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FASTING STUDIES. XII. THE AMMONIA, PHOSPHATE, CHLORIDE AND ACID EXCRETION OF A FASTING MAN.

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Numerous investigations have been made on fasting men and a large amount of valuable information has been collected, though few attempts have been made to correlate the excretion of the substances we wish to consider.

Benedict,[1] in his masterly fasting studies, determined the excretion of phosphates and chlorides. The phosphate output increased to a maximum on the first to fourth day of the fast in various experiments. In general, the chloride excretion was considerable for the first two days, with a marked decrease on the third day. Thereafter, the elimination tended to diminish, but with considerable variation. It was also observed that, where the volume of ingested water was over 1000 cc., the volume of urine was not far from that of the water consumed.

Cathcart,[2] in his work on Beauté, showed an increased excretion of ammonia during the fast, which reached a maximum on the eighth day of the fourteen-day fast and decreased thereafter. The acidity and phosphate output ran parallel, reaching a maximum on the third day and diminishing gradually during the remainder of the fast. The chloride excretion decreased steadily. The amount excreted of all four constituents dropped suddenly to a minimum value, during the starch and cream diet fed subsequent to the fast, and increased toward the normal upon the return to the original high protein diet which was fed previous to the fast. There was a retention of 10 to 11 grams of chlorides.

Van Hoogenhuyze and Verploegh[3] determined the acidity, phosphate and chloride output in a fasting experiment on Tosca. The acidity reached a maximum on the third day of the fast while the phosphate excretion was highest on the fourth. Thereafter there was a general, though irregular, decrease in the output of both constituents. As in Cathcart's experiment, their excretion diminished immediately when food was given. The chloride output decreased suddenly on the first day of the fast and showed marked fluctuations throughout, with the minimum values near the middle of the fast.

Experiments on Succi, in which he fasted thirty days for Luciani[4] and twenty-one days for Ajello and Solaro,[5] show the acidity and phosphate

[1] *Carnegie Publication*, No. **77** (1907).
[2] *Biochem. Z.*, **6**, 109 (1907).
[3] *Z. phys. Chem.*, **46**, 440 (1906).
[4] *Das Hungern.*, *Leipzig* (1890).
[5] *La Riforma Medica*, **9**, [2] 542 (1893).

output running parallel, with a maximum on the fifth day followed by a gradual decrease. The chloride excretion gradually diminished with considerable oscillation. There was a marked retention of phosphates and chlorides during the first feeding days after the latter fast. During the last ten days of the former fast, sugar, gelatine and peptone were fed.

E. and O. Freund[1] report another twenty-one day fast on Succi, in which the acidity increased on the second day and decreased thereafter, while the phosphate excretion was highest on the first day and diminished throughout the fast. The ammonia output decreased during the first three days, then rose to a maximum and diminished to a low value during the remainder of the experiment.

Lehmann, Müller, Munk, Senator and Zuntz[2] publish results of experiments on Cetti, fasting ten days, and Breithaupt, fasting six days. In both cases, the acidity and phosphate output rose to a maximum on the third day and decreased steadily thereafter. The chloride excretion, in the former case, rose to a maximum on the second day and decreased during the remainder of the fast, while in the latter experiment it diminished steadily throughout.

Brugsch[3] determined the various constituents excreted in the last seven days of a thirty-day fast of Succi. He found a high ammonia output (35% of total nitrogen), the chloride and phosphate excretion low, but fairly constant, and the acidity diminishing.

Hoover and Sollman[4] report a case of fasting during hypnotic sleep, in which the phosphate excretion increased to a maximum on the final day and the chloride output decreased to about half its original value during the fast.

Description.

The purpose of this experiment was to determine, if possible, any relations between the ammonia, phosphate, chloride and acid excretion by way of the urine during a seven-day fast and during subsequent low and high protein feeding periods.

The subject (E) was a man weighing 76.6 kilograms at the beginning of the fast and 71.4 kilograms at the end of the period of inanition. He was an instructor in the Chemistry Department of the University of Illinois and had previously[5] been a subject in a fasting experiment of similar length carried out at that university. During the fast, he kept up his daily routine of work as instructor.

