$[\alpha]_D^{20}$ found = +8.58.
$[\alpha]_D^{20}$ for isoleucine = +9.74.

In 20 per cent. hydrochloric acid, 0.1965 gram substance in 13.4543 grams solution (1.46 per cent.; 1.0871 sp. gr.) gave +3.25° (Ventzke) rotation in a 2 dcm. tube at 20°, sodium light.

$[\alpha]_D^{20°}$ found = +35.47.
$[\alpha]_D^{20°}$ isoleucine = +36.80.

It analyzed as follows for nitrogen:

Calculated for $C_6H_{13}O_2N$: N, 10.68. Found: N, 10.89 per cent.

From these results there can be no doubt as to the identity of the two compounds in question. Both of them are well known decomposition products of protein and one of them, leucine, was among the compounds isolated by Suzuki from humic acid.

Compounds from Black Peat.

Compounds from Black Peat.—A sample of black peat described by Jodidi[1] was extracted with 25 per cent. sulfuric acid and the extract treated as above. Upon concentrating the sulfate-free liquid, the characteristic yellow scale formed. It was separated from the mother liquor and purified as in the previous case. The quantity was too small to permit of a separation of the isomers. The material was converted into the copper salt which analyzed as follows:

0.0720 gram substance gave 0.1169 gram CO_2 and 0.0485 gram H_2O.
0.0302 gram substance gave 0.0075 gram CuO.

Calculated for $(C_6H_{12}NO_2)_2Cu$: C, 44.48; H, 7.47; Cu, 19.64.
Found: C, 44.29; H, 7.53; Cu, 19.85.

The substance was evidently a mixture of the two compounds described above.

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FASTING STUDIES. III. NITROGEN PARTITION OF TWO MEN THROUGH SEVEN-DAY FASTS FOLLOWING THE PROLONGED INGESTION OF A LOW-PROTEIN DIET; SUPPLEMENTED BY COMPARATIVE DATA FROM THE SUBSEQUENT FEEDING PERIOD.

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Introduction.

The metabolism of fasting men has been studied by a number of investigators but accurate studies of the nitrogen partition during fasting, in

[1] Mich. Exp. Sta., *Technical Bull.* 4, 25.

which the excretion of creatine and creatinine has been considered, are few in number and have all been made since the introduction of Folin's methods for the determination of these two substances. Benedict's[1] work in which he determined the nitrogen partition as well as the respiratory exchange and the metabolism of chlorine, sulfur and phosphorus is the most comprehensive work upon the metabolism of man during fasting. He also includes data upon the metabolism during the period of regeneration following the fast. Cathcart's[2] work upon Beauté deals with the nitrogen partition and the excretion of the inorganic substances. Data for a period of three days following the fast in which the subject received a nitrogen-free diet and a two-day period on an egg and milk diet are included. In an experiment in which the fasting woman Flora Tosca served as subject, van Hoogenhuyze and Verploegh[3] determined creatinine as well as total nitrogen, urea, uric acid, acidity, phosphates and chlorides. Brugsch and Hirsch,[4] Bonniger and Mohr,[5] and Baumstark and Mohr[6] determined the excretion of total nitrogen and amino acids, and the extent of putrefaction during the sixteen-day fast of a woman (Schenk).

Lehmann, Müller, Munk, Senator and Zuntz[7] report the results of experiments upon Cetti (10 days) and Breithaupt (6 days) in which careful studies were made of the respiratory exchange as well as of the alterations in the composition of the urine, feces and blood: body weight determinations were also made and other physical measurements ascertained. Brugsch[8] investigated the changes in the urinary nitrogen of Succi during a thirty-day fast and demonstrated a decrease in the urea output and an increase in the quantity of ammonia excreted. This latter increase he explained as due to acidosis. Diaber,[9] as the result of a twenty-day fast on Succi, records a decreasing body weight, chlorine excretion and urea output; also a pronounced increase in the excretion of urobilin. Ajello and Solaro[10] record results upon a twenty-one-day fast of Succi including the determinations of urea, acidity, phosphorus, chlorides, indican and blood changes. O. and E. Freund[11] report a twenty-one-day fast upon Succi in which they show a decrease in urea, creatinine (Neubauer) and total sulfur with an increased output of neutral sulfur. Urobilin was

[1] *Carnegie Publication No.* 77 (1907).
[2] *Biochem. Z.*, 6, 109 (1907).
[3] *Z. physiol. Chem.*, 46, 440 (1906).
[4] *Z. exper. Path. und Ther.*, 3, 638 (1906).
[5] *Ibid.*, 675.
[6] *Ibid.*, 687.
[7] *Virchow's Arch.*, 131, supplement (1893).
[8] *Z. exp. Path. Ther.*, 1, 419 (1905).
[9] *Schweiz. Woch. Chem. Pharm.*, 34, 395 (1896).
[10] *La Riforma medica Ann.*, 9, 542 (1893); from *Jahrb. Fortschr. Thier-Chem.*, 24, 544 (1894).
[11] *Wien. klin. Rundschau*, 15, 69 and 91 (1901).

increased in amount from the first day of the fast and from the eighth day sugar was present in the urine. Luciani[1] reports a thirty-day fast of Succi, during the last nine days of which he received peptone, sugar, gelatin, etc. Johansson, Landergren, Sónden and Tigerstedt[2] report the five-day fast of a medical student in which results are given from the examination of the feces and urine as well as from a study of the respiratory exchange. Paton and Stockman[3] report a thirty-day fast upon Jacques in which the urea determinations were made by the hypobromite method.

Description.

Purpose and Plan.—The purpose of this experiment was to study the nitrogen partition of normal fasting men, when performing their customary duties; changes in body weight were also observed. The experiment was conducted upon two men and consisted of three periods, a fore period of four days during which they received a constant diet embracing a uniform water ingestion, a fasting period of seven days and an after-period of eight days.

Subjects.—The subjects of this experiment were two instructors E and H connected with the Chemistry Department of this University. Both subjects were somewhat taller than the average man and were of approximately equal height and build and of a more or less even temperament. Subject E was 23 years old, six feet in height and weighed 74 kilograms. Subject H was 23 years old, six feet one inch in height, and weighed 71 kilograms.

The men were favorably disposed toward fasting, having in previous years practiced it occasionally for intervals of from one to two days. They were at the time engaged in research and realized the importance of careful attention to detail and the strict obeyance of instructions.

The previous dietary history of the two men will be mentioned in brief. Subject E for the six months just preceding the experiment had lived upon a low-protein meat diet of but one meal a day, a diet which would represent not over 30 to 40 grams of protein and 1200–1800 calories of energy per day. On this diet he maintained his weight and performed his university duties. Subject H had lived upon a low-protein non-meat diet during the eight months preceding the experiment, a diet which would give a protein value of about 60 grams and an energy value of approximately 2000 calories per day.

Articles of Diet.—The food used in this investigation was purchased in quantities sufficient to last through the entire experiment. Each article of the diet was sampled and analyzed for total nitrogen.

[1] *Firenze,* 1889; from *Centralb. Physiol.,* 4, 862 (1890).
[2] *Skan. Archiv. Physiol.,* 7, 1 (1897).
[3] *Proc. Roy. Soc. Edinburgh,* 4 (1889).

Crackers.—The graham crackers were obtained from the local dealers. After sampling they were kept in tin boxes.

Milk.—The milk was obtained from the dairy department of this University and analyses made from time to time showed the composition to have remained practically constant.

Butter.—The butter was obtained from the dairy department and was taken from a single "making" of butter, put up in one pound packages and preserved in a refrigerator. An analysis was made upon a sample taken at the time of making.

Water.—The ordinary distilled water was used such as is furnished to the general laboratories.

The Collection and Preservation of Excreta.—The urine was collected in twenty-four-hour periods and preserved in two-liter acid bottles coated on the inside with a thin layer of thymol. This thymol coating was produced by rinsing the bottle with a 10 per cent. alcoholic solution of thymol and allowing the alcohol to evaporate. Powdered thymol was added after the samples for analysis had been taken. When not in use the bottles were kept in a refrigerator.

The feces were collected in period samples and were passed into, and kept in, friction-top pails,[1] which were coated on the inside with a thin layer of thymol in the same manner as described in connection with the urine bottles. Charcoal was given, in gelatin capsules, at the beginning of each period and produced a very distinct separation zone.

Methods of Analysis.—The methods of analysis were as follows: total nitrogen, Kjeldahl; urea, Benedict-Gephart; ammonia, Folin; creatinine, Folin; creatine, Folin, Benedict and Myers.[2]

The Daily Routine.—The daily routine of the subjects was but partially prescribed, but their habits and duties resulted in the performance of a uniform amount of work each day. The prescribed routine was as follows: 7.00 A.M., urinate, defecate and weigh; 7.15 – 7.30, breakfast; 12.00—12.15 dinner; 5.00 P.M., supper; 10.00 P.M., retire.

The body weights were made upon the "stripped" subjects at 7.00 A.M., after they had urinated and defecated, the weights being made upon a platform balance accurate to 0.01 kilogram. The habits of the subjects were such that they defecated regularly at 7.00 A.M. (during the feeding periods), a fact which lends greater significance to the body weights as determined.

The amount of food required by each subject was determined by permitting a free choice of the various articles of food used in the experiment for a period of two days preceding the actual collection of data. The daily food ration was divided into three parts equal and eaten at the

[1] Howe, Rutherford and Hawk, THIS JOURNAL, 32, 1683 (1910).

