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The Sulphur Excretion as Influenced by Copious Water-Drinking with Meals

Chemical Engineering

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THE SULPHUR EXCRETION AS INFLUENCED BY COPIOUS WATER-DRINKING WITH MEALS

938 / 35 / 2n/9

BY

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THESIS FOR THE DEGREE OF BACHELOR OF SCIENCE

IN CHEMICAL ENGINEERING

IN THE

COLLEGE OF SCIENCE

OF THE

UNIVERSITY OF ILLINOIS

Presented June, 1911

1911
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UNIVERSITY OF ILLINOIS

June 1st, 1911

THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Lucius A. Fritze

ENTITLED THE SULPHUR EXCRETION AS INFLUENCED BY COPIOUS WATER-DRINKING WITH MEALS

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF Bachelor of Science in Chemical Engineering

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The Sulphur Excretion as Influenced by Copious Water Drinking With Meals.

Introduction.

The determination of total sulphur in urine presents a number of peculiar features. The sulphur distribution is so varied and the compounds containing it so stable that it requires a vigorous course of oxidation to change it to a sulphate. Along with the oxidation there is required some metal or substance to unite with the sulphate to form a stable body. The sulphur as found in urine is in combination in a number of ways. It is obtained as inorganic sulphates, ethereal sulphates, neutral sulphur, and sometimes in the free state. The amounts of these various forms are indications of the extent of protein catabolism. Folin[1], in his paper on the laws governing the chemical compo-

[1] Folin: Am. J. Physiol. 13, 66, 1905.

sition of urine, claims that the sulphur in urine is frequently used as a measure of the total amount of protein catabolism. It is recognized, however, that the sulphur elimination is much less accurate than the nitrogen as a measure of protein catabolism because of the well known fact that different protein substances contain very different percentages of sulphur. The ethereal sulphates according to Baumann[1a] are derived from aromatic products

[1a] Baumann: Arch. Ges. Physiol. 12, 13.

formed in the intestines by the action of bacteria on the protein

The Rare Book &

of the food. The belief held at the present time, however, is that indican is the only ethereal sulphate having its origin in the putrefaction processes. The "neutral" sulphur is not altogether fully understood, but it is supposed to be more or less derived from the bile acids. Folin, in the article already referred to, gives some valuable suggestions for the determination of the sulphur as sulphate. He suggests that the barium chloride be added to the cold acidified urine very slowly. He claims that among the sulphur constituents of the urine there are very minute quantities of substances which can scarcely be determined separately, but which are included in the total sulphur, and which must therefore be counted as inorganic, ethereal, or as neutral sulphur. The substances which most likely occur as sulphur constituents are hydrogen sulphide and the thiocyanates. These compounds are determined with the ethereal sulphates since the scheme of analysis does not make a complete separation of them before this determination.

According to Benedict[2] the compounds existing in urine which

[2] Benedict: J. Biol. Chem. 6, 363, 1909.

carry unoxidized sulphur are so stable as to require very vigorous oxidizing agents to convert the sulphur into sulphuric acid, yet the subsequent precipitation of the sulphate must take place in the complete absence of oxidizing agents, as well as of numerous special salts which modify the precipitate. Another requirement in the process is that the oxidation takes place in the presence of some substance capable of forming a stable combination with the newly oxidized sulphur. In view of the very questionable

value of the nitric acid process for total sulphur estimation, and on account of the difficulties attending the fusion process, he proposes the method as used in this thesis. The method has it's basis in the endeavor to find a substance, which, while a vigorous oxidizing agent at certain temperatures, should. at the same time, be decomposing into a neutral, non-oxidizing compound. This would form a basis for the oxidized sulphur to form a stable combination. Copper nitrate is the substance found which satisfies these conditions. This salt upon heating is decomposed into nitrogen dioxide and cupric oxide. The nitrogen dioxide being a gas is completely removed. The cupric oxide forms a base for the oxidized sulphur. For a proof of the validity of the method Benedict compares a series of results obtaind by this method with a series obtained by the sodium peroxide method of Folin. The two methods check within the allowable limits of error and the conclusion drawn is that the new method is as satisfactory as the old.