The diet, described in a previous paper,[6] for the preliminary and final

1 *Wiener klin. Rundschau,* **15**, 69, 91 (1901).

2 *Virchow's Archiv.*, **131**, supplement (1893).

3 *Zeit. exp. Path. Ther.*, **1**, 419 (1905).

4 *J. Exp. Med.*, **2**, 405 (1897).

5 Howe, Mattill and Hawk, *J. Am. Chem. Soc.*, **33**, 568 (1911).

6 Sherwin and Hawk, *J. Biol. Chem.*, **11**, 169 (1912).

periods, consisted of 600 grams graham crackers, 1350 grams whole milk, 75 grams butter, 150 grams peanut butter, 1050 cc. water (300 cc. at meal time and 750 cc. between meals). This diet contained 21.86 grams of nitrogen (136 grams protein) with an energy value of about 6000 calories, which gave the subject 1.77 grams protein and 80 calories per kilogram of body weight. The fast was seven days in length, with a daily water ingestion of 1500 cc. Following the fast, was a low-protein period of four days during which 5.23 grams nitrogen or about one-fourth of the regular diet was ingested daily. The subject also received 1500 cc. water daily. The diet for the final period was the same as that for the preliminary period.

The urine was collected in twenty-four-hour periods and kept in a refrigerator in two-liter acid bottles. Thymol was used as a preservative.

The methods of analysis were as follows: ammonia, Folin; total acidity, Folin; phosphates, uranium acetate titration; chlorides, Clark's modification of Dehn's method.

Experimental.

Preliminary Feeding Period.—The subject was placed on a uniform diet which contained 21.86 grams nitrogen per day. The preliminary feeding period lasted four days, during which a satisfactory nitrogen equilibrium was maintained. The urine and the various constituents under consideration were excreted in fairly constant amounts and furnish a normal average for comparison. The ammonia output was noticeably low.

Fasting Period.—During the fast, the subject continued his regular activities as instructor. The amount of water taken daily was 1500 cc. The urine volume was low on the first day, rose on the second, and remained fairly constant with a slight decrease toward the end of the fast. The average was nearly the same as that of the preliminary period.

The ammonia excretion (see table and curves accompanying) increased on the first day of the fast and rose rapidly at first, and then slower, reaching a maximum on the sixth day of 1.463 grams nitrogen, over eight times the average of the preliminary period. On the last day of the fast the value dropped to 1.394 grams.

The acid output rose immediately on the first day of the fast, more than doubled itself on the second day and was still higher on the third. Thereafter, there was a gradual decrease to the end of the fast.

The phosphate excretion increased slightly on the first day, dropped on the second, but reached a maximum on the third. After the third day, the values decreased steadily.

The chloride excretion was less than normal at the outset of the fast and decreased rapidly for three days, after which time there was a slower and less steady diminution.

