termined, given in column 6, differ from the calculated values, given in column 7, by amounts varying from 0.3% to 1%. In view of the indirectness of the oxygen determination a greater degree of accuracy could scarcely be expected.

Summary.

A method has been worked out for the simultaneous determination of hydrogen and oxygen in organic compounds, giving results differing by 0.3% from the calculated values in the case of hydrogen, and by 0.3% to 1% from the calculated values in the case of oxygen.

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[FROM THE LABORATORIES OF THE REFEREE BOARD OF CONSULTING SCIENTIFIC EXPERTS AT THE UNIVERSITY OF PENNSYLVANIA.]

A COPPER BALANCE ON SEVEN[1] EXPERIMENTAL SUBJECTS TO DETERMINE THE EFFECT OF EATING COPPERED VEGETABLES.

BY CARL L. A. SCHMIDT.

The following work constitutes part of the investigation on the "Action of Coppered Vegetables on the Health and Nutrition of Man" conducted under the general direction of Dr. A. E. Taylor of the Referee Board, the general results of which have appeared in Report No. 97 of the United States Department of Agriculture. Since Taylor's conclusions as to the harmfulness of the ingestion of vegetables colored with copper sulfate are almost entirely based on the fact that copper was apparently retained by the individuals eating coppered vegetables, it was thought important to publish the work in detail as a separate article.

The subjects, all of them medical students, were put on a constant diet previously determined by them in an experimental period. A certain amount of peas constituted a part of the daily menu for each individual. The diet was altered in some cases during the course of the experiment by substituting for part or all of the vegetables an increasing amount of peas or string beans so as to increase the amount of ingested copper. The coppered vegetables were purchased in large lots on the open market. For serving, the vegetable was steamed in the can, thrown on a colander, thoroughly mixed, and a sample taken for analysis at the same time that the portions were weighed out by the men. The experiment was divided into three parts—a foreperiod of two weeks during which time the men were given uncolored peas; an experimental period of nine weeks, during which coppered peas and string beans were eaten; and a two weeks' after- or controlperiod of uncolored peas and string beans. One subject was

[1] There were eight experimental subjects. Subject No. 1 was a poor subject for experimentation, being strongly influenced by psychological factors. For this reason the results obtained from him are omitted.

designated as control. He ate the same diet as the others, except, that uncolored peas and string beans were substituted for the coppered vegetables. For five days at the close of the experiment he was given copper sulfate in milk.

Urin was collected daily and determinations of nitrogen, phosphorus, and copper made. Feces were collected in aggregates of two- or three-week periods as indicated in Table I. Separation of the stools corresponding to the different periods was accomplished by giving charcoal on the morning of the new period. Feces from the afterperiod were collected in two lots of one week each so that the final week's collection might be free from any possible contamination of the stool from the previous period of coppered peas. In this way, a comparison between the normal output of copper in the final control week and the two weeks' foreperiod could be made. Feces were thoroughly dried and finely ground.

Copper in the vegetables and feces was estimated by the well-known method of electrolysis from a sulfuric acid solution, the copper being deposited on a platinum gauze electrode and subsequently weighed. One hundred grams of peas or string beans were thoroughly mixed with an excess of sulfuric acid in a porcelain dish, desiccated, and ignited at a low temperature. Addition of a few grams of potassium sulfate prevented any fusion. In determining copper in feces, 30 grams were ashed and an aliquot of the solution taken to represent 25 grams. The analytical factor for the daily input of copper varied between 1.5 and 3, since 100 grams of sample were taken for analysis, while the factor for the output averaged about twenty. The input of copper was determined daily, which might slightly increase the error for the total input. However, errors due to the daily sampling of the vegetable will probably equalize during a long time, while the error in the output copper was minimized by taking the average of several closely agreeing determinations. Estimation of the copper in the urins was carried out on five days' composit samples of 100 cc. each. The urin was oxidized by dropping into a heated mixture of nitric and sulfuric acids; the nitric acid then driven off by heating until white fumes appeared. All organic matter, except a small amount of an aromatic compound, is destroyed. This apparently resists oxidation; it can sometimes be noticed in the distillate from a Kjeldahl nitrogen determination. The sulfuric acid solution was electrolyzed, any traces of copper on the electrode dissolved with nitric acid and estimated colorimetrically as the ferrocyanide. Only faint traces of copper were found; the amount was never greater than that obtained on a blank determination. The elimination of copper in the urin is therefor excluded, a fact similarly observed by Long.[1]

[1] J. H. Long, Report No. 97, United States Department of Agriculture, 1913, p. 423.