In a paper presented by Koch and Upson[3] a treatment of the estimation of the various elements such as nitrogen, phosphorus, sulphur, etc, is dealt with. Nitrogen enters the body and leaves as an unoxidized derivative. Phosphorus enters and leaves as an oxidized derivative, while sulphur enters in an unoxidized form and leaves as the highest oxidation product. In the determination of the sulphur there are certain difficulties to be overcome. (1). The possibility of loss from to rapid combustion especially with dry material, and the danger of spattering with a material in a semi-liquid state. (2). The risk of incomplete combustion,

[3]Koch and Upson: J. Am. Chem. Soc. 31, 1355, 1909.

due to the caking of the fusion mixture. The method used in these... was that of Schmiedeberg. This method gave very good results in cases where large amounts of organic material had to be destroyed but it required time and care. The principle of the method consists in the gradual charring with an alcohol burner of the material in a mixture of seven parts of sodium carbonate and one part of potassium nitrate. The proportion of the potassium and sodium avoids the source of error, pointed out by Folin, in the precipitation of the barium sulphate. The final burning is made with a Barthel alcohol burner at a temperature just below the fusion point of the mixture. After acidifying with hydrochloric acid, a few drops of bromine water are added to remove any nitrous oxide in the solution and the precipitation is done in the usual way.

Weiss[4] compares the results obtained from the analysis of

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[4]Weiss: Biochem. Z. 27, 175-203, 1910.

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the urine of twelve normal men. From 12 per cent to 40 per cent of the sulphur content was found to be neutral sulphur, the general average being 16.5 per cent. Part of the neutral sulphur comes from food protein (exogenous), and a large part from the tissue protein (endogenous), especially in fasting experiments. Conditions causing increased destruction of body protein increase the proportion of neutral sulphur excreted. In pulmonary tuberculosis both the absolute and the relative excretions of neutral sulphur increase. In carcinoma 20 per cent to 55 per cent of the total sulphur was found to be neutral. The excretion of neutral sulphur runs parallel to that of the proteinic acids.

An important fact was pointed out by Tonschegg[5] that in

[5] Monschegg: Arch. Exp. Path. Pharm. [illegible] - 5[illegible] - 17, 191[illegible].

spite of a pronounced decrease in the indican excretion the total output of ethereal sulphuric acid in the urine suffers no diminution when sulphates or elementary sulphur have been ingested. This holds true though accompanied by diarrhea, provided that the latter is not of particularly vigorous form. This fact indicates the possibility that a synthesis of ethereal sulphuric acids occurs in the cells of the intestinal wall when the SO_4 concentration within the intestines increases. With the ingestion of elementary sulphur this formation of ethereal sulphuric acids seems to be preceded by the oxidation of sulphur to sulphuric acid in the cells of the intestinal walls. The findings of Monschegg are of especial interest when taken into consideration in connection with certain results recently obtained in this laboratory. Hattrem and Hawk[6]

[6] Hattrem and Hawk: **Archives of Internal Medicine. (In press.)**

in a study of the intestinal putrefaction processes have determined that the ingestion of a comparatively large volume of water at meal times is instrumental in lowering the output of urinary indican and, furthermore, that such decrease is accompanied by an increased output of total ethereal sulphates. This finding indicates that indican has a different origin from the other ethereal sulphates. Guitaro[7] cites the results obtained in a study of the sulphur con-

[7] Guitaro: Riv. Crit. Clin. Med. 10, 593 - 5,

tents of the urine of children and adults. In children 9 -11

years of age the neutral sulphur was 17.21 per cent of the total urinary sulphur. In adult males it was 19.41 per cent, and in the urine of women it was 21.14 per cent of the total sulphur. This would indicate that the oxidizing processes are strongest in children and the weakest in women.

A new method for the determination of the total sulphur in urine has been proposed by Abderhalden and Funk.[8] When 10 cc. of

[8] Abderhalden and Funk: Z. Physiol. Chem. 58, 331-3, 1897.

urine mixed with a little sodium carbonate and 0.4 grams of lactose are evaporated in a nickel crucible to dryness on the water bath, the residue mixed with 6.4 grams of sodium peroxide, and the mixture fired with a red hot iron nail, the results obtained, proceeding subsequently as usual, are in close agreement with those obtained by the fusion of the urine ash with sodium peroxide, potassium nitrate mixture. Another method has been proposed for the quantitative estimation for sulphur in urine by Shulz.[9] He takes

[9] Shulz: Arch. Ges. Physiol. 121, 1146, 1900.