[2] Methods used are referred to in Howe and Hawk, THIS JOURNAL, 33, 215 (1911)

indicated meal hours. As a result of this selection Subject E consumed a slightly larger amount of food than did Subject H. Subject E took his water *between meals*, 250 cc. in the A.M., 250 cc. in the P.M. and 250 cc. at night while H took his *with meals*, 250 cc. with each meal.

Experimental.

Discussion of the Experiment upon Subject "E."

Preliminary Feeding Period.—Subject E at the beginning of this experiment had been maintaining his weight upon a low-protein meat diet, eating but one meal a day. He was placed upon the cracker-milk diet consisting of 600 grams of graham crackers, 1350 cc. of whole milk, 75 grams of butter and 700 cc. of water per day. Upon this diet the subject gained weight slightly and the nitrogen balance (Table V, p. 583) showed a retention of nitrogen. The preliminary feeding period was eight days in length, data being presented for the final four days of the period.

Nitrogen Partition.—The excretion of total nitrogen remained fairly constant from day to day, the average for the four days preceding the fast being 10.430 grams. The urea-nitrogen excretion paralleled that of the total nitrogen, the average being 9.070 grams of nitrogen per day. The percentage of total nitrogen occurring as urea nitrogen was 86.9 per cent. The ammonia nitrogen remained constant, giving an average of 0.112 gram per day, and representing an average of 1.0 per cent. of the total nitrogen. The creatinine nitrogen was also fairly constant, giving an average of 0.687 gram per day, a value which represented 6.5 per cent. of the total nitrogen.

Fasting Period.—The last food was taken at 5.00 P.M on May 13th and the fast was considered as beginning at 7.00 A.M. on the following day. The ordinary university duties were performed. The subject ingested water according to the following schedule: 7.00 A.M., 500 cc.; 9.00 A.M., 250 cc.; 12.00 M., 250 cc.; 3.00 P.M., 250 cc.; 9.00 P.M., 250 cc., making a total of 1500 cc. of water per day.

Extracts from the Diary of Subject E. May 14.—Slept well. Did not notice any hunger except at the regular meal hours and did not care much for water. *May 15:* Hunger not so noticeable this morning. However, I feel weak when climbing stairs. *May 16:* Slight ache in muscles of legs and back, have dizzy spells at times. *No hunger*, coated tongue and bad taste in mouth. *May 17:* Still have dizzy spells upon sudden movement. *May 18:* Weaker, dreamt of feasting. *May 19:* Rather tired, did not sleep well, again dreamed of eating. *May 20:* Mouth very stale, tongue heavily coated, do not feel much weaker than yesterday, cold feet and hands, otherwise O. K.

Body Weight.—The body weight fell from 74.22 kilograms (Table IV) on the morning of the beginning of the fast to 68.70 kilograms at the end

TABLE I.—SUBJECT E.—GENERAL DATA.

Day of period.	Body weight. kgs.	Volume of urine. cc.	Specific gravity of urine.	Reaction of urine.	Total N. Grams.	Urea N. Grams.	Ammonia N. Grams.	Creatinine N. Grams.	Creatine N. Grams.	Per cent. of total nitrogen. Urea. N.	Ammonia. N.	Creatinine. N.	Creatine. N.
					Preliminary Feeding Period.								
1	74.10	1108	10205	ampho	10.353	9.039	0.105	0.700	...	87.3	1.0	6.8	..
2	74.18	990	10255	ampho	10.495	9.095	0.112	0.666	...	86.7	1.0	6.4	..
3	74.16	1040	1027	ampho	10.417	9.066	0.101	0.700	...	87.0	1.0	6.7	..
4	74.22	1030	1026	ampho	10.456	9.080	0.106	0.683	...	86.8	1.0	6.5	..
Av.					10.430	9.070	0.112	0.687		86.9	1.0	6.6	
					Fasting Period.								
1	73.32	678	10285	acid	10.072	8.732	0.288	0.350	0.269	86.7	2.8	3.5	2.67
2	71.98	1512	1014	acid	15.072	13.504	0.642	0.571	0.073	89.6	4.3	3.8	0.48
3	70.92	1463	10135	acid	14.463	12.692	0.862	0.574	0.089	87.7	6.0	4.0	0.62
4	70.24	960	10195	acid	13.080	11.245	1.201	0.538	0.068	85.9	9.2	4.1	0.52
5	69.61	1148	1013	acid	11.801	9.573	1.266	0.562	0.033	81.1	10.7	4.8	0.28
6	69.12	1137	10125	acid	11.214	8.897	1.373	0.540	0.022	79.3	12.2	4.8	0.20
7	68.70	1170	1013	acid	10.734	8.506	1.371	0.538	0.003	79.2	12.8	5.0	0.03
					Final Feeding Period.								
1	69.82	507	1025	acid	11.404	8.966	1.007	0.590	...	78.6	8.8	5.2	..
2	72.51	527	1028	acid	9.241	7.005	0.758	0.591	0.04	75.8	8.2	6.4	0.32
3	73.38	1558	1018	acid	10.219	7.525	1.413	0.763	...	73.6	13.8	7.5	..
4	73.68	1703	1020	ampho	10.355	8.909	0.301	0.761	...	86.3	2.9	7.4	..
5	73.40	2155	1017	ampho	11.508	9.360	0.988	0.687	...	81.3	8.6	6.0	..
6	73.40	1781	10205	ampho	10.827	9.129	0.510	0.682	...	84.3	4.6	6.3	..
7	73.56	1236	1024	ampho	11.507	10.072	0.239	0.647	...	87.5	2.1	5.6	..
8	73.44	1279	1024	ampho	12.094	10.550	0.310	0.669	...	87.2	2.6	5.5	..

of the seven-day fast, this representing a loss of 5.52 kilograms or 7.4 per cent. of the original body weight. The greatest loss, 1.34 kilograms, occurred on the second day and the smallest loss, 0.42 kilogram, occurred on the seventh day.

Nitrogen Partition.—The average nitrogen output of the preliminary period was 10.430 grams. Under the influence of the first day of fasting this value was reduced to 10.072 grams, thus aggregating a loss of 0.358 gram in the total nitrogen output. A very pronounced *increase* occurred on the second day, the excretion rising from 10.072 grams on the first day to 15.072 grams on the second day or an increase of 5 grams of nitrogen. From the second day on the nitrogen excretion fell, the variation from day to day becoming less pronounced as the fast progressed. The quantity of nitrogen excreted on the last day was greater than the average daily output of the feeding period or of any individual day of the preliminary period.

The urea-nitrogen excretion followed in general that of the total nitrogen. The percentage of the total nitrogen which appeared as urea varied, however, increasing from 86.7 per cent. on the first day to 89.6 per cent. on the second day and then decreasing steadily to the end of the fast, the percentage on the last day (79.2 per cent.) being the lowest value for the period.

The ammonia-nitrogen excretion was higher at all times during the fast than the average of the feeding period, 0.112 gram per day. The minimum excretion was one of 0.288 gram which occurred on the first day and the maximum excretion was one of 1.373 grams which occurred on the sixth day, there being a rapid increase during the first four days, after which the increase was less pronounced. The relative ammonia-nitrogen excretion increased from the average of 1 per cent. of the total nitrogen for the feeding period to 2.8 per cent. on the first day of the fast and then increased steadily to 12.8 per cent. on the last day.

The creatinine-nitrogen excretion dropped from the average feeding value of 0.687 gram to 0.350 gram on the first day of the fast, rising to 0.571 gram on the second day, and from there on remained practically constant throughout the fast. The relative creatinine-nitrogen excretion decreased from the average value of the feeding period (6.6 per cent. to 3.5 per cent.) on the first day of the fast and then increased very gradually for three days, after which it assumed a higher level and remained practically constant on this plane during the last three days of the fast.

On the first day of the fast there was a very marked excretion of creatine, 0.269 gram. The data from the following day present a much lower value, 0.073 gram, which increased on the third day to 0.089 gram and the values then decreased to the end of the fast, the output for the final day being 0.003 gram. The relative creatine output followed that of the absolute excretion, being highest on the first day, falling on the second and rising again on the third day, the lowest percentage occurring on the last day.

Final Feeding Period.—The amounts of food ingested varied from day to day, as shown in Table V, the greatest changes in the diet being in the quantities of milk consumed, although there was an increase in both the crackers and the butter. The nitrogen intake ranged from 13.465 grams on the first day of feeding to 21.107 grams on the second day. There was an increased water ingestion of 300 cc. during this period, the water being taken at 10.00 A.M., 3.00 P.M., and 9.00 P.M., *i. e.*, between meals, as in the preliminary feeding period. As a result of this diet the subject regained 85 per cent. of his body weight in three days and held the same during the remainder of the experiment (see Table IV). The greatest increase in body weight occurred on the second day, when the weight rose from 69.82 kilograms to 72.51 kilograms or an increase of 2.69 kilograms in one day. For further discussion see p. 580.

