0
Water	100 cc.	
	Total,	57.1

In addition, each man took 200 cc. of water at 10 A.M., at 3 and at 8.30 P.M., making a total water ingestion of 900 cc. per day during the preliminary period. During the water period the addition of 500 cc. of water to each meal made the total water ingestion 2400 cc. per day during that time.

Discussion of Results. Subject W.—On the diet as given, some little difficulty was experienced in obtaining nitrogen equilibrium in the preliminary period. Charcoal was taken on the morning of the eighth day, but for the sake of keeping uniformity in the feces data it seemed best not to change the diet. Six days passed and on the morning of the fourteenth day charcoal was again taken and water added to the regular diet. The separation of the preliminary period into two parts proved to be a very important incident in view of what the feces data show (Table III).

TABLE III.—SUBJECT W.

I. Preliminary period. 7 days.		II. Preliminary period. 6 days.		Water period. 10 days.		Final period. 5 days.	
Number of stool.	Fat.	Number of stool.	Fat.	Number of stool.	Fat.	Number of stool.	Fat.
1	12.30	7	4.20	14	1.84	24	3.54
2	3.19	8	7.47	15	10.18	25	6.58
3	4.23	9	8.07	16	4.70	26	3.36
4	4.71	10	10.52	17	9.70	27	8.56
5	12.20	11	3.21	18	3.89	28	5.54
6	5.06	12	5.97	19	7.54	29	4.08
Total	41.69	13	1.90	20	1.74	Total	31.66
Average	5.96	Total	41.24	21	11.59	Average	6.33
		Average	6.89	22	7.85		
				23	8.72		
				Total	67.75		
				Average	6.78		

During the time that intervened between his two experiments Subject W, as has been mentioned, while on an ordinary mixed diet, continued the habit of taking considerable water with his meals. As is evident from the diet of the preliminary period the amount of water taken was small and was, in fact, much less than he was accustomed to use. While this restricted amount of water did not immediately make itself felt in the first few days of the experiment, it did begin to show in the latter part of the preliminary period by a seemingly less complete digestion and absorption of fat. This is evident in an increase in the average daily fecal output of fat during the second part of the preliminary period. The average daily amount of fat excreted in the first part of this period was 5.96 grams as against 6.89 grams in the second part. Since the charcoal separation of this preliminary period into two portions was clear and definit this increase in fat in the feces during the latter part seems to mean a less efficient digestion and utilization of the fat of the food. That this evidence did not appear until some days after the amount of water had been reduced indicates, as in the first experiment, that the beneficial effect which water had upon digestion and absorption did not cease with the withdrawal of water, but was more or less permanent beyond the time during which water was taken with the meals. The evidence given by this finding was entirely unlooked for and seems to be of great importance.

Attention should also be called to the comparison of the fat data of this preliminary period with those of the preliminary period of the first experiment, Table II. The average daily amount of fat excreted in the preliminary period of the first experiment was 10.22 grams as against 5.96 in the second. The average percentage utilization of fat in the former was 94.3 per cent. as against 96.5 per cent. in the latter. These data showing so pronounced an improvement in the digestion and utilization of fat are on an individual living on the same kind of food, but separated by a period of three months in which water drinking with meals was practiced. From these results the conclusion as to the effect of water drinking with meals upon the utilization of fat is further strengthened.

It is further seen in Table III that the average daily excretion of fat in the preliminary period, 6.89 grams, suffered but little change in the water period, 6.78 grams, but was slightly decreased, 6.33 grams, in the final period. Just why this decrease should have come in the final period rather than during the water period is not clear. Perhaps there is a lag in the appearance of the results of water drinking, just as it has been shown that its effects are more or less permanent. In this case the moderate amount of water may have had a stimulatory effect that was not evident during the water period but made itself felt during the period following. The question of individuality probably enters in also. From a study of the data on Subject W during this experiment it may be concluded that the effect of moderate water drinking with meals upon digestion is in the same direction as that of copious water drinking but somewhat less marked.

Subject E.—An examination of Table IV shows the variations in fat excretion from one period to another to be small although similar to those obtained before. The output of fat fell from 6.61 grams per day in the preliminary period to 6.39 grams per day under the influence of moderate water drinking, and again rose to 6.70 in the final period.