5-10 cc. of urine and an equal quantity of fuming nitric acid and digests it in a Kjeldahl flask. The flask is heated vigorously in a slanting position until no more fluid condenses in the neck, ordinarily from 10-15 minutes being required for the process. When cool, water and hydrochloric acid are added, the contents are heated to boiling, transferred quantitatively and precipitated with barium chloride. The analyses were controlled by nitric acid digestions in which the acid fumes were collected in water to intercept any sulphuric acid which might be present.

Konschegg[10] makes several comments on this method. He says

[10]Konschegg: Arch. Ges. Physiol. 123, 274-8, 1902.

the method in the hands of the author shows a loss of sulphur when controlled by alkali nitrate fusion. The addition of 1-2 cc of 20 per cent potassium nitrate solution insures the presence of a sufficient amount of basic radical to prevent volatilization of the sulphuric acid. Analyses given show that the per cent differences between results on six samples of urine by the author's modification of the Shulz method and by the alkali fusion process are very small, the greatest variation being limited to the second decimal place.

A new method has been proposed by Hess[11] for the determination

[11]Hess: Berl. Klin. Wochschr. 45, 1452-3, 1908.

of "neutral" sulphur in urine. At least 500 cc of urine are acidified with pure hydrochloric acid (5-10 cc HCl to every 100 cc of urine) then treated with a slight excess of barium chloride and heated to boiling, subsequently being kept fully six hours on the water bath. This process should be carried out in a flask fitted with an apparatus similar to Peligot's, containing pyrogallic acid and lye, to prevent oxidation by the air. It is filtered cold after standing 24 hours. The water-clear filtrate, containing neutral sulphur, is made stongly alkaline with pure sodium hydroxide and chlorine gas is passed through the solution until it is saturated. During the operation the beaker should be covered with a watch glass. Some hours later the fluid is acidified with pure hydrochloric acid, the chlorine is driven off by heating, and the fine precipitate is allowed to settle. This is the neutral sulphur as barium sulphate,

and is then weighed. The daily output normally on a mixed diet varies from .0044 to .0128 grams. Pathologically the output may vary markedly from these values. It is highest (up to .0811 grams) in organic diseases of the central nervous system and in chronic tuberculosis (up to .0637 grams).

The sodium peroxide method as proposed by Folin was found to give inaccurate results by Gill and Grindley.[12] The fusion gave

[12] Gill and Grindley: J. Am. Chem. Soc. 31, 52-9, 1909.

off hydrogen sulphide in many cases when acidified. By adding small amounts of sodium peroxide to the hot fusion a product was obtained which did not give off hydrogen sulphide when acidified, but the total sulphur obtained even by this modified Folin method gave results lower than those obtained by Konschegg's method, in which fuming nitric acid and potassium nitrate were used. The authors consider that their work throws considerable doubt upon the true values of the data obtained in the past experiments as to sulphur metabolism where the alkali fusion methods have been used to determine the amount of sulphur in the urine.

In reply to this criticism Folin[13] cites results of analyses

[13] Folin: J. Chem. Soc. 31, 284, 1909.

made by him using the sodium peroxide method, and criticises the fuming nitric acid method. He claims that Gill and Grindley did not follow his directions carefully for it would have been impossible for them to obtain hydrogen sulphide from a sodium peroxide fusion. He considers that the higher results obtained by the nitric acid method are due to compensating errors, and that the acid method is

less reliable than the sodium peroxide method as described by him.

Hawk,[14] in his work on the time relations of protein metabolism

[14]Hawk: J. of Physiol. 10, 115, 1903.