Nitrogen Partition.—The total nitrogen excretion (Table I, p. 573) instead of varying with the fluctuations in the diet remained fairly constant. The lowest excretion of nitrogen, 9.241 grams, occurred on the second day when the ingestion of food was the greatest, 21.107 grams of nitrogen (Table V), and the highest excretion (12.094 grams) occurred on the eighth day of feeding when the ingestion was lowest, 14.043 grams of nitrogen. This was the same diet as that fed during the preliminary feeding period. The urea-nitrogen excretion varied with the total nitrogen; relatively, however, there were more marked changes (Table I, p. 573). The percentage of the total nitrogen which appeared as urea nitrogen decreased during the first three days of the feeding period from 78.6 per cent. to 73.6 per cent., these values all being lower than the lowest percentage output of the fasting period. From this point there was a general tendency toward a rather pronounced increase, the final day of the feeding period showing an output of 87.2 per cent. of the total nitrogen in the form of urea. This figure was about normal (86.9 per cent.) for this subject. The ammonia-nitrogen excretion also showed fluctuations during this period, being low when the urea was high and *vice versa*. A low (0.301 gram) ammonia excretion occurred on the fourth day, being preceded by a high excretion. This high excretion of 1.413 gram of ammonia nitrogen was the maximum for the period, the minimum (0.239 gram) occurring on the seventh day. For the first two days the creatinine-nitrogen excretion remained near the level of that of the fasting period, the output being 0.538 gram on the last day of the fast as against an average of 0.590 gram for the first two days of the feeding period. On the third and fourth days there was a distinct rise to an average of 0.762 gram, after which the excretion fell, the average output for the fifth and sixth days being 0.684 gram. The average daily excretion for the final four days of the period (0.671 gram) was very close to the average daily output (0.687 gram) for the preliminary period.

Discussion of the Experiment upon Subject H.

Preliminary Feeding Period.—Subject H, previous to the experiment, had been living upon a more normal diet than Subject E. The diets differed in that Subject H had ingested no meat for a period of eight months, his diet being distinctly so-called "vegetarian" in character. The diet in the preliminary feeding period was also slightly different and consisted of 450 grams of graham crackers, 1350 cc. of whole milk, 75 grams of butter and 750 cc. of water, furnishing a nitrogen value of 12.138 grams per day. On this diet he maintained an almost constant body weight and the nitrogen balance (see Table V, p. 583) showed but slight variations.

Nitrogen Partition.—The excretion of total nitrogen remained nearly constant throughout the preliminary feeding period. The average excretion for the four days was 9.814 grams per day. The absolute urea-nitrogen excretion followed that of the total nitrogen, the average being 8.520 grams per day. Relatively, the variations ranged between 83.4 per cent. of the total nitrogen on the first day and 84.6 per cent. on the second day, the average for the period being 84.3 per cent. The average ammonia-nitrogen excretion for the period was 0.271 gram per day. The relative excretion of ammonia nitrogen varied slightly, decreasing from a value of 3.5 per cent. on the first day to 2.5 per cent. on the fourth day, the average for the period being 2.7 per cent. The creatinine-nitrogen excretion remained practically constant throughout the four days, the average being 0.639 gram per day. Relatively the average excretion was 6.5 per cent of the total nitrogen.

Fasting Period.—The fast of Subject H began under the same conditions with regard to the day, etc., as that of Subject E, see p. 572. Subject H, however, consumed 250 cc. more water per day than did Subject E.

Extracts from the Diary of Subject H.—*May 14:* Feeling well, slight lassitude. *May 15:* Felt well all day, *no hunger*, tongue coated, bad taste in mouth. *May 16:* Felt well all day, slightly weak, *no hunger. May 17:* Quite weak this morning, slept well, dizzy spell in afternoon, *no hunger. May 18:* Felt well all day and although I am weak, it is not as great an exertion to walk as it was at first. *No hunger. May 19:* Feeling well, in very good spirits only weak when it comes to any exertion. *No hunger. May 20:* Weak, slept well. *No hunger.*

Body Weight.—The body weight fell from 70.30 kg. on the morning of the day the fast began to 64.94 kg. at the end of the fast or a loss of

TABLE II.—SUBJECT H.—GENERAL DATA.

Day of period.	Body weight. kgs.	Volume. of urine. cc.	Specific gravity of urine.	Reaction of urine.	Total N. Grams.	Urea N. Grams	Ammonia N. Grams.	Creatin-ine N. Grams.	Creatine N. Grams.	Per cent. of total nitrogen. Urea N.	Ammonia N.	Creatin-ine. N.	Creatine. N.
					Preliminay Feeding Period.								
1	70.36	770	1031	ampho	9.852	8.218	0.344	0.630	...	83.4	3.5	6.4	..
2	70.44	800	1027	ampho	9.987	8.452	0.259	0.636	...	84.6	2.6	6.4	..
3	70.36	770	1029	ampho	9.615	8.121	0.238	0.647	...	84.5	2.6	6.7	..
4	70.30	790	1029	ampho	9.801	8.286	0.243	0.641	...	84.5	2.5	6.5	..
Av.					9.814	8.520	0.271	0.639		84.3	2.7	6.5	
					Fasting Period.								
1	69.50	651	10235	acid	8.338	6.999	0.330	0.547	0.012	83.9	3.9	6.6	0.14
2	68.22	1150	1014	acid	11.097	9.565	0.632	0.580	0.037	86.2	5.7	5.2	0.33
3	67.12	1320	10145	acid	12.821	10.898	1.097	0.555	0.066	85.0	8.6	4.3	0.52
4	66.53	890	10195	acid	12.116	9.705	1.434	0.539	0.089	80.1	11.8	4.4	0.73
5	65.90	1132	10145	acid	11.176	8.727	1.541	0.520	0.036	78.1	13.8	4.6	0.32
6	65.28	1098	10115	acid	10.407	7.892	1.432	0.554	0.053	75.8	13.8	5.3	0.51
7	64.94	939	10165	acid	10.158	7.700	1.411	0.509	0.033	75.8	13.9	5.0	0.33
					Final Feeding Period.								
1	65.72	530	1018	acid	10.996	8.628	1.025	0.355	0.207	78.5	9.3	3.2	1.88
2	68.98	550	1019	acid	10.919	8.647	0.919	0.349	0.273	79.2	8.4	3.2	2.5
3	69.50	870	10275	ampho	9.687	6.684	1.610	0.611	0.025	69.0	16.6	6.3	0.26
4	69.69	1035	1025	ampho	8.665	7.119	0.340	0.615	0.033	82.2	3.9	7.1	0.38
5	70.28	1491	1020	ampho	9.244	7.707	0.223	0.646	...	83.4	2.4	7.0	..
6	69.43	1386	1021	ampho	8.602	7.060	0.256	0.606	...	82.1	3.0	7.0	..
7	68.56	1213	10235	ampho	9.199	8.019	0.228	0.549	...	87.2	2.5	6.0	..
8	68.10	856	10295	ampho	10.104	8.313	0.242	0.589	...	82.3	2.4	5.8	..

5.36 kg. in the seven days. The loss in body weight on the first day, 0.80 kg., was followed by a much greater loss, 1.28 kg., on the second day, after which the losses from day to day became less and less as the fast progressed until the end of this period, when the minimum loss, 0.34 kg., occurred.

Nitrogen Partition.—The average output of nitrogen during the preliminary period was 9.814 grams per day. Under the influence of the first day of fasting 8.338 grams were excreted, this being the minimum daily output recorded during the course of the experiment. During the two following days, the nitrogen excretion increased to the maximum, 12.821 grams, and then decreased gradually throughout the remainder of the fast. An excretion of 10.158 grams of nitrogen occurred on the last day; this value was higher than the total nitrogen excretion for any day during the feeding period (see discussion of data, p. 588).

The urea-nitrogen excretion followed that of the total nitrogen. The percentage of the total nitrogen which was excreted as urea nitrogen fell below the average of the feeding period (84.3 per cent.) on the first day (83.9 per cent.), then rose on the second day (86.2 per cent.) and decreased from this point to the end of the fast. The highest percentage (86.2 per cent.) occurred on the second day and the lowest (75.8 per cent.) on the sixth and seventh days.

The absolute excretion of ammonia nitrogen increased from day to day until the fifth day and then decreased slightly during the next two days, the excretion being 0.330 gram for the first day, 1.541 gram for the fifth day and 1.432 and 1.411 gram for the final days. Relatively, the percentage of the total nitrogen excreted as ammonia nitrogen increased from day to day, the lowest percentage being 3.9 on the first day and the highest being 13.9 on the last day of the fast.

The creatinine-nitrogen excretion dropped on the first day from the average of the feeding period (0.639 gram) to 0.547 gram and fluctuated but slightly during the remainder of the fast. The relative creatinine-nitrogen excretion decreased from 6.5 per cent. on the first day to 4.3 per cent. on the third day and then increased to 5.3 per cent on the sixth day and fell slightly to 5.0 per cent. on the last day of the fast.

Creatine appeared on the first day of the fast and the data from the creatine nitrogen show an increase for the first four days from 0.012 gram of nitrogen on the first day to 0.089 gram on the fourth day and then a decrease during the remainder of the fast, to 0.033 gram on the seventh day. The percentage of the total nitrogen excreted in the form of creatine nitrogen varied, the maximum being 0.73 per cent. on the fourth day and the minimum 0.14 per cent. on the first day.

Final Feeding Period.—The general conditions as outlined for Subject E obtained for Subject H during this period. Subject H, however, selected a diet furnishing less nitrogen, he consumed less milk and also took larger quantities of water, see Tables V and VI, pp. 583 and 587. With this diet, Subject H made very rapid gains in body weight and recovered his original weight in five days. At this point, the original uniform diet was ingested, which dietary change was accompanied by a loss in weight, due probably to the fact that the low ration was insufficient for this organism just recovering from a seven-day fast. The largest gain in weight occurred during the second day, the body weight increasing from 65.72 kilograms to 68.98 kilograms or a gain of 3.26 kilograms in one day.