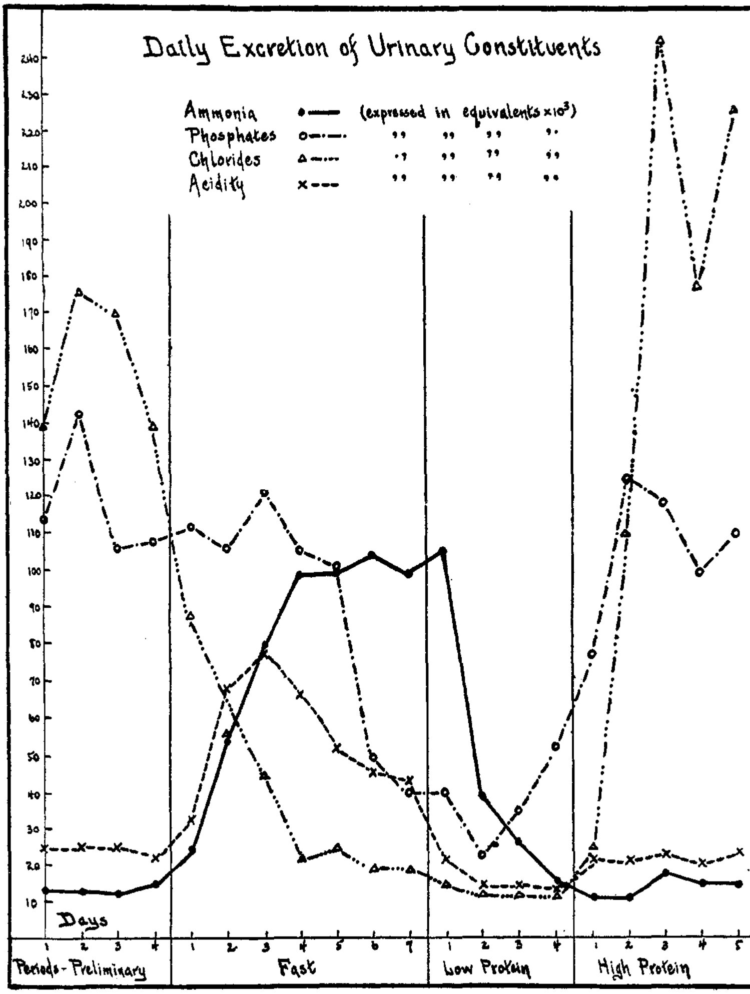
GENERAL DATA.

Subject E.

Day of Exp.	Vol. urine. Cc.	Ammonia N. Grams.	Acidity. Cc. N/10 NaOH.	P_2O_5. Grams.	Chloride. Grams. NaCl.	NH_3. % total N.	$\frac{P_2O_5}{Acidity}$.
Preliminary Period.							
1	1200	0.185	245.2	2.683	7.996	1.05	0.109
2	1350	0.177	245.2	3.358	10.232	1.00	0.137
3	1210	0.158	246.7	2.503	9.801	0.89	0.101
4	1150	0.209	217.2	2.527	8.000	1.18	0.116
Av.	1228	0.182	238.6	2.768	9.007	...	0.116
Fasting Period.							
1	990	0.344	328.9	2.616	5.035	2.39	0.079
2	1345	0.747	677.1	2.509	3.231	4.21	0.037
3	1540	1.113	770.4	2.851	2.539	5.70	0.037
4	1340	1.388	664.2	2.490	1.253	8.62	0.037
5	1410	1.390	525.0	2.376	1.474	10.46	0.045
6	1015	1.463	462.4	1.186	1.132	11.22	0.026
7	1120	1.394	438.9	0.955	1.137	10.98	0.022
Av.	1251	1.120	552.4	2.140	2.257	...	0.040
Low-Protein Final Period.							
1	900	1.479	214.8	0.951	0.874	13.19	0.044
2	1000	0.552	140.6	0.537	0.714	6.40	0.038
3	1240	0.370	143.4	0.822	0.710	4.54	0.057
4	1330	0.217	136.2	1.246	0.762	2.51	0.091
Av.	1118	0.655	158.8	0.889	0.765	...	0.058
High-Protein Final Period.							
1	970	0.161	212.0	1.830	1.404	1.14	0.086
2	1420	0.158	205.5	2.934	6.322	1.04	0.142
3	2530	0.241	228.0	2.778	14.063	1.73	0.122
4	1550	0.207	199.6	2.332	10.220	1.49	0.117
5	2175	0.204	228.7	2.587	13.029	1.29	0.113
Av.	1729	0.194	214.8	2.492	9.008	...	0.116

Low Protein Feeding Period.—At the close of the fasting period, the subject was placed on a diet similar to that of the preliminary period but only one-fourth that amount, so that, for four days after the fast, the subject received 5.23 grams nitrogen and about 1800 calories daily. The urine volume was low on the first day but increased gradually giving an average a little below normal for the period.

The ammonia nitrogen was higher on the first day of the low protein feeding period than on the last day of the fast and was, indeed, slightly higher than the maximum value for the fast. A marked decrease was observed on the second day, the value dropping from 1.479 grams to 0.552 gram with a continued decrease each day following. The minimum



value, however, was somewhat higher than any value in the preliminary period.

