LV. THE INFLUENCE OF EXCESSIVE WATER INGESTION ON PROTEIN METABOLISM.

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A number of observations have been made upon the effects of increased ingestion of water on the output of nitrogen. As the various results obtained by previous workers are referred to later, it will suffice here to state that there is almost unanimous agreement that if water be freely exhibited to an animal in nitrogenous equilibrium there accompanies the consequent diuresis an increased excretion of nitrogen. As to whether this increased nitrogenous excretion signifies a mere mechanical flushing out of waste products or an accelerated catabolism of protein, opinion has been sharply divided. Fowler and Hawk [1910] undertook an investigation on the subject and extended the field of observation to include creatine and creatinine. They found regarding these, that the addition of three litres of water per day produced a decrease in the output of creatinine and led to the appearance in the urine of considerable amounts of creatine.

The primary object of the present research was to investigate the accuracy of these findings. In view of the fact, however, that most of the earlier workers conducted their experiments on animals and limited their observations either to the total nitrogen or the urea, it was thought that further information might be obtained by making a more complete urinary analysis in the human subject and by varying the protein intake in the different experiments.

METHOD.

The subjects of the experiments, normal healthy males, were given a fixed creatine-free diet for several days, until the daily excretion of the various nitrogenous constituents of the urine had become approximately constant.

Thereupon, in addition to the diet, a given quantity of water was drunk each day for three days, whereafter, the diet was continued for several days more without the extra water. Some of the water was taken with the meals and the rest in the intervals between. The protein content of the food was varied in the different experiments from 27 to 319 grams per day. The urine was collected in 24 hour samples, the collection being completed at 9 a.m. before either food or water had been taken for the day.

In one of the experiments, No. IV, the water taken during the preliminary period was fixed in amount. The daily requirements however, as indicated by the inclination of the subject to drink, were not uniform. In all the others therefore, to ensure that the conditions on the pre-water days would be as natural as possible, the amount taken was not restricted. It was considered that this procedure would give a more perfectly normal period for comparison with the period of excessive consumption, and that the volume of the urine would indicate the average daily intake, with sufficient accuracy for the purposes of the research.

The methods of analysis adopted were: total nitrogen—Kjeldahl: ammonia—Folin: urea Exp. II, III and IV,—Folin, I and V,—the urease method described by Plimmer and Skelton [1914]: amino acids—Sörensen's formalin titration method: creatine and creatinine—Folin.

PROTOCOLS.

Experiment I. Very low protein diet.

Diet. In grams, apple 100, banana 150, potato 400, butter 75, sugar 75, cocoa 10, bread 200, containing approximately protein 27 g., fat 70 g., carbohydrate 325 g.

Subject aet. 33; weight at beginning of exp. 67·8, at end 65·8 kilos.

The diet was continued for a preliminary period of eight days. On the 9th, 10th and 11th days three litres extra of water were drunk. Owing to the rapid loss of weight of the subject the experiment was stopped on the 12th day.

The results of the first six days are omitted, the only points of interest being a continuous loss of weight, an excretion of nitrogen in excess of the intake and a uniform decrease of creatinine from 1·367 to 1·320 grams per day.

TABLE I.

Day of exper.	Weight kilos	Urine c.c.	Tot. N. grs.	Urea N. grs.	NH_3 N. grs.	Amino Acid N. grs.	Creatinine grs.	Creatine grs.	Per cent. of Tot. N. as urea	Remarks
7	65·97	412	6·300	4·151	0·259	0·113	1·320	—	65·9	Fixed diet
8	65·97	405	6·300	4·157	0·185	0·143	1·328	—	65·9	„
9	65·80	2830	8·610	6·629	0·231	0·163	1·330	—	76·9	3 lit. H_2O extra
10	66·02	3545	6·325	4·534	0·266	0·161	1·315	—	71·6	„
11	65·80	3602	6·125	4·388	0·280	0·108	1·313	—	71·6	„

Experiment II. Moderately low protein diet.

Diet. In grams, oatmeal 100, bread 300, butter 80, apple 70, cocoa 10, sugar 100, milk 300 c.c., containing approximately protein 48 g., fat 93 g., carbohydrate 346.

Subject aet. 22; weight at beginning of exp. 57·8, at end 57·7 kilos.

The diet was continued for a preliminary period of seven days. On the 8th, 9th and 10th days three litres extra of water were drunk. On the 11th, 12th and 13th days the diet was continued without extra water. The first five days are omitted from the table, as they show no points of interest. The first post-water day is also omitted, as the analysis showed the day's collection to have been contaminated.