Nitrogen Partition.—The excretion of nitrogen varied within rather narrow limits as compared with the fluctuations in the quantity of nitrogen ingested (see Tables II and V, pp. 577 and 583). The urea-nitrogen excretion followed that of the total nitrogen. Relatively, the urea nitrogen increased from day to day; the lowest percentage (69.0) occurred on the third day, an output which was the lowest recorded at any time during the experiment. The highest percentage (87.2 per cent.) occurred on the seventh day of the feeding period. The excretion of ammonia nitrogen decreased with the ingestion of food but exhibited marked fluctuations. On the third day the excretion rose from 0.919 gram to 1.610 grams, this increase of 0.691 gram being greater than that occurring on any day during the fast. An equally pronounced drop resulted on the following day, the excretion being 0.340 gram. The excretion for the fifth day was 0.223 gram and the ammonia-nitrogen output remained practically at this level during the remainder of the period. The percentage of the total nitrogen excreted as ammonia showed similar fluctuations, being high on the third day, 16.6 per cent., decreasing on the fourth day to 3.9 per cent., and continuing on a similarly low level. Upon the ingestion of food, the creatinine-nitrogen excretion fell from 0.509 gram to 0.355 gram and remained low for two days. The excretion regained approximately the preliminary feeding level on the third day and maintained this level during the remainder of the experiment. Similar changes occurred in the relative creatinine-nitrogen excretion, which dropped to 3.2 per cent. on the first day and rose to 6.3 per cent. on the third day. This level was virtually maintained throughout the succeeding experimental days. The excretion of creatine noted during the fast continued during the first four days of feeding, the highest creatine-nitrogen excretion of the experiment, 0.273 gram, occurring on the second day of the feeding period. The excretion decreased on the third day and disappeared on the fifth day. It is an interesting fact that an average of over 2 per cent. of the total nitrogen output was excreted as creatine upon the first two days of the feeding period.

General Discussion.

The data from the fasts of two men, Subjects E and H, have been presented. These men had been, previous to the beginning of the experiment, living upon different diets; the one, Subject E, upon a low-protein meat diet of but one meal a day, and the other, Subject H, upon a medium low-protein non-meat diet taken in the normal manner, *i. e.*, three meals a day. The men were very similar as to physique and temperament and their daily duties were, in a sense, similar. Thus, we approach a fast with two subjects whose dietary habits are different, through a preliminary feeding period in which the same diet was fed but in which water was taken in the case of Subject H, with meals, and in the case of Subject E, between meals. Under these conditions, they experienced a fast of seven days, the results of which are very similar as regards body weight changes and the partition of urinary nitrogen. Following the fasts came feeding periods during which the diet varied quantitatively but not qualitatively.

Body Weights.—The possibility of error in the determination of the body weights was reduced to a minimum, since practically all the interfering factors, such as the retention of urine or feces, changes in the weight of the clothes etc., were eliminated, consequently the fullest credence may be given the results. The daily variations in the body weights of the two subjects were very similar. Subject E sustained the greatest total loss, 5.52 kilograms, for the seven days, against a loss of 5.36 kilograms for Subject H during the same time. Subject H, however, experienced the greatest percentage loss, this being 7.6 per cent. of the body weight as compared with a loss of 7.4 per cent. for Subject E. The actual daily losses as well as the daily percentage losses for the two subjects show but slight variations, the former values being slightly higher for subject E, whereas, the latter values were a trifle higher for Subject H. In both cases a smaller loss in body weight occurred on the first day as compared with the losses on the second and third days. After the second day, which was the day of maximum loss, the loss per day became gradually less, the minimum occurring on the last or seventh day in each case. The percentage loss per day shows similar variations to that of the absolute loss.

An interesting comparison may be made of the daily losses for the first seven days of the more important fasts recorded in the literature. A consideration of the appended table (Table III, p. 581) will show that changes in body weight similar to those just discussed have occurred in previous fasts although the gradual decrease in the daily loss was not so regular as in the experiments reported by us. The experiments in which the losses in body weight more nearly approach those of our ex-

Table III.—Losses in Body Weight.

	Johanson, Landergren, Sonden and Tigerstedt.[1]	Lehmann, Müller, Munk, Senator, Zunz.[2]		Landergren.[3]	At Paris.[4]	At Milan.[4]	Luciani.[5]	Ajello and Solaro.[6]	Paton and Stockman.[7]	Benedict.[8] S. A. B.			Cathcart.[9]	Howe, Mattill and Hawk.	
	J. A.	Cetti.	Breithaupt.	XI.	Succi.	Succi.	Succi.	Succi.	Jacques.	73.	75.	Average.	Beauté.	E.	H.
Wt. on last day of food	67.80	57.00	60.07	78.60	..	..	63.30	63.60	62.01	59.13	59.52	..	65.61	74.22	70.30
Loss 1st day	1.63	0.55	0.55	0.60	..	..	0.90	..	1.33	1.17	0.04	1.05	1.04	0.90	0.80
Loss 2nd day	0.92	0.94	0.63	1.77	3.60	1.55	1.40	1.80	0.94	0.69	0.72	1.00	0.85	1.34	1.28
Loss 3rd day	0.75	1.08	0.77	1.00	0.40	0.80	1.20	1.20	0.51	0.84	0.69	0.79	0.95	1.06	1.10
Loss 4th day	1.03	0.98	0.95	0.80	1.00	0.75	+0.10	0.80	+0.01	0.78	0.89	0.88	0.81	0.68	0.59
Loss 5th day	0.68	0.85	0.20	..	0.60	0.50	0.60	0.70	0.26	0.67	0.45	0.56	0.55	0.63	0.63
Loss 6th day	..	0.25	0.52	..	0.30	0.85	0.65	0.90	0.62	..	0.39	0.39	0.58	0.49	0.62
Loss 7th day	..	0.00	..	..	0.10	0.55	..	0.70	+0.20	..	0.50	0.50	0.60	0.42	0.34

[1] *Skan. Archiv. Physiol.*, 7, 29 (1897).
[2] *Virchow's Archiv.*, 131, suppl. (1893).
[3] *Skan. Archiv. Physiol.*, 14, 152 (1903).
[4] Taken from *Carnegie Pub.*, 77, 1907.
[5] *Das Hungern, Leipzig*, 1890.
[6] *La Riforma Medica*, 9, 2, 542 (1893).
[7] *Proc. Roy. Soc. Edinburgh*, 16, 121 (1888–1889).
[8] *Carnegie Pub.*, 77 (1907).
[9] *Biochem. Z.*, 6, 109 (1907).

periments in regularity were two of the fasting tests in which Succi served as subject.

Due to the varying diet, the changes in the body weights of Subjects E and H during the period of regeneration were variable. The most significant results are those of the first three days which indicate a small increase in the body weight on the first day of food followed on the second day by a very pronounced increase amounting to 2.69 kilograms and 3.26 kilograms for Subjects E and H respectively. On the third day, a much smaller increase in body weight occurred and the data show the daily increases for the remaining days of the period to have been on this latter plane. During the last three days of the regeneration period, Subject H ingested the constant diet previously fed during the preliminary period, a diet which apparently was not sufficient for him under the circumstances, as indicated by a daily loss in weight. Subject E also returned to the original diet for a single day during which a small loss in weight was recorded.

Table IV.—Changes in Body Weight and Creatinine Coefficients.

	Subject E.				Subject H.			
Day of period.	Body weight. Kg.	Daily loss or gain in weight. Kg.	Per cent.[1] daily loss or gain in weight.	Creatinine coefficient. Mg.	Body weight. Kg.	Daily loss or gain in weight. Kg.	Per cent.[1] daily loss or gain in weight.	Creatinine coefficient. Mg.
	Preliminary Feeding Period.							
4	74.22	..	..	9.2	70.30	..	..	9.1
	Fasting Period.							
1	73.32	— 0.90	— 1.21	4.8	69.50	— 0.80	— 1.14	7.9
2	71.98	— 1.34	— 1.79	7.9	68.22	— 1.28	— 1.82	8.5
3	70.92	— 1.06	— 1.43	8.1	67.12	— 1.10	— 1.56	8.3
4	70.24	— 0.68	— 0.92	7.7	66.53	— 0.59	— 0.84	8.1
5	69.61	— 0.63	— 0.85	8.1	65.90	— 0.63	— 0.90	7.9
6	69.12	— 0.49	— 0.66	7.8	65.28	— 0.62	— 0.88	8.5
7	68.70	— 0.42	— 0.57	7.8	64.94	— 0.34	— 0.48	7.8
Total.......		— 5.52	— 7.44			— 5.36	— 7.62	
	Final Feeding Period.							
1	69.82	+ 1.12	+ 1.49	8.4	65.72	+ 0.78	+ 1.11	5.4
2	72.51	+ 2.69	+ 3.62	8.1	68.98	+ 3.26	+ 4.64	5.1
3	73.38	+ 0.87	+ 1.17	10.4	69.50	+ 0.52	+ 0.74	8.8
4	73.68	+ 0.30	+ 0.40	10.3	69.69	+ 0.19	+ 0.27	8.8
5	73.40	— 0.28	— 0.38	9.3	70.28	+ 0.59	+ 0.84	9.2
6	73.40	0.00	....	9.3	69.43	— 0.85	— 1.21	8.7
7	73.56	+ 0.16	+ 0.22	8.8	68.56	— 0.87	— 1.24	8.0
8	73.44	— 0.12	— 0.16	9.1	68.10	— 0.46	— 0.65	8.6

[1] The per cent. *loss* in body weight during the fasting period is calculated on the basis of the body weight on the last day of the preliminary period, whereas the *gain* during the final feeding period is calculated on the basis of the weight on the last day of the fast.

TABLE V.—NITROGEN BALANCES.