Again it appears that the effect of drinking water in moderate amounts with meals is in the same direction as when large amounts are used, although the differences observed are of a smaller order of magnitude; as with the copious amounts, absolutely no harmful effects were to be observed. With moderate amounts of water the inconvenience of disposing of an unusual quantity of liquid after the meal was removed, and the lethargic effects of a full meal, such as were noted under the experiment on copious water drinking, were also avoided.

TABLE IV.—SUBJECT E.

Preliminary period. 7 days.		Water period. 10 days.		Final period. 4 days.	
Number of stool.	Fat.	Number of stool.	Fat.	Number of stool.	Fat.
1	3.79	9	3.33	20	2.79
2	1.51	10	5.85	21	5.60
3	8.96	11	3.96	22	7.75
4	11.63	12	8.20	23	7.88
5	3.92	13	9.78	24	2.77
6	8.66	14	6.50		
7	5.20	15	7.07	Total	26.79
8	2.63	16	3.63	Average	6.70
		17	2.90		
Total	46.30	18	9.59		
Average	6.61	19	3.10		
		Total	63.91		
		Average	6.39		

The results just given were obtained on subjects one of whom (W) had lately become accustomed to drinking with meals; the other of whom (E) habitually took considerable water with his meals. In each case the organism, though accustomed to the presence of water in the alimentary tract during digestion, responded to an increase in its amount by a better utilization of the fat of the food. The results obtained, therefore, probably represent the minimum rather than the maximum effect that may be obtained by moderate water drinking with meals, and are such as might safely be expected in any individual, but especially in one not accustomed to drinking water under these conditions.

The Effect of Copious Water Drinking with Meals upon an Habitual Water Drinker.

At this point an answer was sought to the question as to whether a very large water ingestion with meals would show its effect upon digestion even though relatively large amounts of water were habitually taken at meal time. For this investigation Subject E seemed very well fitted; during the experiment on moderate water drinking he had frequently made it his boast that he was not drinking more water with his meals during the water period than was his custom. It seemed advizable therefore to try upon E the effect of such amounts of water as would be copious for his digestive mechanism.

Description.—Continuing with the same diet as in the final period of the previous experiment, a period of six days was made the preliminary period for this experiment. During the five days following this period an addition of one and one-third liters of water was made to the water ingestion of each meal. This is a larger amount of water than was used in the first experiment on copious water drinking, where only 1000 cc. additional were taken with each meal. A final period of three days closed the experiment. On the very first day of this large water ingestion Subject E records that he had no trouble in drinking all of the water, nor was any discomfort experienced throughout the experiment.

TABLE V.—SUBJECT E.

Preliminary period. 6 days.		Water period. 5 days.		Final period. 3 days.	
Number of stool.	Fat.	Number of stool.	Fat.	Number of stool.	Fat.
1	2.75	8	3.17	14	5.58
2	4.28	9	1.84	15	3.87
3	10.87	10	11.35	16	8.69
4	5.90	11	4.46	17	0.82
5	7.44	12	7.33	Total	18.96
6	8.59	13	3.53	Average	6.24
7	1.97	Total	31.68		
Total	41.80	Average	6.34		
Average	6.97				

Discussion of Results.—Table V shows that the average daily excretion of fat in the preliminary period, 6.97 grams, fell to 6.34 grams in the water period, and the daily average value for the final period, 6.24 grams, was even slightly less than for the water period. The effect of copious water drinking with meals is seen to be in the same direction when the organism is accustomed to water drinking as when it is not, except that when water drinking with meals is habitual the results are less striking than otherwise.

Inferences and Discussion.

All of the observations made have pointed to a decreased elimination of fat in the feces when water was taken with meals, indicating a more complete utilization of the fat of the food than without the water ingestion, and in most instances the evident better digestion continued for several days beyond the period during which an increased water ingestion was practiced. A large amount of water was more efficient in this regard than a small one and a more pronounced result was obtained in persons not used to water drinking with meals than in those for whom it was habitual.