TABLE II.

Day	Body wt. kilos.	Urine c.c.	Tot. N. grs.	Urea N. grs.	NH_3 N. grs.	Amino Acid N. grs.	Creatinine grs.	Creatine grs.	Per cent. of Tot N. as urea	Remarks
6	57·7	600	9·07	6·10	0·342	0·1834	1·350	—	67·3	Fixed diet
7	57·7	670	8·77	5·95	0·347	0·1788	1·421	—	67·8	„
8	58·4	2980	9·83	6·90	0·459	0·3633	1·360	—	70·2	3 lit. H_2O extra
9	58·2	3105	8·96	6·12	0·465	0·1827	1·360	—	68·3	„
10	58·2	3400	9·24	6·41	0·459	0·1970	1·377	—	69·4	„
12	57·7	880	9·38	6·44	0·336	0·1970	1·343	—	67·6	Fixed diet
13	57·7	870	9·46	6·59	0·403	0·1480	1·350	—	69·7	„

Experiment III. Moderately high protein diet.

Diet. In grams, sugar 100, "plasmon" 60, cheese 200, dried skimmed milk 50, bread 400, cocoa 20, butter 50, apple 100, containing approximately protein 160 g., fat 73 g., carbohydrate 359 g.

Subject same as Exp. II, aet. 22; weight at beginning of exp. 57·2, at end 57·4 kilos.

On the 5th, 6th and 7th days three litres extra of water were drunk. On the third post-water day the diet was stopped. The table gives the results from the third day.

TABLE III.

Day	Body wt. kilos	Urine c.c.	Tot. N. grs.	Urea N. grs.	NH_3 N. grs.	Amino Acid N. grs.	Creatin-ine grs.	Creat-ine	Per cent. of Tot. N. as urea	Remarks
3	57·2	1220	17·53	13·98	0·468	0·1274	1·377	—	79·7	Fixed diet
4	57·3	1040	17·47	14·03	0·392	0·1060	1·381	—	80·3	„
5	58·8	3450	19·04	14·54	0·609	0·1000	1·332	—	76·4	3 lit. H_2O extra
6	58·8	3895	19·06	15·87	0·566	0·1081	1·405	—	83·3	„
7	58·9	2730	18·72	14·99	0·549	0·1172	1·343	—	80·1	„
8	57·6	1350	19·49	16·26	0·426	0·2744	1·400	—	83·4	Fixed diet
9	57·4	1220	20·12	16·67	0·440	0·0792	1·340	—	82·9	„

Experiment IV. Very high protein diet.

Diet. In grams, dried skimmed milk 600, cheese 300, bread 300, water 3 litres containing approximately protein 319 g., fat 153 g., carbohydrate 179 g.

Subject aet. 21; weight at end of exp. 57 kilos.

In this experiment the diet was continued for 15 days. On the 5th and on the 9th, 10th and 11th days 9·6 litres of water were drunk in addition to the water used in preparing the food.

TABLE IV.

Day	Urine c.c.	Tot. N. grs.	Urea N. grs.	NH_3 N. grs.	Amino Acid N. grs.	Creatin-ine grs.	Creatine grs.	Per cent. of Tot. N. as urea	Remarks
1	1930	39·82	33·59	0·250	0·241	1·660	—	84·4	Fixed diet
2	1980	40·24	33·83	0·241	0·249	1·480	—	84·1	„
3	2180	42·19	33·90	0·246	0·264	1·563	—	80·4	„
4	2260	43·88	35·03	0·206	0·324	1·554	—	79·8	„
5	7400	40·19	36·41	0·477	0·103	1·533	—	90·6	9·6 litres H_2O
6	2150	34·60	31·71	0·202	0·348	1·588	—	91·4	Fixed diet
7	2110	38·19	35·36	0·283	0·267	1·650	—	92·6	„
8	2040	40·22	36·58	0·299	0·398	1·604	—	91·0	„
9	8370	40·19	37·05	0·576	0·268	1·586	—	92·2	9·6 litres H_2O
10	8500	40·57	36·68	0·563	0·436	1·459	—	90·4	„
11	8070	39·24	36·12	0·499	0·387	1·547	—	92·1	„
12	2440	34·16	31·28	0·252	0·348	1·687	—	91·6	Fixed diet
13	2150	36·34	33·46	0·333	0·279	1·605	—	92·1	„
14	2160	36·89	33·96	0·345	0·285	1·619	—	92·1	„
15	2300	40·40	37·18	0·361	0·363	1·642	—	92·0	„

Experiment V.