The acidity dropped immediately after the fast to a value lower than any observed in the preliminary period, and decreased further giving a minimum value for the experiment on the fourth day. The excretion of phosphates diminished slightly on the first day and showed a considerable decrease on the second, after which there was a steady and rapid increase to a value about half that of the preliminary period. The chloride excretion dropped considerably on the first day and decreased somewhat on the next and stayed about this minimum value for the remainder of the period.

Final Period.—The final period consisted of five days, during which the food intake was increased to the amount eaten in the preliminary period. The urine volume was again low on the first day but rose rapidly to a maximum value on the third day, and then decreased to a value above the normal. The average was considerably above that of the preliminary period.

The ammonia output continued to decrease for the first two days, but showed a marked rise on the third from 0.158 gram to 0.241 gram, an increase of over 52%. On the fourth and fifth days, the values were decreased to somewhat above the average of the preliminary period.

The acid output increased on the first day of the final period to a value somewhat below the normal, and showed no great variation throughout the period.

The phosphate excretion increased rapidly, giving values on the second and third days above the normal. For the remainder of the period, the output was somewhat below the average of the preliminary period.

The output of chlorides rose rapidly for the first three days of the final period, and reached a maximum of 14 grams on the third day. The values for the last three days showed considerable variation and were all higher than the average of the preliminary period.

General Discussion.

The urine volumes varied within normal limits throughout the experiment. There was, however, a considerable diminution on the first day of each period (see Table p. 140). The volumes were less than a liter on these days only. In each case, there was a marked retention of water; for the daily water intake (water + water in milk) was 2225 cc. for the preliminary and final periods, 1500 cc. for the fast, and 1890 cc. for the low-protein period. The uniformly observed decrease in the urine volume on the first day of each period is remarkable owing to the difference in the conditions of the experiment at these times, but may indicate a retention due to a change in the metabolic activities of the body both on beginning and discontinuing the fast. A similar retention occurred in the previous fast on this subject.[1] On the first day of that fast, with an intake of 1000 cc. water, the excretion was only 678 cc., and on the first two days following the fast, with water ingestions of 1000 cc. and 1750 cc., the excretions were 507 cc. and 527 cc., respectively. Uniform weather conditions precluded the possibility of great variations in loss of water by other channels than the kidneys.

On the third day of the fast, the urine volume was 1540 cc., which was 40 cc. more than the water intake. This was the only time during the fast when the volume of liquid excreted was larger than the volume ingested. Our data, in this respect, are comparable with Benedict's[2] observation

[1] Howe, Mattill and Hawk, THIS JOURNAL, **33**, 568 (1911).

[2] *Carnegie Publication*, No. **77** (1907).

that, when the volume of ingested liquid exceeds 1000 cc., the urine volume is not far from that of the liquid ingested. The average urine volume was higher for the fast than for the preliminary period.

In the low protein period, there was a steady increase from the minimum on the first day. The volumes in the final period increased daily from the minimum on the first day to the maximum for the experiment on the third day, when the volume of urine was again greater than that of the ingested liquids.

The ammonia output rose immediately on the first day of the fast and increased rapidly, both absolutely and relatively, to a maximum value on the sixth day, which was eight times the average for the preliminary period (see Table p. 140). There was a slight decrease on the seventh day. The relative ammonia values increased in like manner from 1% of the total nitrogen in the preliminary period to 11% on the sixth day. A similar increase occurred in the former fast by this same subject, giving in that instance a value of nearly 13% on the seventh fasting day.[1] These results are similar to those reported by Cathcart, in which he found an increase to 15% of the total nitrogen on the eighth day of the fast. In striking contrast, E. and O. Freund in their work on Succi found scarcely any increase in the ammonia excretion.

Our ammonia nitrogen value rose to 13% on the first day of the low protein period and decreased steadily throughout the period. Cathcart feeding a nitrogen-free diet observed a marked increase in three days from 7% to 11% of the total nitrogen. In both our investigation and that of Cathcart the relative value was near the normal in the final period.