takes up the matter of the nitrogen, sulphur and phosphorus ingestion and excretion. The results given were obtained from two subjects on a known diet for a period of nine days. The author says: "In general the cause of the sulphur excretion followed that of the nitrogen. A notable difference, however, was in the greater regularity in the position of the points of maximum excretion in the case of the sulphur." The relative excretion of sulphur of both subjects was fairly constant during the whole experiment. With one subject the sulphur excretion of every day of the experiment showed two well defined maxima; in the case of the other subject there were no such points of maximum excretion. The sulphur excretion of subject one was lowest during the first period of the day and the lowest period of subject two was during the night. After the ingestion of extra protein the sulphur excretion of subject one began to rise in three hours and the rise of subject two took place at once. The maximum sulphur excretion occured with each subject six to nine hours after the ingestion of the extra protein. The normal rate of excretion was regained in 24 hours. The ratio between nitrogen and SO_3 was the lowest on the day following the ingestion of extra protein, due to the fact that the SO_3 reached the normal level more quickly than the nitrogen.
In their article on the elimination of nitrogen, sulphates and Phosphates after the ingestion of proteid food Sherman and Hawk[15] show the relation of these elements as excreted to the composition of the

food ingested. The purpose of the experiments was to study the

[15]Sherman and Hawk: Am. J. Physiol. 4, 25, 1900.

time of appearance and the extent and duration of some of the changes in the urinary excretion which result from the ingestion of a rather large quantity of protein food when the body is kept in a uniform and perfectly normal condition of nutrition. The diet furnished about 15 grams of nitrogen and 2600 calories of energy, and was divided into three nearly equal meals at about the ordinary intervals. After the diet had been maintained until the elimination of nitrogen became fairly regular, most of the fat of a single morning meal was replaced by an isodynamic amount of protein, so that about ten grams of extra nitrogen was taken without any change in the supply of potential energy available to the body. When the effect of the change seemed to have disappeared a similar pair of experiments was made in which the protein was simply added to the morning meal thus increasing the nitrogen about ten grams and the fuel value about 400 calories. In general the excretion of sulphates ran closely parallel to that of nitrogen both on the normal days and on those affected by the extra ingestion of the protein. On close examination it, however, appears that the relative increase of the SO_3 as compared with that of nitrogen is somewhat less in the first period following the ingestion and a little greater in the third. After reaching the maximum the rate of excretion of the sulphates falls rapidly reaching the normal after about 27 hours. In the summary the following is given: As measured by three hour periods, the rate of excretion of nitrogen and sulphates run closely parallel and normally show a tendency to

rise during the morning reaching a maximum after the midday meal with a slight fall in the following period and another rise after the evening meal. During the night the excretion usually reaches the minimum. The increased excretion of sulphates was proportional to that of nitrogen, and followed the same general course. It appeared, however, to begin a little later and certainly regained the normal a little earlier.

In experiments in which the metabolism was increased by muscular work, Gairatt[16], found the increased excretion of sulphates to be proportional to that of urea but of less duration and of greater intensity, requiring only one half as long either to reach the maximum or to regain the normal.

Hawk and Chamberlain[17], in their work on the influence of a small increased proteid ingestion investigated first, the parallelism of the rates of excretion of the nitrogen and sulphates and the independent course of the phosphate excretion, and second, the time interval between the ingestion of extra proteid food and the maximum rate of the nitrogen, sulphate and phosphate excretion following this ingestion etc. Comparing the excretion of sulphates with that of nitrogen for each subject it was found that the rate of excretion of sulphates followed more closely that of nitrogen than did the rate of phosphate excretion. There was, however, no exact parallelism. The daily maximum of the nitrogen excretion was usually in the second or third period, while the

[16] Gairatt: J. of Physiol. 23, 150, 1898.

[17] Hawk and Chamberlain: Am. J. of Physiol. 10, 269, 1904.

maximum rate of excretion of sulphates usually appeared in the fourth or fifth period. Furthermore, while the rate of nitrogen excretion rose immediately after the morning meal, that of the sulphate excretion fell. Thus the maximum rate of sulphate for the day on which extra proteid food was ingested occurred with one subject during the fifth long (three hour) period and with the other during the third. In the case of the latter subject, the rate of excretion of sulphates followed, in general, that of nitrogen on the day following the extra ingestion, and fell to the normal one day later. With the first subject the rate of excretion of sulphates regained its normal after four days, as was also true of the nitrogen.