Diet creatine-free, containing protein 110, fat 67, carbohydrate 325 g.

In this experiment nitrogenous equilibrium had not been attained before the increased water intake. Its results are omitted in discussing the effects of water on the excretion of total nitrogen.

TABLE V.

Urine c.c.	Tot. N. grs.	Urea N. grs.	NH_3 N. grs.	Amino Acid N. grs.	Creatinine grs.	Creatine grs.	Per cent. of Tot. N. grs.	Remarks
1298	10·885	8·458	0·322	0·243	1·330	—	77·7	Fixed diet
898	12·810	9·940	0·280	0·261	1·340	0·02	77·6	,,
3741	13·583	10·962	0·378	0·257	1·340	0·02	80·7	3 litres H_2O extra
4020	13·02	10·596	0·406	0·229	1·385	—	81·4	

Results.

Total Nitrogen. In all except Exp. IV the increased ingestion of water causes a distinct rise in the excretion of total nitrogen. There is, however, in the degree of the increase a lack of uniformity, which corresponds to the divergent results obtained by different workers. Thus Forster [1878] found an increase of 90 %, Heilner [1906] 40 %, Voit [1860] 25 %, Mayer [1880] 9 %, Gruber [1901] 7 %, Fränkel [1877] 6–12 %, and Salkowski and Munk [1877] about 3 %. Dubelir [1891], Seegen [1871] and Straub [1899] found no distinct increase. Heilner [1906] suggests that the results vary with the amount of food. Voit and Forster worked with fasting dogs, while Dubelir and others used dogs on an ample diet of flesh. In the present series of experiments the greatest increase was obtained in Exp. I, where the protein was abnormally low, whereas in Exp. IV with the high protein intake there was no increase. In Table VI the whole series is compared.

TABLE VI.

No. of exper.	Protein in diet. approx. g.	Excretion of Tot. N. on last pre-water day, g.	Excretion of Tot. N. on first water day, g.	Increase in Tot. N. g.	Increase per cent.
1	27	6·30	8·6	2·3	36·5
2	48	8·77	9·83	1·06	12·1
3	160	17·47	19·04	1·57	8·9
4	319	40·22	40·19	*nil*	*nil*

It is thus seen that the percentage increase of total nitrogen excreted on excessive water ingestion tends to vary inversely with the amount of protein fed.

In Exp. IV there is evidence of a marked retention of nitrogen on the cessation of the excessive water intake. After the single water-drinking day the amount excreted fell from 40·19 to 34·69 grams. After the three-day period of increased water intake it fell from 39·24 to 34·16, and only returned to its former level on the fourth day.

There would appear to be two distinct factors involved, one, whose action is immediate and whose influence is most manifest in protein deficiency, tending to cause a flushing out of nitrogenous end products, and another, whose influence is more prolonged, tending to cause a retention of nitrogen.

Ammonia. There is in every instance a distinct rise in the ammonia output which coincides exactly with the increased water intake. This is in keeping with the results of Fowler and Hawk [1910], who argued that the water stimulated gastric secretion and the increased ammonia represented the amount necessary to neutralise the excess of HCl thereby produced. If this argument were well founded the increase in ammonia output should be more or less parallel with the increase in water drunk. As appears in Table VII, there is no evidence of such parallelism.

The increase of water passing through the system must accelerate the flow of blood through the liver and of lymph through the tissues. In the process of deaminisation, as the NH_2 moiety is detached it would tend to be carried off by the accelerated flow of blood and lymph and excreted as ammonia instead of being converted to urea. If this supposition be correct, the increase in ammonia would be in some degree proportional to the extent of deaminisation taking place in the tissues, which in turn would be influenced by the amount of protein in the food. The following table shows that the increase in ammonia more or less corresponds to the amount of food protein being catabolised.

TABLE VII.

No. of exper.	Protein in diet, g.	NH_3N. Aver. excret. on non-water days, g.	NH_3N. Aver. excret. on water days, g.	Increase g.	Percentage increase
1	27	0·222	0·259	0·037	16·6
2	48	0·345	0·461	0·116	33·6
5	110	0·301	0·392	0·091	30·2
3	160	0·430	0·575	0·145	33·7
4	319	0·273	0·529	0·256	93·8

The increase in ammonia evidently represents a mere flushing-out process, and the amount of the increase depends upon the amount of ammonia being liberated in the liver and other tissues.