This increased elimination of ammonia during the fast has been frequently observed by various investigators and explained as due to acidosis, caused by the formation of the acid products of fat metabolism. In fasting, the body must draw on its own stores of glycogen and fat for energy and, as the fat is metabolized, the acid products formed, diacetic and β-hydroxybutyric acids, accumulate so rapidly that a condition of acidosis is established which calls for an increased formation of ammonia to aid in maintaining the normal reaction of the body fluids. In this consideration, it is interesting to compare the figures we have obtained for ammonia, acidity and phosphate, as excreted in the urine during and following the fast. An inspection of the curves in the figure will make the relations clearer.

The values of the three constituents just mentioned were increased at the beginning of the fast. The ammonia output rose rapidly to a maximum on the sixth day; the acidity increased to a maximum on the third day and then gradually decreased; the phosphate output increased in general to the third day and then decreased, the relative decrease being

[1] Howe, Mattill and Hawk, THIS JOURNAL, 33, 568 (1911).

closely parallel to that of the acidity. Cathcart,[1] Van Hoogenhuyze and Verploegh,[2] Benedict,[3] Luciani,[4] Ajello and Solaro,[5] Munk[6] and others report somewhat similar results for the constituents which they determined.

The explanation of the relations above mentioned may lie in the complicated mechanism by which the body attempts to maintain a uniform reaction of its fluids. As soon as fasting starts, the glycogen and fat stores of the body are called upon to furnish the necessary energy for the body. At first, the glycogen is used in large amounts, the fats being called upon more and more as the fast progresses. Thus, on the first day, the acids formed by fat metabolism were produced in small amounts only, which called for but a slight increase in the ammonia formation and a slightly increased acid and phosphate output.

Henderson's work[7] on the relation of phosphate to the maintenance of neutrality of the body fluids shows that they play an extremely important role. As acids are formed in the body and pass into the blood or lymph, an equilibrium is immediately established, some of the di-sodium phosphate present changing to monosodium phosphate. An excess of monosodium phosphate in the blood is excreted by the kidneys, due either to an increased tendency to dialyze as shown by Maly,[8] or to a function of the kidneys, or both. Thus, an increased phosphate excretion with increased urine acidity follows an increased formation of acids in the body. Just such a condition was met with in our experiment. The acids formed by fat metabolism caused increased excretion of phosphates with increased urine acidity.

After the glycogen stores were depleted, the fat metabolism became more marked, as shown by a greater increase in the ammonia output on the second day, when the excretion was more than doubled. The ammonia formation did not, however, keep pace with the acid formation as indicated by the increased acidity of the urine on the second day of the fast. The relatively low value of the phosphate output is unexplained. The third day showed an increased excretion of all three constituents. Thereafter, the ammonia increased, but the acidity and phosphate output decreased, indicating that the production of ammonia had caught up with the acid production within the body and was gradually exceeding

[1] *Biochem. Z.*, **6**, 109 (1907).

[2] *Z. Physiol. Chem.*, **46**, 440 (1906).

[3] *Carnegie Publication*, No. **77** (1907).

[4] *Das Hungern.*, *Leipzig* (1890).

[5] *La Riforma Medica*, **9**, [2] 542 (1893).

[6] *Virchow's Archiv.*, **131**, supplement (1893).

[7] Henderson, *J. Biol. Chem.*, **9**, 403 (1911). Fitz, Alsberg and Henderson, *Am. J. Physiol.*, **18**, 113 (1907).

[8] *Ber.*, **9**, 164 (1876).

it. With the increase of ammonia, there would be a smaller amount of free acids to be eliminated and therefore a decreased urine acidity. The ammonia excretion reached a maximum on the sixth day and decreased on the seventh, which may have been caused by decreased fat metabolism, due to a lessening of the fat stores of the body.