Day of period.	Subject E.				Subject H.			
	Nitrogen in urine. Grams.	Nitrogen in urine + feces. Grams.	Nitrogen in food. Grams.	Nitrogen balance. Grams.	Nitrogen in urine. Grams.	Nitrogen in urine + feces. Grams.	Nitrogen in food. Grams.	Nitrogen balance. Grams.
				Preliminary Feeding Period.				
1	10.353	12.485	14.043	+1.556	9.852	11.828	12.138	+0.310
2	10.495	12.627	14.043	+1.416	9.987	11.953	12.138	+0.185
3	10.417	12.549	14.043	+1.494	9.615	11.681	12.138	+0.457
4	10.456	12.588	14.043	+1.455	9.801	11.762	12.138	+0.371
Av.,	10.430	12.396		+1.480	9.814			+0.331
				Fasting Period.				
1	10.072	10.182	....	—10.182	8.338	8.483	....	—8.483
2	15.072	15.182	....	—15.182	11.097	11.242	....	—11.242
3	14.463	14.573	....	—14.573	12.821	12.966	....	—12.966
4	13.080	13.190	....	—13.190	12.116	12.261	....	—12.261
5	11.801	11.911	....	—11.911	11.176	11.321	....	—11.321
6	11.214	11.320	....	—11.320	10.407	10.552	....	—10.552
7	10.734	10.840	....	—10.840	10.158	10.303	....	—10.303
				Final Feeding Period.				
1	11.404	14.697	13.465	—1.231	10.996	13.265	14.988	+1.723
2	9.241	12.534	21.107	+8.573	10.919	13.188	18.800	+5.612
3	10.219	13.512	19.817	+6.305	9.687	11.956	14.100	+2.144
4	10.355	13.648	18.820	+4.828	8.665	11.934	14.100	+2.166
5	11.508	14.801	18.800	+3.999	9.244	11.513	18.800	+7.287
6	10.827	14.120	17.740	+3.620	8.602	10.871	12.793	+1.922
7	11.507	14.800	15.242	+0.442	9.199	11.468	12.138	+0.690
8	12.094	15.387	14.043	—1.344	10.104	12.373	12.138	—0.235

A consideration of the nitrogen balances during the feeding periods (Table V, p. 583) and the changes in body weights (Table IV, p. 582) present some very interesting facts. On the first day, Subject E showed a negative nitrogen balance of 1.231 grams with an increase in weight of 1.12 kilograms, denoting a storage of material other than protein and probably in large part water. Subject H, on the other hand, had a distinct positive nitrogen balance, 1.723 grams, with a much lower gain in body weight, 0.78 kilogram. The total nitrogen excretion in both cases was quite similar, *i. e.*, 11.404 grams for Subject E and 10.996 grams for Subject H. Upon the following day with an ingestion of a diet containing 21.107 grams of nitrogen and 3750 cc. of water, Subject E showed a nitrogen balance of +8.573 grams and a gain of 2.69 kilograms in body weight. On the other hand, Subject H, upon an ingestion of 18.800 grams of nitrogen and 3566 cc. of water yielded a nitrogen balance of +5.612 grams, and gained 3.26 kilograms in body weight. Benedict[1] observed a pronounced nitrogen storage under similar conditions after

[1] *Carnegie Pub.*, **77**, 534.

the third day. The urine volumes of the two subjects were almost identical, 527 cc. for Subject E and 550 cc. for Subject H.

The phenomena just mentioned, we have interpreted as indicating a marked retention of water which must have been held as water of combination resulting from the renewed activities of the cells inasmuch as throughout the fast the subjects ingested a uniform and liberal quantity of water each day. A certain small part of this marked increase in weight was, of course, due to the rapid formation of glycogen, since the glycogenic function is markedly stimulated when food is taken after a fast.[1] The quantities formed, however, according to Benedict's figures, which show a maximum formation of 217 grams of glycogen on the third day, are not such as would account for any large part of the observed excessive increase in body weight. It must be further borne in mind that the general anabolic activities of the organism are, of course, greatly stimulated during such a "post-fasting" feeding period. Pashutin[2] has recorded data from lower animals which show a marked increase in weight on the first and second days of feeding after fasting. Benedict, in his experiments in feeding after fasting, does not record any such marked increases in body weight as those reported by us. His subjects received but small amounts of food during the early days (three) of the regeneration period and gave negative or low positive nitrogen balances for that period. The conditions were different in the case of our subjects inasmuch as these men ingested the *maximum amount of food for any individual day upon the second day after the fast.* It is of interest that the subjects experienced no discomfort following the ingestion of the excessive diet so soon after the close of the fast. Furthermore, no undesirable results of any kind were noted in connection with this unusual dietary régime. It is, of course, customary to use great care in the feeding of fasting subjects immediately subsequent to the fast. Pre-digested material is many times ingested in small quantity and under certain conditions nothing other than simple fruit juices are permitted the person who is in the initial stages of "breaking" the fast. With these facts in mind it is of added significance that the subjects of our experiments experienced no ill effects from the ingestion of the customary *full ration* immediately subsequent to the fast. *In fact, H ingested upon the first day of feeding, 15 grams of nitrogen as against 12 grams during the period preceding the fast.*

As a result of the ingestion of food as already indicated Subject E *regained 90 per cent. of his lost body weight in four days* and then maintained a practically constant body weight during the remaining four days of

[1] Benedict, *Carnegie Pub.*, 77, 531; Schultz and Mangold, *Arch. ges. Physiol.*, 114, 419 (1906).

[2] *Loc. cit.*

the experiment. After five days of feeding, Subject H had practically *regained his original body weight, i. e., 99.6 per cent.* He had, however, regained only about one-third of the nitrogen lost during the fast. On the sixth day of feeding H returned to the same diet as that consumed before the fast. Under these conditions he gradually lost weight and the nitrogen balance indicated that the body was not receiving sufficient nitrogen to maintain its functional activities and normal body weight. This phenomenon of increased food requirement has been recorded by Pashutin[1] in experiments on rabbits. Howe and Hawk, in the case of the dog, found that the ingestion of the normal quantity of food resulted in a very slow restoration of the lost nitrogen and that the body weight remained practically constant although when the quantity of food ingested was increased, the nitrogen loss and the weight were restored rapidly. This same condition appears to exist in our experiment, for upon the increased diet, the body weight increased regularly and large amounts of nitrogen were retained, but upon return to the diet of the preliminary period, there was a loss of nitrogen and a decrease in body weight.

The process of regeneration, then, seems to have been in a general way accompanied by a rapid formation of glycogen, the retention of water and a pronounced retention of nitrogen during the earlier days, each of these factors becoming gradually less in evidence as the feeding period progressed. The pronounced retention of nitrogen may be considered to represent the formation of the nitrogenous tissue through the instrumentality of the marked stimulation of the anabolic changes subsequent to the fast. The body weight was restored very quickly but there were not concomitant changes in body weight for the amounts of nitrogen stored. For example, on the second day of the feeding period, Subject H ingested 18.8 grams of nitrogen, gained 3.26 kilograms in body weight and stored 5.612 grams of nitrogen, whereas, on the fifth day on a nitrogen ingestion, identical with that mentioned above, he stored 30 per cent. more nitrogen (7.287 grams), notwithstanding the fact that he gained less than 1/5 as much in body weight (0.59 kg.) as upon the second day. Upon the reduction of the diet to that consumed before the fast the body weight decreased about as rapidly, on the average, as it did during the fast, when no food was received. The nitrogen balance during this time tended toward a negative condition showing that the requirements of the body were greater than before the fast and that more nitrogen must be ingested to maintain the body in nitrogen equilibrium and at a constant weight.

Ingestion and Elimination of Water.—Data regarding the quantity of urine voided per day and the specific gravity of these samples are given in Tables I and II, pp. 573 and 577. The volume of the urine excreted

[1] *Loc. cit.*

is influenced among other things by the amount of water consumed, the work performed and the weather conditions. In these experiments, the quantity of water consumed was maintained as a constant for each man, the amount and nature of the work performed from day to day was uniform and the weather conditions during the fast were fortunately very regular. The extreme variation in the temperature[1] was 7.5° F. (60° F.–67.5° F.) or, excluding the first day, 4.7° F. (60° F.–64.7° F.). The humidity[2] was also quite constant after the first two days when the mean humidity was 80 and 74.3, respectively. The average for the remaining five days was 63.6, the extremes being 66 and 59.6 on the sixth and seventh days respectively.

Under these very favorable conditions the urine volumes of the two subjects were relatively similar, being low on the first day of the fast and rising to a higher plane on the second and third days, dropping on the fourth day and then rising again on the fifth to remain nearly constant through the remainder of the fast. The cause for the decreased volume on the fourth day is not apparent, for the conditions existing on this day were almost identical with those of the previous day and yet both subjects showed the same phenomenon. A comparison of these data with those of other investigators,[3] especially those of Benedict, who has made the most accurate determinations, is hardly possible on account of the differences in the water ingestion. The factor of work which enters into our experiment increased in general the insensible elimination of water and the specific gravities and urine volumes were thereby affected.