The results of our experiments warrant more than a negative conclusion. The ingestion of water along with the food secures a better utilization of the fat of the food as shown by a diminished excretion of fat in the feces. It is possible to explain this result on the basis of four different facts.

(1) **The Stimulating Action of Water upon the Gastric Secretion and Independently upon the Secretion of Pancreatic Juice and Bile.**

The facts observed by Pavlov and his co-workers[3] mentioned above, and the findings of Foster and Lambert[4] as to the stimulating action of water upon the gastric secretion in dogs have also been observed in human beings with gastric and esophageal fistulas. In some of the older investigations it was shown[16, 17, 18] that a purely psychic secretion, such as is noted in dogs, is not as pronounced in man as in these animals. A pleasant taste of food in the mouth caused a flow of gastric juice in some instances, but whether, in general, such a secretion of gastric juice in man arizes indirectly through stimulation carried by the blood or by the nerves, or whether it is due directly to the contact of substances with the mucous membrane of the stomach is uncertain. The observations of Bogen,[19] Kaznelson,[20] and Sommerfeld[21] upon patients with gastric fistulas have clearly demonstrated a psychic secretion. Most varied stimuli through taste, smell, and sight of food, and through sounds associated with the preparation of food called forth a secretion of gastric juice. In the subject examined by Lavenson[22] no psychic secretion was demonstrable but water was found to be a definit though not powerful stimulus. Sommerfeld[21] was able to show that water had a stimulating action upon the gastric secretion, and further, that the mere drinking of water, after the manner of sham feeding, caused a flow of gastric juice. It is claimed that saliva is not a factor in inducing gastric secretion. The evidence adduced by Hemmeter[23] as to a salivary hormone producing increased flow of gastric juice could not be verified by Loevenhart and Hooker.[24] The stimulating factor may be mastication itself, including the taste phenomena and also the desirability of the food.

In human beings as well as in the lower animals investigated the acidity of the gastric juice is found to vary with the kind of food. The findings of Foster and Lambert[4] on dogs with accessory stomach indicate not only a more voluminous but also a more acid secretion when water is taken with food and they suggest an automatic control in the stomach, such that the chyme, no matter what its state of dilution, always has the same optimum acid concentration. The increased acidity noted in the accessory pouch may not actually exist in the stomach proper; here, by dilution, the acid concentration may remain unchanged. Certain other experiments reported from this laboratory[24a] apparently confirm this view. If the stimulation of water is entirely a chemical one, however, it is difficult to see why the mucosa of the pouch, which is not in contact with the water, should respond as readily as the stomach itself, even though it has the same nerve and blood supply. Any effect which the accessory pouch shows may possibly be less marked than the one actually secured in the stomach proper.

Whether an increased acidity and digestive power of the gastric juice is of immediate importance in the digestion of fat is not clear. The cleavage of fat by gastric lipase is very minimal in the normal acid reaction of the stomach except when the fats are in the form of a natural emulsion. London and Versilova[25] showed that in dogs the cleavage of fat fed in such a form (egg-yolk) rose as high as 32 per cent. in the stomach, due in part to gastric lipase and in part to regurgitated duodenal juice. A similar observation has been made recently by Levites.[26] Kaznelson[20] found a lipase in the gastric juice of her patient. According to Lavenson's observations[22] a regurgitation of bile and pancreatic juice in the stomach occurred with great constancy when oil was given.

No absorption of fat takes place in the stomach. In the experiments of London and Versilova[25] where one-third of the fat administered was split in the stomach no absorption took place until this material reached the ileum. As a result of their findings Camus and Nicloux[27] and also Stire[28] emphasize the unimportance of gastric lipase. The fats undergo practically no change in the stomach, and when they do it is as a result of a regurgitated duodenal secretion.

In view of these facts the importance, for fat digestion, of the greater quantity of gastric juice, or a greater acidity, or both, as a result of the stimulating action of water with the food is to be sought rather in the effect upon the secretion of the bile and the pancreatic juice.

(2) The Acid Chyme as an Excitant for the Flow of Pancreatic Juice and Bile.