In his work on the metabolism of nitrogen sulphur and phosphorus in the human organism Sherman[18], discusses the relative income and outgo of these three elements and also the change that each undergoes. The sulphur or phosphorus balance, like the nitrogen balance, may be found by comparing the amounts ingested in the food with those eliminated through the kidneys and intestines. By far the greater part of the sulphur of the food enters the body in organic combination in protein substances. When protein matter is oxidized in the body most of the sulphur is changed to sulphuric acid, the greater part of which appears in the urine as normal inorganic sulphates. A smaller part of the sulphuric acid is found in the form of ethereal sulphates, that is combined with organic radicals. Not all of the urinary sulphur, however, exists in the form of sulphates. About 15 to 20 per cent is usually found in less complete oxidized forms, this portion being called unoxidized

[18] Sherman: U. S. Dept. Ag. Bull. 121, 1902.

or neutral sulphur. In the paper there are discussed the results of ten digestion experiments besides the metabolism of the three main elements. In each experiment the diet was uniform and the urine for each twenty-four hours was collected and examined. Comparing the"sulphate" and "total" sulphur in the different experiments, it would appear that from 83.1 to 89.6 per cent of the sulphur in the urine was in the form of sulphates. The effect of the loss of sleep on the elimination of the three elements was studied. The loss of sleep tends to cause an increased elimination of each element, but for lack of time the sulphur determination was not considered thoroughly. Upon the change of diet the "lag" of elimination of sulphur, nitrogen and phosphorus increased simultaneously. All these experiments had to do with the increased elimination brought about by the ingestion of extra protein with a single meal. In the summary the following conclusions are made: The digestibility of the protein of the bread and milk diet as found in nine of the ten experiments agreed closely with the results calculated. Marked loss of sleep for three successive nights resulted in a small increase in the amounts of nitrogen, sulphur and phosphorus excreted. The increase of sulphur was proportional to that of nitrogen and the increase of phosphorus was very slightly larger, the relative difference being no greater than might be attributed to the usual daily variations. The increased elimination resulting from loss of sleep did not appear until the third day, while changes resulting from alterations of the diet were always perceptible on the first day. In general, the metabolism and "balance" of sulphur ran approximately parallel with that of nitrogen.

In his interesting paper on the investigation of the influence

of copious water drinking, Hawk[19], discusses the effect of water

[19]Hawk: Univ. of Penn. Med. Bull. 18, 7, 1905.

on the excretion of nitrogen, sulphur and phosphorus. At the time of nitrogen equilibrium there was practically a condition of sulphur equilibrium as well. When a large amount of water was ingested it was observed that an immediate rise in the amount of SO_3 excreted in the urine occurred. The excretion for the two days following the water period ran high, and following this the SO_3 equilibrium was again established. As in the case of the nitrogen elimination the total influence upon the elimination of SO_3 of the ingestion of the water may be measured only by taking into consideration the data of the excretions from the beginning of the water period to the time when SO_3 equilibrium was re-established. Calculating upon this basis, we find that the average normal output of SO_3 observed during the preliminary period, upon the constant diet, was increased by the water ingestion on an average 0.195 grams daily for a period of four days, the total increase being 0.77 grams for the four day period. The excretion of SO_3 followed that of nitrogen as measured in the twenty-four hour periods, in beginning its increased elimination upon the day of increased water ingestion and also in regaining its original equilibrium plane at the expiration of the second day after the close of the water period. The excretion of the SO_3 differed from that of the nitrogen in the fact that the increase in SO_3 on the first day of the copious water drinking was 15.6 per cent above the normal, whereas the increase in nitrogen was but 12.8 per cent. The most stiking contrast between the course of the excretions, however, was seen in the fact that the total increase in

the excretion of nitrogen, due to the influence of water, was 6.0 per cent above the normal, whereas the total increase in the excretion of SO_3 was 11.4 per cent above the normal output of the preliminary period. In conclusion, the author says: "The course of the SO_3 excretion while somewhat irregular, still showed a tendency to run parallel with that of nitrogen. A daily addition of 4500 cc of water for a period of two days caused an increase of 0.265 grams or 15.6 per cent in the SO_3 excretion by the urine on the first day, and one of 0.195 grams or 11.6 per cent, on the second day. When the amount of water added daily during a period of forty-eight hours was 3100 cc, the increase upon the first day varied from 0.205 grams or 10 per cent, to 0.085 grams or 4.1 per cent. On the second day there was an increase of 0.128 grams or 6.2 per cent. In one experiment the SO_3 excretion was decreased on the days in which 3100 cc of water was added to the diet, the decrease varying from 0.309 grams or 20.9 per cent to 0.016 grams or 0.81 per cent for the first day, and from 0.258 grams or 14.4 percent to 0,124 grams or 6.3 per cent for the second day.