In Table VI, p. 587, are given data regarding the percentage of the ingested water which was eliminated by way of the kidneys. We see, in the first place, that this percentage was throughout the fast and the preliminary feeding period rather higher for E than for H. For example, E's preliminary water value was about 55 per cent., whereas H's was about 40 per cent. When we pass to the fast we find the water percentages for E are still above those for H. Upon the first day of the fast, 67.8 per cent. of the ingested water (1000 cc.) was eliminated by E, whereas H eliminated but 37.2 per cent. of the volume (1750 cc.) ingested, and a similar relation, although somewhat less accentuated, held for each individual day of the fasting period. Benedict[4] found, when the volume of water taken by fasting men was small, that "the urine volume may exceed it several times," whereas, "when the volume of drinking water is over 1000 cc., the volume of the urine is not far from

[1] The data regarding the weather were obtained from the weather records of the Ill. Agr. Exp. Sta. through the kindness of Prof. J. G. Mosier.

[2] The humidity used was the average of the three daily observations taken at 7.00 A.M., 2 P.M. and 9 P.M.

[3] *Loc. cit.*

[4] *Carnegie Pub.*, **77**, 351.

that of the water consumed." In our experiments we found only one instance in which the volume of urine exceeded that of the ingested water and that was an excess of only 12 cc. on a basis of 1500 cc. It will be further noted in our experiments that the largest water ingestion was not followed by the most complete water elimination as found by Benedict. For example, Subject H ingested 1750 cc. of water per day as against 1500 cc. for Subject E and eliminated from 12–35 per cent. *less* than Subject E. In other connections one of us[1] has shown that under conditions of *normal nutrition*, a copious water ingestion will be followed by the elimination of a *higher percentage* of that water in the urine than would be excreted upon a lower-water ration. This has been shown to hold true for several subjects. The situation is materially altered, however, when we cause a man to ingest water *unaccompanied by food*.

TABLE VI.—PERCENTAGE WATER ELIMINATION.

	Subject E.				Subject H.			
Day of period.	Urine volume. cc.	Water ingested. cc.	Water + water in milk[2] ingested. cc.	Per cent. of ingested water eliminated through kidneys.	Urine volume. cc.	Water ingested. cc.	Water + water in milk[2] ingested. cc.	Per cent. of ingested water eliminated through kidneys.
				Preliminary Feeding Period.				
1	1108	700	1875	59.1	770	750	1925	40.0
2	990	700	1875	52.8	800	750	1925	41.6
3	1040	700	1875	55.5	770	750	1925	40.0
4	1030	700	1875	55.0	790	750	1925	41.0
				Fasting Period.				
1	678	1000	..	67.8	651	1750	..	37.2
2	1512	1500	..	100.8	1150	1750	..	65.7
3	1463	1500	..	97.5	1320	1750	..	75.4
4	960	1500	..	64.0	890	1750	..	50.9
5	1148	1500	..	76.5	1132	1750	..	64.7
6	1137	1500	..	75.8	1098	1750	..	62.7
7	1170	1500	..	78.0	939	1750	..	53.7
				Final Feeding Period.				
1	507	1000	2175	23.3	530	1100	2492	21.3
2	527	1750	3750	14.0	550	2000	3566	15.4
3	1558	1250	3120	49.9	870	1250	2424	35.9
4	1703	500	2545	66.9	1035	1000	2175	47.6
5	2155	500	2066	104.3	1491	750	2316	61.3
6	1781	1000	2827	63.0	1386	750	1925	72.0
7	1236	1000	2392	51.7	1213	750	1925	63.0
8	1279	1000	2175	58.8	856	750	1925	44.5

It seems rather clear that the whole question of the percentage of water which will be eliminated by a man by way of the kidneys is influenced,

[1] Hawk, University of Pennsylvania, *Medical Bulletin*, 18, 7 (1905); Rulon and Hawk, *Archives Internal Medicine*, 1911,

[2] Water content of milk taken as 87 per cent.

to a surprising degrée, by the factor of individuality. This has been clearly demonstrated by one of us[1] in experiments which go to show that two men of approximately the same age, weight and lung capacity, maintained upon the same constant diet and drinking precisely the same volume of water daily may nevertheless excrete surprisingly different volumes of urine. Therefore, in the fasting study under consideration in the present paper it may, of course, very well be that the element of individuality is the basic factor leading to the uniformly higher percentage water elimination for one subject above that noted for the other subject.

The changes in the urine volumes during the feeding period were most marked during the first two days, when they were very low. In the case of Subject E, with water ingestions (water plus the water in the milk) of 2175 cc. and 3750 cc., the urine volumes were but 507 and 527 cc. or but 23.3 per cent. and 14.0 per cent. of the water ingested. The same phenomenon existed with H. On water ingestions of 2492 cc. and 3566 cc., but 530 cc. and 550 cc. of urine were excreted, these values being only 21.3 per cent. and 15.4 per cent. respectively of that ingested. This condition represents a very marked retention of water, as already mentioned. There was also a noticeable retention of water in the case of Subject H upon return to the original diet during the last three days. This retention was accompanied by a negative nitrogen balance.

Distribution of Nitrogen. Total Nitrogen.—The excretion of total nitrogen has been studied in nearly all the experiments reported upon fasting man. A summary of the total nitrogen data for the first seven days of fasting from the more important fasts is contained in Table VII, p. 589. These data, with the exception of those for Subjects E and H, were collected from subjects *not performing manual labor.*[3] A comparison of the data there presented shows in general a drop in the excretion of nitrogen on the first day of the fast above that excreted on the last day of food, followed by a rise in the excretion, which reached its maximum on the second or third day. Prausnitz[3] made a study of the nitrogen excreted during the first two days of fasting and he found in general, an increased elimination on the second day. Neither in these experiments by Prausnitz nor in those of the investigators tabulated did the maximum excretion exceed that of the last feeding day by any great amount. In fact, in the majority of the cases reported, it was less than that of the feeding period. The average results of Benedict's[4] experiments have also been incorporated in the table and these show similar

[1] Hawk, *Am. J. Physiol.*, 10, 125 (1903).

[2] The subject J. A. did light muscular work in a respiration apparatus.

[3] *Z. Biol.*, 29, 151 (1893).

[4] *Loc. cit.*

Table VII.—Excretion of Nitrogen.

Investigator.	Johansson, Landergreen, Sonden and Tigerstedt.[1]	Lehman, Müller, Munk, Senator, Zuntz.[2]		Luciani.[3]	Ajello and Solaro.[4]	O. & E. Freund.[5]	Cathcart.[6]	Benedict.[7]			van Hoogenhuyze and Verploegh.[8]	Brugsch and Hirsch.[9]	Howe, Mattill and Hawk.	
				Succi.										
Subject	J. A.	Cetti.	Breithaupt.				Beauté.	S.A.B. 73.	S.A.B. 75.	(Average).	Tosca.	Schenk.	E.	H.
Last food.	22.41	13.49	13.02	17.85	8.99	——	16.45	12.50	19.50	——	13.99	——	10.43	9.81
Day of fast. 1	12.04	13.55	10.01	15.19	8.72	17.00	10.51	10.29	12.24	10.03	8.76	8.41	10.07	8.34
2	12.72	12.59	9.92	12.13	8.45	11.20	14.38	11.97	12.45	12.76	8.38	6.59	15.07	11.10
3	13.48	13.12	13.29	15.25	9.05	10.55	13.72	11.54	13.02	13.08	10.73	7.78	14.46	12.82
4	13.56	12.39	12.78	14.08	8.51	10.80	13.72	10.39	11.63	11.44	9.40	7.86	13.08	12.12
5	11.34	10.70	10.95	14.12	9.87	11.19	(11.30)	9.98	10.87	10.43	7.87	7.82	11.80	11.18
6	...	10.10	9.88	11.13	8.62	8.79	10.77	...	10.74	10.74	7.73	7.13	11.21	10.41
7	...	10.89	...	10.31	7.62	9.74	9.67	...	10.13	10.13	6.11	6.20	10.73	10.16

[1] *Skan. Archiv Physiol.*, **7**, 29 (1897).
[2] *Virchow's Archiv*, 131, suppl. (1893).
[3] *Das Hungern, Leipzig* (1890).
[4] *La Riforma Medica*, **9**, 2, 542 (1893).
[5] *Wiener klin. Rundschau*, **15**, 69 and 91 (1901).
[6] *Biochem. Z.*, **6**, 109 (1907).
[7] Benedict, *Carnegie Pub.*, **77** (1907).
[8] *Z. physiol. Chem.*, **46**, 415 (1905–06), woman.
[9] *Z. exp. Path. Therap.*, **3**, 638 (1906), woman.

variations in the total nitrogen excretion. The findings obtained from our experiment upon fasting men at moderate work show like changes to those already mentioned, there being an increase in the amount of nitrogen excreted on the second or third day and a gradual decrease to the end of the experiment. The excretion on the last day (seventh) of the fasts tended to approach a value very similar to that obtained by other investigators upon *men* (see Table VII, p. 589) *i. e.*, approximately *10 grams of nitrogen per day.* An interesting comparison may be made here with the findings from certain of Voit's experiments.[1] In these experiments he fed a 35 kg. dog with various diets having a wide nitrogen range and subjected the animal to a subsequent fast in each instance. His data indicate that the urea output for the seventh day was uniform no matter what the character of the diet which preceded the fast.

In our experiments the excretion of nitrogen after the first day was on a plane above that of the normal feeding period. This is a condition which did not exist in any of the experiments which have been heretofore reported. The explanation of this finding might at first thought be taken as an index of the effect of muscular work. However, upon comparison with the data of previous fasts and experiments reported in which muscular work has been a factor,[2] we are led to the belief that *this phenomenon was not due to the effect of work but rather was a result of fasting after a low-protein diet.* The preliminary diet of E contained 88 grams of protein, whereas that of H contained 76 grams. While these diets are not pronouncedly low-protein in character they are sufficiently low when considered in connection with the previous dietary history of these subjects to permit us to say that these men had been existing upon a low-protein diet for some time previous to the commencement of the fast.