Urea. The rise in the urea output is most marked. Both in absolute amount and in percentage of total nitrogen excreted as urea, the increase is maintained throughout the water and the post-water periods. Thus, in Exp. I. on the third water day the total nitrogen falls from 6·3 grams on the pre-water days to 6·125, while the urea rises from 4·154 to 4·388. In Exp. II the average urea output of the post-water days is 6·52 as against 6·03 on the pre-days. In Exp. III the figures are: pre-water 14 g., post-water 16·46 g. In Exp. IV, on the last day of the records, i.e. the fourth day after the period of increased water intake, the total nitrogen output is 40 g. as compared with an average of 41·53 g. in the preliminary period, while the urea, instead of decreasing with the total nitrogen, rises from 34·09 to 37·184 g. The prolongation of the increased excretion of urea beyond the period of increased diuresis is evidence that the increase cannot be accounted for by a mere mechanical flushing out of urea accumulated in the tissues.

Table VIII shows the percentage of total nitrogen excreted as urea in the preliminary period and in the period following the beginning of the increased consumption of water including both the water and the post-water days.

TABLE VIII.

	Average daily excretion in preliminary period			Average daily excretion on water and post-water days			
No. of exper.	Tot. N. g.	Urea g.	Per cent. of Tot. N. as urea	Tot. N. g.	Urea g.	Per cent. of Tot. N. as urea	Increase per cent.
1	6·3	4·154	65·9	7·02	5·184	73·8	7·9
2	8·92	6·025	67·5	9·374	6·492	69·3	1·8
3	17·50	14·005	80·0	19·286	15·666	81·2	1·2
	41·532	34·09	82·8	38·27	35·07	91·6	8·8
5	11·848	9·199	77·6	13·30	10·779	81·0	3·4

It will be seen that the greatest increase in the excretion of urea occurs in Exp. I, where there is a protein deficiency, and in Exp. IV, where the protein intake is excessive.

Amino acids. In Exps. I and II on the low protein diets there is an immediate rise in the excretion of amino acids on the first day of increased water ingestion. In Exp. III with a moderately high protein intake there is a slight drop, and in Exp. IV, where the protein intake is higher, the drop is marked. Table IX compares the series.

TABLE IX.

No. of exper.	Protein intake, g.	Amino acid N. Average in prelim. period	Amino acid N. First water day	Difference
1	26	0·128	0·163	+·035
2	48	0·181	0·363	+·182
5	110	0·255	0·257	+·002
3	160	0·117	0·100	−·017
4	319	0·270	0·103	−·168

While there is an absence of uniformity in the results, they are such as suggest the condition, that with a deficiency of protein in the food the stimulated diuresis due to increased water intake causes an initial washing out of the amino acids, while with an excess of protein the increased water intake causes an initial retention of these. As these results are not maintained, they would appear to be due to the sudden alteration in the amount of fluid passing through the system.

Faecal nitrogen. Only in Exp. I was the collection and analysis of faeces made. The daily evacuation was fairly regular, taking place in the forenoon. The first day's collection was discarded, as they belonged more properly to the preceding period. Those of the first water day were regarded as belonging to the preliminary period and those of the first post-water day as belonging to the water period. The results obtained were:

Preliminary period		Increased water period	
Dried weight	Total nitrogen	Dried weight	Total nitrogen
19·42 g.	0·927 g.	12·9 g.	0·542 g.

These results are in agreement with those of Fowler and Hawk [1910]. Too much importance should not be attached to the results of a single experiment, but even keeping in view the fact that the faecal nitrogen is not all derived from food residues, the results seem to suggest that the increased consumption of water is productive of a more complete utilisation of the ingested protein.