The formation of secretin from prosecretin is the result of an acid reaction of the duodenum; the larger the amount of acid the greater the stimulation given to the secretory action of the pancreas, and the flow of bile is regulated by the same mechanism. That the efficiency of bile in aiding the digestion of fats by pancreatic lipase is due to the bile acids has again been shown recently by Terroine.[29] The same investigator has also shown[30] that at an optimum concentration of pancreatic juice and of esters or whatever other substances are undergoing cleavage, the hydrolysis is activated proportionately by increasing quantities of bile salts.

Fat in small amounts is a regular constituent of pancreatic juice and especially of the bile, and an increase in these secretions should cause increased elimination of fat in the feces unless a compensation was found. This increased excretion is not found, but, on the contrary, a constant *decrease* is observed under the influence of water drinking with meals. It follows from this that the digestibility of fat during the period of water drinking with meals was increased even beyond what the data indicate, since part of the excreted fat might come from the larger amounts of digestive juices secreted under the stimulating influence of water. And furthermore inasmuch as the fat values for the stools derived by the Kumagawa-Suto technic include any cholesterol present, the increased output of biliary cholesterol during the water period would also be a factor tending toward an apparently augmented output of fecal fat during this interval.

(3) Heightened Peristalsis and Increased Blood Pressure as Factors in the More Complete Digestion and Utilization of Fat.

Peristalsis is known to increase with the volume of material within the intestin. Whether a large amount of a liquid mass is as efficient in this regard as an equal bulk containing less water is uncertain. The effect of dilution and increased peristalsis brought on by purgatives was shown by Ury[31] not to increase the amount of soluble foodstuffs or of their products in the feces. His observations were limited to soluble protein and to sugar.

Water begins to pass the pylorus very soon after its ingestion, and is quickly absorbed. During the time that this water is in the tissues and is flowing in the blood stream, the increased volume causes a rise in blood pressure similar to the rise regularly following a meal. In duplicate feeding experiments on a dog with gastric fistula Dobrovolskii[32] found that after bleeding there was an almost complete stoppage of the process of digestion during the first 3 hours, due in part to the decreased blood pressure. No measurements of blood pressure were made during these experiments on water drinking; it seems reasonable to assume, however, that a greater blood pressure and a consequent stronger and more rapid heart beat might also be factors in the more complete absorption of the fat of the food when water is taken with meals.

Both of these factors, peristalsis and blood pressure, are to be investigated as to the effect which large volumes of water with meals have upon them.

(4) Dilution as a Factor in More Complete Utilization.

More important than any of the other factors, probably, is this one of dilution. Like all other chemical reactions, those brought about by enzymes are reversible. Governing these is the general principle expressed in LeChatelier's theorem which states that when a system in equilibrium is subjected to a constraint by which the equilibrium is shifted, a reaction takes place within the system which opposes the constraint, *i. e.*, one by which its effect tends to be destroyed. Processes within the system tend to counteract the effect of external changes. Thus the dilution of any solution in which the reaction $AB \rightleftarrows A + B$ had come to equilibrium would result in the formation of further amounts of A and B in order to increase the total concentration of dissolved material by way of counteracting the effect of dilution. The reaction would be driven toward the right and would be brought more nearly to completion. Looked at in another way such a reversible reaction as given above reaches an equilibrium whose constant is expressed by the equation $C_a \times C_b/C_{ab} = K$. The numerical value of this fraction as expressed by K remains unchanged whatever the total concentration of the solution may be. If the solution is diluted, causing a reduction in all three of the terms C_a, C_b and C_{ab}, the values C_a and C_b must diminish relatively less rapidly than C_{ab} in order that K should remain the same. And in order to accomplish this some of the substance whose concentration is C_{ab} is transformed into the substances whose concentrations are C_a and C_b. A concentrating of the solution would have the opposit effect. Now all of the hydrolytic cleavages occurring in digestion are of the type $AB \rightleftarrows A + B$, *i. e.*, a single substance is broken up into two or more products, and such reactions are brought the more nearly to completion the greater the dilution in which they take place. The corresponding synthetic reactions are accomplished by beginning with a high concentration of the corresponding decomposition products.