We do not wish to be understood as claiming that the nitrogen level of the seventh fasting day was regulated in any marked degree by the fact that the subjects of our experiments came into the fast from a low-protein plane. The experiments of Voit, just referred to, would go to show that a similar level would have been reached upon this day even had the preliminary diet of these men contained 100 per cent. more nitrogen than that actually ingested. It is evident from a consideration of certain data obtained in this laboratory[3] that the claim of Voit does not always hold in the case of the dog. For example, in the investigation cited, the nitrogen excretion of the dog upon the seventh day of one fast was 2.56 grams whereas the nitrogen excretion of a subsequent fast was 1.13 grams upon the seventh day. This lack of agreement is

[1] *Z. Biol.*, 2, 307 (1866).

[2] Frentzel, *Arch. ges. Physiol.*, 68, 212 (1897); Lusk, *Science of Nutrition*, 2nd Ed., 79; Luciani, *Das Hungern*, (1890); Pettenkofer and Voit, *Z. Biol.*, 2, 459 (1866).

[3] Howe and Hawk, *Loc. cit.*

all the more remarkable when it is recalled that the dog in question was in each instance brought into nitrogen equilibrium previous to the fast through the feeding of the same uniform diet. This lowered nitrogen output of the second fast we have interpreted as *indicating an immunity or resistance acquired as a result of the first fast* which permitted the animal to make a more economical utilization of its reserves when again subjected to the complete withdrawal of food.

Subject H showed a slight positive nitrogen balance during the preliminary period, an average of 0.331 gram of nitrogen per day, and Subject E showed a somewhat larger positive balance, an average of 1.480 grams per day. While the subjects maintained their body weights upon the low-protein diets and were in good health, they did not give evidence of having any marked fat deposits. The previous dietary condition of the subjects may, then, be offered as the explanation of the nitrogen excretion which during the fast was above the plane assumed during preliminary feeding. In other words, the two men possessed a minimum storage of fat and glycogen, two factors which permitted an early consumption of the readily available protein substances of the body.

Urea Nitrogen.—During the fast the urea nitrogen fluctuated both absolutely and relatively with the variations in the total nitrogen excretions. The relative urea-nitrogen excretion rose with the increased total nitrogen excretion and fell with the decrease in the latter. This was to be expected,[1] but in addition there was a gradual decrease in the percentage of urea nitrogen excreted as the fast progressed, which is common to the urea excretion for fasting men as has been shown by previous investigators.[2] This is brought out in a very striking manner in our experiments when it is considered that *the nitrogen excretion during the fast remained above, while the urea percentage after the third day fell below,* the level of that of the preliminary feeding period. The decrease in the urea output has been explained from the standpoint of acidosis.[3] As a result of feeding, the normal conditions were again resumed and the relative urea-nitrogen excretion tended toward that of the preliminary period.

Ammonia Nitrogen.—The ammonia-nitrogen excretion increased both absolutely and relatively throughout the fasts. The relative increase in ammonia corresponded to the decreasing relative urea-nitrogen output and is explained in the same manner, *i. e.*, as due to the condition of acidosis which resulted from fasting. In the case of the subjects of this experiment, acetone and diacetic acid were found to be present in the urine during the final days of the fast, the acidosis according to

[1] Folin, *Am. J. Physiol.*, 13, 66 (1905).

[2] Cathcart, *Loc. cit.*; Brugsch, *Loc. cit.*; E. and O. Freund, *Loc. cit.*

[3] Bonniger and Mohr, *Loc. cit.*

qualitative tests being rather more pronounced in the case of H than in that of E. A consideration of the ammonia data will also show the output of ammonia to have been higher for H than for E during this period. This acidosis is believed to have been brought about through the formation of an excess of certain organic acids through the abnormal metabolic régime of fasting, the acids thus formed being ultimately neutralized by a portion of the ammonia which would in the natural course of events have passed by way of the portal circulation to the liver and assisted in the formation of urea. Instead of fulfilling this normal function, however, under the influence of the pronounced acidosis which accompanied fasting a certain portion of this ammonia combined with the organic acids and was eliminated from the body by way of the kidneys. Acidosis, therefore, was accompanied by an increased output of ammonia and a decreased urea excretion.

The ammonia-nitrogen excretion during the feeding period presented a peculiar phenomenon. We refer to the *marked increase in the ammonia output upon the third day.* This increase in the case of E was one of over 60 per cent., whereas in the case of H it was practically a 100 per cent. increase. There was a decreased output of urea in each instance but this decrease in the case of E fell far short of compensating for the high ammonia elimination. There was an accompanying increase in the output of creatinine.

A consideration of the data upon the percentage output of urea, ammonia and creatinine during the third and fourth days of feeding brought out a very striking condition in the metabolic processes of both subjects. Upon the third day occurred the high ammonia excretion and an increase in the quantity of creatinine excreted, while upon the following or fourth day of feeding, the nitrogen partition was very close to that of the preliminary feeding period denoting a return to the normal conditions of metabolism upon this day. It appears, then, that for a period of three days after the fast the metabolic processes were more or less in a chaotic condition due to the fact that the tissues were striving strenuously to make good the devastations brought about by the previous withdrawal of food. The anabolic processes were, therefore, stimulated far beyond the normal limits and all the varied activities of the organism were pushing forward the regeneration of the tissues and organs with the greatest acceleration. Through the very efficient coördination of the various functions of these two organisms, coupled with the further fact that a large quantity of food material was passed into them immediately after the fast ended, the activities which were making for a rapid regeneration were greatly aided. In a rough way we may consider that the normal rhythm of the metabolic functions was reassumed by the end of the third day. We are borne out in this assertion by the fact that the data obtained from an examination of the urine of the fourth day were approximately such as were secured from the normal metabolism of these individuals.

The excessive output of ammonia upon the third day was perhaps due to an attempt on the part of the organism to bring all the different phases of its metabolism to an approximately normal plane at the same time and for this reason all traces of acidosis were eliminated in the final effort. This process resulted in a much more pronounced ammonia excretion than had been in evidence during the two previous days. However, we cannot consider this very high ammonia excretion of the third day as due entirely to acidosis inasmuch as there was not a corresponding drop in the output of urea. This phenomenon may, perhaps, be explained upon the supposition that the acidoses of the previous days were somewhat more pronounced than the data indicate due to the fact that the ammonium compounds were not excreted as rapidly as formed during that period. This accumulation of ammonium compounds of various sorts may then be considered to have been retained in the body and excreted *in toto* upon the third day. This explanation seems the most logical for a condition such as we have here, *i. e., a very high ammonia excretion unaccompanied by an increased total nitrogen output or a compensating decrease in the urea excretion.*

As further evidence in favor of the belief that the plane of normal metabolism for these men had been reached by the fourth day we would cite the further facts that the creatinine coefficient was practically normal at this time and furthermore the subjects had regained at least 85 per cent. of the weight lost during the seven days of fasting.

If we examin the data presented by Cathcart[1] upon the professional faster, Beauté, we will note a similar attempt on the part of his subject to return to the plane of normal metabolism upon the fourth day of the feeding period subsequent to the fast. Our subjects, however, returned much more nearly to the normal plane than did Carthcart's subject. Carthcart makes no point of the fact that the metabolic régime of his subject came very near that of the normal at this time. In fact, he had no grounds for making an interpretation of this sort, inasmuch as just at this time he changed the diet of his subject from starch and cream to eggs and milk. It may be asserted that the change in diet was responsible for the pronounced alteration in the metabolic relations. In our experiments, however, there was no such change in the character of the diet and yet there was a very evident attempt on the part of the organism to assume normal relationships upon the fourth day.

The increased excretion of ammonia by Beauté upon the third day of the feeding period was not so pronounced as was this increase in the

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

case of E and H. However, the elimination was about three times as great as that excreted upon the following day or during the preliminary period. Furthermore, this was followed by a marked drop in the total nitrogen excretion with a return to a distribution of nitrogen very near the normal for this subject on the fourth day. A similar increased excretion of ammonia upon the fourth day of feeding has previously been reported from this laboratory[1] in connection with an experiment upon a dog subjected to "repeated" fasting.

It is a very interesting fact that the data from E and H and Beauté should be so very similar during the first part of the period of regeneration when it is appreciated that the diet in our experiment was a *high-protein* one, whereas, that of Beauté was practically *non-nitrogenous*. The data from Beauté if considered apart from the data on E and H might, perhaps, logically be interpreted as arising from the ingestion of a nitrogen-free diet, whereas the data from E and H if considered apart from Cathcart's report might be interpreted as brought about through the ingestion of a highly nitrogenized diet. Taking the two experiments under consideration, *i. e.*, Carthcart's and our own, we find that the main feature in common during the period in question was that the subjects in each instance were *ingesting food subsequent to a fast*. In one instance the food was *highly nitrogenous*, in the other it was *principally fat and carbohydrate*. However, the findings in the two cases, as already mentioned, were very similar *i. e.*, a very high ammonia excretion and low urea excretion upon the third day, followed by a return to conditions of nitrogen distribution closely approaching normal upon the fourth day. We would, therefore, give these findings a broad general interpretation and consider them as arising from the *ingestion of food by a fasting organism*. The character of the diet apparently had no very important bearing upon the situation.