Description of Experiment.

In the present experiment two subjects H and W were used. They were young men aged twenty and twenty-five respectively, and were engaged in active university work throughout the course of the investigation. The men were brought into nitrogen equilibrium by a preliminary period on a uniform diet which remained the same throughout the entire experiment. The preliminary period was followed by a five day water period during which time 1000 cc of water were ingested in addition to and with each regular meal. Following this period was the final period of three days for H, and five for W, in which the additional 1000 cc of water with meals was

not taken. The conditions in this period were the same as in the period before copious water drinking.

Daily Schedule, Diet, etc. The following was the daily schedule:

6:30 A.M. arose.

7:15 A.M. urinated, defecated, and weighed.

7:20 A.M. breakfast.

10:00 A.M. 200 cc water taken.

12:00 M. lunch.

3:00 P.M. 200 cc water taken.

5:30 P.M. dinner.

6:15 P.M. one cigar. (W only).

8:30 P.M. 200 cc water taken.

Each meal consisted of:

Graham crackers---------------150 grams.

Peanut butter----------------- 20 grams.

Butter------------------------ 25 grams.

Milk--------------------------450 cc.

Water-------------------------100 cc.

The graham crackers were purchased in quantity in original packages and carefully sampled before beginning the experiment. The peanut butter was bought in one pound jars, mixed in a large dish, sampled, and returned to the jars. The butter was put up and sampled from a single "making" at the university creamery. The milk was obtained in quart bottles and always well shaken and mixed before use. Occasionally during the experiment the milk was analysed for a check. The water used was obtained from the university supply and was softened by adding five litres of saturated lime water to thirty litres of the tap water. It was

allowed to settle then filtered and placed in carboys for use.

The analysis of the foods were as follows:

Graham crackers	1.391 per cent nitrogen.			
Peanut butter	4.412	"	"	" .
Butter	0.079	"	"	" .
Milk	0.509	"	"	" .

For the analysis of the University of Illinois water see Fowler and Hawk[1]. The water was treated in exactly the same manner as in that experiment and the composition of the supply is

[1]Fowler and Hawk: J. Exp. Med. 12, 388, 1910.

practically constant.

Collection and Analysis of Urine: The urine was collected in twenty-four hour periods and kept in a refrigerator until analyzed. The experimental day began and ended at 7:15 A.M. The samples for analysis were immediately measured and the analysis started at the close of each experimental day.

Method.

The method used in the total sulphur determination is the one proposed by Benedict[20]. The procedure is as follows:10 cc of urine

[20]Benedict: Loc. cit.

are measured into a small evaporating dish and 5 cc of Benedicts sulphur reagent added. (This reagent is made by adding 200 gr. crystallyzed copper nitrate to 50gr. $KClO_3$ in 1000 cc of water.) The contents of the dish are evaporated over a free flame which is regulated to keep the solution just below the boiling point, so there can be no loss through spattering. When dryness is reached the

flame is raised and the dish is heated until the contents became blackened. The dish is then heated to redness for ten minutes. This heating decomposes the last traces of chlorate or nitrate. The dish is then allowed to cool. The contents of the dish are dissolved in 1:4 hydrochloric acid. The solution thus obtained is then transferred to an Erlenmeyer flask, diluted with 150 cc distilled water and ten cc of a ten per cent barium chloride solution added. After the barium sulphate has settled it is filtered upon a Gooch crucible and weighed. The method was followed in detail up to the point where the barium chloride was added. Here at first, a slight modification was attempted. Instead of adding the barium chloride to a cold quiet solution, drop by drop, the author agitated the solution by means of a paddle. The apparatus used was a funnel flatened and bent at the end, and held in the solution by means of an iron clamp. The funnel was revolved by means of a string attached to a small water motor. By this means the solution was kept in constant agitation. The barium chloride was poured into the funnel and entered the solution through a hole in the paddle. A series of analyses were made, on the same urine, to compare the results of the two methods. The results obtained are found in Table I.