The failure to provide for the removal of the end-products, either by dilution or by dialysis, has often been shown to prevent a reaction from going to completion. In experiments upon the saponification of fats by pancreatic juice obtained by fistula from a dog Terroine showed[33] that the addition of oleic acid or of sodium oleate to olein emulsions rendered saponification of the latter more difficult. The addition of glycerol to suspensions of olive, castor, and cottonseed oils had the contrary effect of making them much more readily saponifiable, but this was due to the physical effect of the glycerol in making the emulsions more complete and more permanent by virtue of decreasing the surface tension. In his investigations upon human pancreatic juice Bradley[34] found that the undiluted juice acted on ethyl butyrate less rapidly than when diluted

1 : 10. The optimum dilution was found to lie between 1 : 15 and 1 : 20. In experiments on the effect of bile salts on pancreatic juice Terroine[35] found that the action was a physico-chemical one, directly upon pancreatic juice, that if the time of the action was prolonged digestion was retarded, and that if still more prolonged digestion was inhibited. Shorter periods resulted in maximum digestion.

In the light of these facts the better absorption and more complete utilization of the fats attendant upon water ingestion with meals are a result of the greater completeness of the hydrolytic cleavage under the influence of dilution and of the accompanying more rapid removal of the end-products.

Summary.

Experiments were performed on men living on a uniform diet; a preliminary period of small water ingestion was followed by a period of large water ingestion with meals, and this, in turn, by a final period with the original conditions.

When one liter of water additional was taken with meals the average daily excretion of fat in the feces was much reduced below that found when a minimum amount of water was taken with meals; one and one-third liters had a like effect; a similar but less marked reduction was observed when 500 cc. of water were taken with meals.

The decreased excretion of fat observed during water drinking with meals was usually evident for a number of days after water had ceased to be taken in large or moderate amounts with meals indicating that the beneficial influence of water was not temporary but was more or less permanent.

A slight gain in weight accompanied the water drinking and this gain was not subsequently lost.

After several months of moderate water drinking with meals a pronounced improvement in the digestibility of fat was observed, the percentage utilization having risen from 94.3 to 96.5.

The better digestion and absorption of fat was probably due to the following factors:

(1) Increased secretion of gastric juice and independently of pancreatic juice as a result of the stimulating action of water.

(2) Increased acidity of the chyme bringing about a more active secretion of pancreatic juice and bile.

(3) Increased peristalsis due to larger volume of material in the intestin and increased blood pressure due to rapidly absorbed water.

(4) A more complete hydrolysis of the fats by lipase due to increased dilution of the medium and consequent more rapid absorption.

REFERENCES.