TABLE I.—INPUT AND OUTPUT OF COPPER. (GRAMS.)

Subject No. 8.

1911.	Input.	Output.	Balance.
Mar. 1–14	..	0.031	...
Mar. 15–Apr. 4	0.3555	0.274	...
Apr. 5–19	0.3262	0.351	...
Apr. 20–May 2	0.3183	0.292	...
May 3–16	0.4487	0.513	...
May 17–23	..	0.019	...
May 24–30	..	0.018	...
Total	1.449	1.498	—0.049

Subject No. 7.

1911.	Input.	Output.	Balance.
Mar. 1–14	..	0.026	...
Mar. 15–Apr. 4	0.3555	0.289	...
Apr. 5–19	0.3262	0.294	...
Apr. 20–May 2	0.3183	0.389	...
May 3–16	0.4809	0.454	...
May 17–23	..	0.043	...
May 24–30	..	0.019	...
Total	1.481	1.514	—0.033

Subject No. 2.

1911.	Input.	Output.	Balance.
Mar. 1–14	..	0.037	...
Mar. 15–Apr. 4	0.3555	0.310	...
Apr. 5–18	0.2293	0.220	...
Apr. 19–May 2	0.1753	0.187	...
May 3–16	0.2737	0.294	...
May 17–23	..	0.018	...
May 24–30	..	0.015	...
Total	1.034	1.081	—0.047

Subject No. 6.

1911.	Input.	Output.	Balance
Mar. 1–13	..	0.029	...
Mar. 14–21	..	...	...
Mar. 22–Apr. 4	0.2337	0.175	...
Apr. 5–18	0.2293	0.248	...
Apr. 19–May 2	0.1753	0.258	...
May 3–16	0.2737	0.292	...
May 17–23	..	0.022	...
May 24–30	..	0.015	...
Total	0.912	1.039	—0.127

Subject No. 5.

1911.	Input.	Output.	Balance.
Mar. 1–14	..	0.036	...
Mar. 15–Apr. 4	0.3555	0.314	...
Apr. 5–20	0.3262[1]	0.330	...
Apr. 21–25	...	...	...
Apr. 26–May 2	0.1758	0.190	...
May 3–16	0.4487	0.424	...
May 17–23	..	0.055	...
May 24–30	..	0.019	...
Total	1.306	1.368	—0.062

Subject No. 3.

1911.	Input.	Output.	Balance.
Mar. 1–14	..	0.028	...
Mar. 15–27	..	0.035	...
Mar. 28–Apr. 11	0.2512	0.165	...
Apr. 12–25	0.1985	0.220	...
Apr 26–May 2	0.0880	0.100	...
May 3–16	0.2737	0.215	...
May 17–23	..	0.016	...
May 24–30	..	0.018	...
Total	0.811	0.797	+0.014

Subject No. 4.

1911.	Input.	Output.	Balance.
Mar. 1–14	...	0.016	...
Mar. 15–Apr. 4	...	0.047	...
Apr. 5–18	...	0.027	...
Apr. 19–May 2	...	0.057	...
May 3–16	...	0.041	...
May 17–23	0.157[2]	0.125	...
May 24–30	...	0.014	...
Total	0.157	0.327	—0.170

[1] No peas eaten on Apr. 20.

[2] 0.0314 gram Cu as $CuSO_4$ in milk for 5 days.

TABLE II.—RÉSUMÉ.

Actual copper balances.

A.

Period of eating vegetable colored with $CuSO_4$.	Subject No. 8. M. W.	Subject No. 7. J. F. W.	Subject No. 2. M. R. B.	Subject No. 6. W. C. T.	Subject No. 5. C. P. K.	Subject No. 3. O. H. C.	Subject No. 4. E. S. F.
Input of Cu in vegetable, grams	1.449	1.481	1.034	0.912	1.306	0.811	0.157[1]
Other input of copper, gram	0.140	0.117	0.167	0.125	0.148	0.100	0.016
Total input of copper, grams	1.589	1.598	1.201	1.037	1.454	0.911	0.173
Total output of copper, grams	1.430	1.426	1.011	0.973	1.258	0.700	0.125
Retention of copper, gram	0.159	0.172	0.190	0.064	0.196	0.211	0.048

B.