From the first series of determinations it will be seen that the results of the new method are higher than those of the Benedict method. In the second set the results are just reversed. The conclusion drawn from this is that the difference in weight is due to experimental error. Since the results of the Benedict method were just as reliable as the results from the modification, the direct Benedict method was followed.

In Table II are given the data obtained by the author in testing

the accuracy of his manipulation of the method. The results check very well considering the opportunity for error in sulphate determinations. At first there was a constant error through loss by spattering. The use of a larger evaporating dish was attempted but this failed to reduce the error. Finally, sodium chloride, as suggested by Denis,[21] was added to each determination with the re-

[21]Denis: J. Biol. Chem. 8, 401-3, 1910.

sult that spattering was reduced considerably. With these slight changes the method as described was followed.

Denis[22], has commented on Benedict's method, claiming that the

[22]Denis: Loc. cit.

spattering at first, due to the ignition of the potassium chlorate, always causes mechanical error. In forty attempts made by him to determine the total sulphur in urine by this method, he invariably obtained results that were low and, at the same time, did not check. He therefore condemns the original method and proposes a modification which is simply the original method with a solution of sodium chloride added to the oxidizing reagent and the use of twenty-five cc of urine instead of ten cc. In reply to this criticism Benedict[23]- points

[23]Benedict: J. Bio. Chem. 8, 499-501, 1910.

out the fact that the condemnation was all oral and no data were published. He says the main criticism as given was the spattering caused at first by ignition. By the use of a watch crystal this loss by spattering can be reduced. The results obtained by the use of this method show that the reliability of it warrants its use.

The adaptibility of the methcd is further verified by Schmidt[24].

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[24]Schmidt: J. Bio. Chem. 8, 423-5, 1910.

He has run a great many sulphur determinations using the Benedict method and the results obtained are very favorable. He says Benedict's method gave results which not only checked with themselves but also checked with the peroxide method. Any slight loss due to spattering, which may sometimes take place in oxidizing with copper nitrate, can be prevented by covering the evaporating dish with a watch glass. The only case where the Benedict method is liable to give poor results is with urines containing albumin.

Sulphur in the Human Organism.

Before attempting to discuss the results of the experiment it may be of value to first see what the sulphur content means. Small quantities of sulphur, in the form of sulphates, enters the body in the food and drink, but the greater part concerned in metabolism enters the body in organic combination. This sulphur is found chiefly in protein. The metabolism of sulphur, then, is part of the protein metabolism, and in many respects runs parallel to that of nitrogen. It has been found that on a mixed diet there would be consumed not far from one gram of sulphur for each 100 grams of protein. From this we can say that in ordinary health the sulphur requirement is complete when the protein requirement is covered.

In the body when the protein is oxidized the sulphur becomes converted into sulphuric acid. This acid must at once be neutralized, foreven in small quantities it is very injurious to the cells. The greater part of this sulphate found in metabolism is found in the urine as inorganic sulphates. A small part, however, is found as the ethereal or organic sulphate. The ethereal sulphate consists

of a molecule of sulphuric acid in which one hydrogen is replaced by potassium and the other by an aromatic body. The amount of this form of sulphur present depends, according to some authorities, wholly upon the amount of putrefaction in the intestines and this, of course, depends on the character of the food. On the other hand, there is strong evidence in favor of the belief that indoxyl potassium sulphate is the only ethereal sulphate arising from intestinal putrefaction.

All the sulphur eliminated does not belong to the inorganic or ethereal form. A part of it is given off in a less completely oxidized form, this form being called the "neutral" sulphur. The true source of this is not definately known, but it is supposed to have its origin in the bile acids.

Discussion of the Results.

The daily ingestion of the sulphur was not determined thus making it impossible to obtain a sulphur balance. However, since the nitrogen balance is known, it will be possible to base our sulphur calculations on this.

Subject H was brought to nitrogen equilibrium in the preliminary period, through the ingestion of 16.046 grams of nitrogen daily[1]

[1]Unpublished results of Wills and Hawk.

On the first day of the water period the excretion of urinary nitrogen rose very slightly (0.124 grams), but in the remaining days of the period it fell below the equilibrium plane so that the average for the period was 12.945 grams per day. This value was 1.091 grams per day less than when the body was in equilibrium. In the final period the excretion of nitrogen continued low with an average

excretion of only 12.300 grams per day.