1. Carrington, "Vitality, Fasting and Nutrition," p. 397 (Rebman, N. Y., 1908). 2. Fischer, "Physiology of Alimentation," p. 183 (Wiley, 1907). 3. Pavlov, "The Work of the Digestive Glands," translated by Thompson, second edition, p. 112 (Griffin & Co., London, 1910). 3*a*. Heidenhain, quoted by Pavlov, *Ibid.*, p. 130. 3*b*. Sanotskii, quoted by Pavlov, *Ibid.*, p. 112. 4. Foster and Lambert, "Some Factors in the Physiology and Pathology of Gastric Secretion," *J. Exp. Med.*, 10, 820 (1908). 5. Pavlov, *Ibid.*, p. 144. 6. Togami, "Influence of Certain Foods and of Emotion on Pancreas Secretion," *Z. physik-diät. Ther.*, **12,** 453 (1908); through *Biochem. Centr.*, 8, 202. 6*a*. Hawk, *Arch. Int. Med.*, **8,** 382 (1911). 7. Wohlgemuth, *Berl. klin. Wochschr.*, **47,** 92 (1910). 7*a*. Fairhall and Hawk, unpublished. 8. von Mering, "Ueber die Funktion des Magens," *Verhandl. des Congresses f. innere Med.*, **12,** 471–82 (1893). 8*a*. Cohnheim, *Münch. med. Wochschr.*, **54,** 2581 (1907). 8*b*. Kaufmann, *Z. Heilk.*, **28,** 203 (1907). 9. Ruzicka, "Ein Selbstversuch über Ausnutzung der Nährstoffe bei verschiedenen Quantitäten des mit dem Mahle eingeführten Wassers," *Arch. Hyg.*, **45,** 409–16 (1902). 10. Lusk, "Science of Nutrition," second edition, p. 46 (Saunders, 1909). 10*a*. Strasburger, *Z. klin. Med.*, **46,** 413–44 (1902). 11. Schmidt and Strasburger, "Die Faeces des Menschen," p. 1 (Berlin, 1905). 11*a*. Howe and Hawk, unpublished. 11*b*. Hattrem and Hawk, *Arch. Int. Med.*, **7,** 610 (1911). 12. Howe, Rutherford and Hawk, "The Preservation of Feces," THIS JOURNAL, **32,** 1683–6 (1910). 13. Schmidt and Strasburger, *Ibid.*, p. 17. 13*a*. Mendel and Fine, *J. Biol. Chem.*, **10,** 309 (1911). 14. Kumagawa and Suto, "Quantitative Bestimmung des Fettes und der unverseifbaren Substanzen in tierischem Material," *Biochem. Z.*, **8,** 213–347 (1908). 15. Inaba, "Fettbestimmungen der Fäces und einigen Nahrungsmittel nach der Methode von Kumagawa-Suto," *Biochem. Z.*, **8,** 348–55 (1908). 15*a*. Shimidzu, *Biochem. Z.*, **28,** 237–73 (1911). 16. Hornborg, dissertation Helsingfors, 1903. Quoted by Hammarsten (Mandel), "Textbook of Physiological Chemistry," p. 440 (Wiley, 1911). 17. Umber, "Die Magensaftsekretion des Menschen bei Scheinfütterung und Rektalernährung," *Berl. klin. Wochschr.*, **42,** 56 (1905). 18. Cade and Latarjet, *Compt. rend. soc. biol.*, **57,** 496 (1904). 19. Bogen, *Arch. ges. Phys.* (*Pflüger*), **117,** 150 (1907). 20. Kaznelson, "Scheinfütterungsversuche am erwachsenen Menschen," *Arch. ges. Phys.* (*Pflüger*), **118,** 327–52 (1907). 21. Sommerfeld, "Zur Kenntniss der Magensaftsekretion," *Arch. Kinderheilk.*, **49,** 1–15 (1909). 22. Lavenson, "Observations on a Child with Gastric Fistula," *Arch. Int. Med.*, **4,** 271–90 (1909). 23. Hemmeter, "Die Wirkung der Totalexstirpation sämmtlicher Speicheldrüsen," *Biochem. Z.*, **11,** 238–59 (1908). 24. Loevenhart and Hooker, *Proc. Soc. Exp. Biol. Med.*, **5,** 114–7 (1908). 24*a*. Wills and Hawk, *Proc. Am. Soc. Biol. Chem.*, II, p. **xxix** (1911). 25. London and Versilova, "Die Spaltung emulgierter Fette im Darmkanal des Hundes," *Z. physiol. Chem.*, **56,** 545–50 (1908). 26. Levites, "Verdauung der Fette im tierischen Organismus," *Z. physiol. Chem.*, **57,** 46–8 (1908). 27. Camus and Nicloux, *Compt. rend. soc. biol.*, **68,** 619–22 (1910). 28. Stire, *Cent. ges. Phys. Path. Stoffw.*, **4,** 889–90 (1909). 29. Terroine, *Compt. rend. soc. biol.*, **68,** 439–41 (1910). 30. Id, *Ibid.*, **68,** 518–20, 666–8 (1910). 31. Ury, "Das Vorkommen von gelösten Substanzen in den Fäces bei gesteigerter Darmperistaltik," *Salkowski Festschr.*, 385–96; through *Jahresb. Tierchem.*, **34,** 518 (1904). 32. Dobrovolskii, *Z. physiol. Chem.*, **56,** 408–16 (1908). 33. Terroine, *Biochem. Z.*, **23,** 404–28 (1909). 34. Bradley, "Human Pancreatic Juice," *J. Biol. Chem.*, **6,** 133–71 (1909); *Ibid.*, **8,** 251 (1910). 35. Terroine, *Compt. rend. soc. biol.*, **68,** 754–5 (1910).