Entire time of experiment.	Subject No. 8. M. W.	Subject No. 7. J. F. W.	Subject No. 2. M. R. B.	Subject No. 6. W. C. T.	Subject No. 5. C. P. K.	Subject No. 3. O. H. C.	Subject No. 4. E. S. F.
Input of Cu in vegetable, grams	1.449	1.481	1.034	0.912	1.306	0.811	0.157
Other input of copper, gram	0.202	0.169	0.241	0.185	0.219	0.182	0.204[2]
Total input of copper, grams	1.651	1.650	1.275	1.097	1.525	0.993	0.361
Total output of copper, grams	1.498	1.514	1.081	1.039	1.368	0.797	0.327
Retention of copper, gram	0.153	0.136	0.194	0.058	0.157	0.196	0.034

Table I shows the input of copper, the output in the different periods, and the corresponding balances, as well as the grand totals for the entire time of the experiment. It will be seen at once that during the foreperiod there was an output of copper in the feces of all subjects, although apparently no copper was given. This can only be accounted for by the fact that small amounts of copper were present in the foods eaten. That many cereals and vegetable products contain small traces of copper has been shown by other observers.[3] All subjects including the control showed an output of copper during the foreperiod, the amounts being nearly the same in all cases. If some of the feces of the second period had been carried over into the first, one would naturally expect that the amounts would vary; similarly some of the first period's feces would, in some subjects, be carried over into the second period, in which case either no copper ought to appear in those feces (*i. e.*, if there was no copper normally ingested in foods) or only that resulting as normal elimination. The amount of copper normally ingested was too small for direct estimation. The average daily ingested matter (including water) for subject No. 8 for the two weeks of foreperiod was 3070 grams. During that time the output copper in the feces was 0.031 gram. This would allow less than 1 mg. of copper per kilo of ingested matter.

The analytical balances of all subjects, except No. 3, are negative as shown in Table I. A small retention is observed in No. 3. In order that a true balance may be obtained, an allowance must be made for normally

[1] Balance for week when $CuSO_4$ was taken in milk.

[2] Calculated from entire time subject was control.

[3] *Ibid.*, page 214 and 424.

ingested copper. This can be done by assuming that the output during the foreperiod corresponds to the normal input during that time. That this assumption is very nearly correct is shown by the fact that the average daily elimination of copper in the final control week of all subjects corresponds very closely to that of the two weeks of foreperiod.

Taking the output of copper in the foreperiod of each subject as the basis of normal input copper, we can arrive at a balance which probably represents more nearly the true copper balance. Part A of Table II shows the copper balance during the period when vegetables colored with copper sulfate were eaten, allowances being made for a normal input of copper, while part B of the same table shows the balance for the entire time of the experiment. All subjects show a plus balance. This clearly indicates a retention of copper.

The plus balances obtained cannot be wholly due to analytical error, since it is not conceivable that the analytical error, which is largely due to sampling, should always be in the same direction for the entire time of the experiment. It may also be said that during the course of the experiment the diet was changed by substituting for part or all of the vegetables, coppered peas and string beans, and hence the normal input of copper as calculated from the foreperiod is incorrect. This is true only to slight extent since the amount of vegetables daily ingested is small as compared to the total of other ingested matter. The figures, as given, may not show the absolute copper balance, but since in all the subjects the balance is decidedly positive and of the same order of magnitude, the only conclusion is that there was a retention of copper in all individuals. That there was a retention even after the two weeks of afterperiod is also shown. That the copper so retained will probably be held in the body for some time and but slowly eliminated, is indicated by the fact that the average daily elimination of copper in the final control week is nearly the same for all subjects as that of the two weeks foreperiod.

Conclusions.

(1) Seven subjects on an experimental diet, a part of which consisted of coppered vegetables, showed a distinct retention of copper.

(2) In order to obtain a true balance, an allowance must be made for copper normally ingested in foods. This value can be obtained by assuming that the output of copper in the foreperiod represents the copper ingested during that time.

(3) The copper so retained in the body will probably be only slowly eliminated.

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