Creatinine Nitrogen.—With the beginning of the fast, the creatinine-nitrogen excretion decreased slightly below the average of the feeding period and remained fairly constant at this new level throughout the fast with a slight tendency to decrease. Such a condition as we have described was to have been expected according to the ideas of Shaffer[2] and Folin[3] to the effect that the creatinine is an index of the muscular efficiency of the individual. The creatinine-nitrogen excretions in our experiment were nearly the same for the two men, denoting what was outwardly apparent, and equal muscular efficiency at the beginning of, and corresponding changes during, the fast. Relatively to the total nitrogen excretion, the creatinine-nitrogen excretion increased. Benedict[4]

[1] Howe and Hawk, THIS JOURNAL, 33, 215 (1911).

[2] *Am. J. Physiol.*, 16, 252 (1906).

[3] Folin, *Loc. cit.*

[4] *Loc. cit.*

has presented data showing a decrease in the creatinine nitrogen as a result of fasting. His data also show an increasing creatine-nitrogen excretion such that he considers the total excretion of creatine plus creatinine as *practically constant* during a fast. In our own experiments in the case of E there was a *gradually decreasing* output of total creatinine (creatinine + creatine) after the second day, whereas in the case of H there was less regularity with a general tendency toward a decrease. Cathcart,[1] Benedict and Diefendorf[2] and van Hoogenhuyze and Verploegh[1] have also noted a gradual decrease in the creatinine excretion. Other experiments reported from this laboratory[3] have furnished further evidence that the creatinine excretion appears to be a function of the amount of active muscular tissue in the body.

Creatine Nitrogen.—There was a general tendency for the creatine-nitrogen excretion to increase gradually during the first part of the fast, this initial increase being followed by a decreased output. A marked exception to this gradual increase was observed in the case of E, who eliminated his maximum creatine output upon the *first day of fasting.* Benedict in his experiments noted, in general, a rise in the creatine excretion as the fast progressed. On the other hand, the data submitted by Cathcart show a rise and a fall in the creatine excretion during the first seven days of fasting after which the daily excretion remained fairly constant.

We have already mentioned the fact that the sum of the excreted creatine and creatinine was fairly constant for each subject for each day of the fast. There was, however, a tendency for this total to decrease slightly as the fast progressed, thus causing the average excretion for the first four days to be appreciably higher than the average for the last three days. In the case of H, these averages were 0.606 gram and 0.568 gram, respectively, whereas in the case of E the values were 0.630 gram and 0.566 gram. It is significant that the average daily total creatinine output for the final part of the fast was practically the same for each subject, *i. e.*, 0.566 gram as against 0.569 gram.

The total creatine-nitrogen excretion of E during the fast was 0.557 gram. If we consider this creatine as representing disintegrated muscular tissue we find by calculation[4] that it is equivalent to sufficient muscular tissue to liberate 20.06 grams of total nitrogen. As a matter of fact, however, the total nitrogen output for this period aggregated 86.436 grams for this subject. In other words, less than 25 per cent. of the total nitrogen output can be accounted for on the basis of the creatine eliminated.

[1] *Loc. cit.*

[2] *Am. J. Physiol.*, 18, 362 (1907).

[3] Howe and Hawk, *Loc. cit.*

[4] Fowler and Hawk, *Loc. cit.*; Howe and Hawk, *Loc. cit.*

This condition was more pronounced in the case of H. The discrepancy becomes all the more evident when we recall certain other experiments made in this laboratory[1] which have demonstrated conclusively that the creatine content of muscle may be decreased 66 per cent. under the influence of fasting although retaining its nitrogen content but slightly decreased. It is evident, therefore, that a certain part of the creatine quota of the present study, which we have calculated as representing katabolized flesh, may logically be inferred as *having been removed from muscular tissue which was still functioning in the body* and which, therefore, aided in no way toward increasing the output of total nitrogen. Certain other experiments made in this laboratory have shown from an entirely different standpoint[2] that it is probably possible to remove creatine from muscular tissue without of necessity associating such removal with the katabolism of the creatine-bearing tissue.

The discrepancy mentioned as occurring between the total nitrogen output and the creatine output, considering for the moment that creatine represents disintegrated muscular tissue, might perhaps be partly explained on the basis that at least a portion of the excess of nitrogen arose from the katabolism of nitrogenous, non-creatinized substance within the organism. When katabolized, such constituents of tissue and fluids would aid in the augmentation of the total nitrogen output but would have no part in the creatine increase. One would expect, however, that the amount of nitrogen having this origin would be at the most a minimal quantity. It must also be borne in mind that the mere fact that the nitrogen content of fasting muscle is so near that of the normal is no absolute proof that muscle at some time, either before, during or after the removal of a portion of its creatine may not have lost a part of its nitrogen quota, a loss subsequently made good through synthetic processes.[3]

Summary.

Two men (instructors) of similar weight and general characteristics were fed for a period of seven days upon a uniform diet embracing a constant-water ration. Previous to this time they had been living upon low-protein diets for a period of at least six months, one upon a low-protein meat diet and the other upon a low-protein non-meat diet. After the preliminary feeding period they fasted seven days, receiving, however, a constant quantity of water each day. They meanwhile performed their usual college duties. Following the fast they were under observation for a period of eight days, during which time they ingested varying amounts of food without changing the quality of the diet as ingested in

[1] Howe and Hawk, *Loc. cit.*

[2] Fowler and Hawk, *Loc. cit.*

[3] Paton, *J. Physiol.*, 39, 485 (1910).

the preliminary feeding period. The distribution of the urinary nitrogen, the changes in body weight, the alterations in the urine volume, including the relative percentage of the ingested water which was eliminated in the urine, were all carefully determined. The observations made upon the men in this experiment exhibited very uniform relative variations during the various phases of the study.

The withdrawal of food from these men was accompanied by the following findings:

1. An increase in the excretion of total nitrogen followed by a decrease.

2. A urea excretion which followed that of total nitrogen, although the percentage of the total nitrogen which appeared as urea decreased.

3. A small decrease in the quantity of creatinine excreted.

4. The excretion of creatine.

5. A fairly constant output of total creatinine (creatine + creatinine) for each subject for each day of the fast.

6. An acidosis. This acidosis was indicated by a marked increase in the ammonia output and the appearance of acetone and diacetic acid in the urine.

7. *With both subjects the excretion of total nitrogen during the fast remained above the plane of the normal preliminary feeding period*, with the exception of the first day of fasting. This unique finding we have interpreted as being due to the fact that the subjects *began the fast from a low-protein plane*. In spite of this unusual condition as regards nitrogen output, however, the nitrogen excretion on the seventh day was very close to that for the seventh day of fasting *men* as determined by other investigators, *i. e.*, *ten grams*.

8. The largest water ingestion was *not* followed by the most complete elimination by the kidneys. A subject ingesting 1750 cc. per day eliminated 12–35 per cent. *less* than a subject ingesting only 1500 cc. per day.

9. A decrease in body weight, aggregating 7.44 per cent. for one subject and 7.62 per cent. for the other subject.

10. No discomfort with the exception of a slight feeling of hunger on the first or second day.

The ingestion of food by the subjects subsequent to the fast was accompanied by the following findings:

1. A marked retention of water during the first two days.

2. A pronounced retention of nitrogen from the second to the sixth day inclusive.

3. *A return to the normal metabolism upon the fourth day*, as indicated by the nitrogen partition. This return to the normal was preceded by an extremely excessive output of ammonia upon the third day. The return to the normal we have interpreted as having been brought about

simply through the *ingestion of nutritive material,* the character of the diet having no important bearing.

4. It was necessary to place the subjects upon a higher nutritive plane after the fast than before the fast in order to maintain body weight and nitrogen equilibrium.

5. Contrary to the custom usually followed in "breaking" a fast our subjects ingested the *maximum amount of food* for any individual day upon the *second day after the fast.*

URBANA, ILL.

NOTES.

Note on the Determination of Halogens in Organic Compounds.—To Stefanov's method for the determination of halogens in organic compounds[1] which is based on the reducing action of nascent hydrogen, formed by the action of sodium on ethyl alcohol, Bacon[2] proposed a considerable modification in the procedure by which he uses the action of sodium ethylate on the halogen compounds. We tried this modified method using monobromobenzene and α-bromonaphthalene, both of which were carefully purified. For monobromobenzene the calculated percentage of bromine is 50.93. A Carius determination on the sample we used yielded 50.83 per cent. Br. With Bacon's method about forty trials were made, all of the results being low, and in no case were duplicates obtained which agreed, which showed that the reduction was incomplete and irregular. The results for α-bromonaphthalene were low also, but much nearer the calculated percentage. We thought that perhaps an increase in the amount of nascent hydrogen might effect a complete reduction, so $1\frac{1}{2}$, 2, $2\frac{1}{2}$ times the amounts of sodium and alcohol recommended by Bacon were used. The results obtained with monobromobenzene were 35.36, 50.96, 48.65, 49.19, 49.40, 47.43, 47.18 per cent. Br. It will be noticed that the second result is very near the theoretical, but we were unable to get a similar result again.

The sodium used was Merck's, and the alcohol 99.5 per cent.

In acidifying with nitric acid we noticed that if an excess were used a vigorous reaction occurred with the production of nitrous fumes, and the liquid was changed to a greenish yellow color, which persisted even if boiled. The compounds produced affected the end point in two ways, (1) the ferric thiocyanate seemed to become reduced, with the result that more potassium thiocyanate had to be added, thus lowering the result for bromine, (2) the end point was harder to judge. This difficulty, however, is easily eliminated by acidifying only slightly.

We are forced to conclude, therefore, that this method is not of general

[1] *Ber.*, 39, 4056 (1906).

[2] THIS JOURNAL, 31, 49 (1909).