At the beginning of the fast, there was a marked decrease in the ratio of phosphates to acidity, indicating that less than usual of the urinary acidity was due to acid phosphates. The ratio decreased to a lower figure as the fast progressed, which may be accounted for by the rapid increase of acidosis in the body and the increased excretion of free organic acids in the urine. This explanation is confirmed by the fact that β-hydroxybutyric and diacetic acids were present in considerable amounts during the last days of the fast, when the phosphate-acidity ratio was at its minimum value. Undoubtedly, also, a deficiency of phosphates, caused by their continuous excretion, was instrumental in bringing about a retention which continued through practically all of the low protein period.

With the intake of food, the ratio of phosphates to acidity increased quickly, due to a decreased excretion of organic acids as indicated by a comparison of values for the first and third days of low protein ingestion. These show a decreased phosphate excretion, accompanied by an increase in the ratio of phosphates to acidity. The ratio swung to a value above normal on the second day of high protein feeding and then returned to a normal figure, indicating that the body fluids had returned to their usual condition of equilibrium where the monosodium phosphate excreted is the chief factor causing urinary acidity.

On the first day of the low-protein feeding period, the ammonia output increased considerably, exceeding slightly the maximum value of the fast. This may have been due to an attempt of the organism to reduce the acidosis of the body. As a result of this high ammonia formation, the acidity dropped to less than half the value of the previous day. This decrease in acidity was undoubtedly also accentuated by the ingestion of food, which furnished bases for neutralization of acid metabolic products and calories which alleviated the condition of acidosis.

The diet of the low-protein period seemed sufficient to overcome much of the acidosis, as the ammonia output decreased nearly two-thirds on the second day and diminished thereafter to the end of the period. The acidity of the urine decreased in general with a slight increase on the third day. The phosphate output diminished nearly one-half on the second day, and increased slowly to the end of the period. This retention of phosphates has been noted by Cathcart, Benedict, and Van Hoogenhuyze and Verploegh, and may indicate a tendency on the part of the body to regain its normal amount of phosphates. The ratio of phosphates to acidity increased toward the normal.

With the high-protein ingestion, there was an immediate decrease in the ammonia excretion. The output of the first two days was rather below the normal average, but on the third day the excretion increased more than 50% with a return to slightly above normal for the remainder of the period. The high value on the third day has been noted before in experiments,[1] in this laboratory, on this and two other subjects[2] and in certain other data, especially in Cathcart's work on Beauté. In the latter experiment, a slight rise occurred on the third day of normal feeding following two days of non-nitrogenous food ingestion. This rise in the ammonia following fasting has been explained in a previous article, by one of us,[2] as due to an attempt on the part of the body to free itself of the last traces of acidosis so that it may return to a normal metabolic régime. This explanation gains support from the fact that on this day there was also a high excretion of acids and phosphates.

The increase in the ammonia output on the third day after the fast, as mentioned above, was less in our present experiment on Subject E than in the previous experiment, made upon this same subject. In the former instance he passed immediately to a high protein diet at the conclusion of the fast, whereas in this instance he was on a low protein diet for four days before ingesting the high protein diet. The difference in the dietary conditions and the added fact that the organism may conduct itself differently metabolically in a repeated fast[2] may account for the variation noted.

By examining the data for the low-protein period, we note that no increase occurred but, instead, a continuous decrease. The non-appearance of the rise in the ammonia excretion on the third day of this period may have been due to the fact that the food ingested, being but one-fourth the amount of the normal diet, was low not only in nitrogen but also in calorific value and the body was still forced to derive a part of its energy from its own fat supply, thus allowing a continued, though decreased, formation of acids from fat metabolism and a continued state of acidosis.

The chloride output throughout the experiment seemed to bear no relation to the excretion of the other constituents considered. It decreased rapidly during the first four days of the fast and remained fairly constant for the remainder of the period with a slight diminution. In the low-protein feeding period, the excretion dropped to less than one-tenth of the normal output, showing a considerable retention. A similar retention has been observed in a number of experiments as previously noted.

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[1] Howe, Mattill and Hawk, THIS JOURNAL, 33, 568 (1911).

[2] Howe and Hawk, *Ibid.*, 33, 215 (1911).