Creatine and creatinine. In Exps. II, III and IV there is on the water days an apparent decrease in the excretion of creatinine. On these days, however, the bulk of the urine was enormously increased and the concentration of the creatinine correspondingly decreased. It was necessary, therefore, to depart from the routine mode of analysis on the water days. In some instances 30 c.c. of urine were taken for analysis instead of 10 c.c., and in other instances, after development of the colour, dilution was carried only to 250 c.c. instead of to 500 c.c. Both of these deviations, which are recommended by Folin [1904] in such cases, in his original description of the colorimetric method are productive of error, showing a reduction in the amount of creatinine found which is quite fictitious. In Exps. I and V the urine from the beginning was diluted to 5,000 c.c. and the dilution and method of analysis rigidly adhered to throughout the experiment. In these, on the water days there appears no decrease in the amount of creatinine excreted. It may be concluded, therefore, that the decrease found in the earlier experiments should be ascribed to faulty methods of analysis. On no occasion was the increased consumption of water followed by the appearance of creatine in the urine. The influence of excessive water ingestion on the excretion of creatine and creatinine has already been dealt with [Burns and Orr, 1914] and need not be further discussed here.

Discussion of Results.

The source of the increased excretion of nitrogen on excessive consumption of water has been much discussed. V. Noorden [1907], after a review of the literature, says: "It all turns on the flushing of nitrogenous end products out of the system." He bases his conclusion largely on the work of Neumann [1899], who found that, as the consumption of water rose, the urinary nitrogen rose, the rise being most marked on the first day and rapidly disappearing, and that on a return to a normal consumption of water there occurred a retention of nitrogen. The initial increased output and subsequent retention have been here verified, but the compared results obtained on the varying protein intakes do not support the view that the increase represents a mere flushing out of end products. If that view were correct, the greatest increase would appear where there existed the greatest amount of end products, which one would expect to be during the period of highest protein intake. We have seen however that it is just in this case that no increase takes place, a result which is in agreement with the findings of those who worked with dogs on a heavy flesh diet, and that, on the contrary, the highest increase is found in Exp. I, where the protein was abnormally low, viz. 27 grams per day, the diet consisting mostly of carbohydrate and fat, in which case one would expect the minimum accumulation of nitrogenous end products liable to be flushed out. While it is not to be doubted that there occurs a flushing-out process, to which the increase of ammonia appears entirely due, the whole of the influence exerted by the water cannot be ascribed to this mechanical cause. The results obtained on the varying protein intakes, together with the fact that the increased percentage of urea continues on the post-water days, after the mechanical flushing-out process has ceased, indicate that the water has a direct influence on the protein metabolism.

Voit [1860] believed that the influence of the water is to produce an increased protein catabolism, a view supported by Forster [1878] and others. In conditions of protein deficiency as in fasting dogs with which Voit worked, such a conclusion appears reasonable. In Exp. IV, however, where the protein of the food is excessive in amount, there appears on the addition of the extra water no increase in the urinary nitrogen, but there occurs a most marked rise both in the absolute amount of urea and in the percentage of total nitrogen excreted as urea, a result obtained in all the experiments. While it is doubtful, therefore, whether in conditions of protein sufficiency excessive consumption of water causes an increase in the amount of protein catabolised, as indicated by the urinary nitrogen, there appears to result in every case a stimulation of the catabolic processes, leading to a more complete disintegration of the protein molecule and the production of those end products whose immediate destination is excretion.

The subsequent marked retention of nitrogen in Exp. IV is in agreement with the results obtained by Neumann [1899] and Fowler and Hawk [1910]. In view of the fact that the retention takes place on the highest protein diet (319 grams per day), when there would be a surplus of circulating protein and non-protein nitrogenous material within the economy, it is improbable that the amount retained, viz. about 8 grams after the one day's water drinking, and in addition to that about 14 grams after the three day's period, would represent an addition to either of these bodies. It is more probable that the retained nitrogen represents an increase in tissue protein. If this supposition be regarded as well founded, we would be able to conclude that the influence of the water is to stimulate both the analytic and the synthetic phases of protein metabolism.

An observation made on the body temperature in Exp. I lends support to this view. During the preliminary period the average temperature was 97·1° F., and during the water days the average was 97·6° F. Heilner [1907] observed an increased production of heat in rabbits on increased water ingestion.

SUMMARY.

The excessive ingestion of water produces :

1. An increased excretion of urinary nitrogen which is most marked on a low protein diet.

2. A retention of nitrogen on the return to normal consumption of water in the case of excessive protein intake.

3. An increase in the percentage of total nitrogen excreted as urea.

4. A marked increase in the excretion of ammonia.

5. No excretion of creatine and no decrease in the excretion of creatinine.

6. A decrease in the faecal nitrogen which is interpreted as indicating a more complete utilisation of the food protein.

It is suggested that the results indicate that the influence of the increased water consumption is to accelerate both the catabolic and the anabolic phases of protein metabolism.

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