The sulphur excretion tallies very closely with the output of nitrogen. On the first day of the water period the sulphur output rose slightly (0.014 gram), but in the remaining days of the period it fell below the average for the preliminary so that the average for the period was 2.255 grams per day. This value is 0.082 gram per day less than when the body was in equilibrium in the preliminary period. In the final period the sulphur excretion remained low, being only 2.147 grams per day.

Subject W ingested 16.046 grams of nitrogen daily, and excreted in the urine and feces an average of 16.919 grams during the preliminary period. This shows a body loss of 0.873 gram of nitrogen per day. On the first and second days of the water period it is more striking for, upon the ingestion of the additional three litres of water per day, there followed a further loss of 1.332 grams of nitrogen. On the third day, however, the excretion was back, practically, to the level of the preliminary period, while on the last two days it fell below.

With the sulphur the results again check closely with those of the nitrogen. The sulphur output for the preliminary period runs parallel to the nitrogen excretion. On the first day of the water period the sulphur elimination decreased 0.042 gram below the average of the preliminary period. On the second day, however, the output rose to 2.427 grams, while on the third day the elimination was 2.719 grams. This makes the sulphur output run parallel to the nitrogen just one day late, a retardation probably due to the sulphur "lag" so common in metabolism. On the third day the highest excretion was reached with an output of 2.719 grams. For the two following days the elimination decreased, the excretion on the

fifth day being below the average for the preliminary period by 0.128 gram. In the final period the average daily excretion was below the average for the water period by 0.113 gram.

With both subjects there was a large increase in the excretion of sulphur and nitrogen during the first part of the water period, followed by a fall in the latter part of the period which continued also in the final period. This may be taken as an indication that the primary influence of the ingestion of the large volume of water was to stimulate protein catabolism, thus causing an increased output of these elements. This primary stimulation was then followed by a more efficient functioning of the various organs concerned, with the ultimate result that there was a far more satisfactory absorption of the protein constituents. This view is further verified by the fact that this lowered output of urinary sulphur and nitrogen is accompanied by a corresponding lower output of the fecal nitrogen[2] The output of the fecal nitrogen for the preliminary

[2]Unpublished resuts of Matill and Hawk.

period was 2.385 grams which was lowered to 1.422 grams under the influence of the water ingestion, and remained low (1.762 grams) in the final period.

The sulphur output for both subjects is found to reach its maximum on the third day, and then decreases for the remainder of the period. This may be interpreted as an immediate attempt on the part of the body to compensate for the catabolic processes in force during the water period by opposing anabolic processes which appear immediately upon cessation of the copious water ingestion. A marked cellular activity particularly of a catabolic character has been stimulated continuously during the water period of five

days, during which time more water, than the body has ever been accustomed to, has been ingested. The stimulation and the subsequent reaction have been severe, a large part of the waste material has been excreted and the cells free from this encumbrance, are in a better condition to do more effective work than before. The large water ingestion is now minimized and the strengthened cells begin at once a course of reconstuction, thus causing the anabolic or building up process which exceeds the catabolic or breaking down. This anabolism naturally takes up the excess sulphur and nitrogen making a decrease in the excretion of these elements. When the copious water has been decreased to the normal supply the excretion of both sulphur and nitrogen tend to rise to the normal equilibrium of the preliminary period.

Table [illegible].

Checks on New Method.

Urine cc.	Crucible Weighed(1).	Crucible Weighed(2).	$BaSO_4$	Sulphur	Average
10	15.[illegible]891	15.9366	.0475	.0066	
10	14.6642	14.7116	.0474	.0066	
10	15.6161	15.6653	.0492	.0063	
10	14.3160	14.3638	.0478	.0066	
	Average Benedicts Method -----				.0066
10	15.2690	15.3213	.0523	.0072	
10	14.3875	14.4396	.0521	.0072	
10	14.4295	14.4823	.0528	.0073	
10	15.5375	15.5895	.0520	.0072	
	Average New Method ----------				.[illegible]
10	15.2170	15.2724	.0554	.0077	
10	14.8455	14.9000	.0545	.0076	
10	15.3560	15.4120	.0560	.0077	
	Average Benedicts Method ----				.0077
10	14.9304	14.9855	.0551	.0076	
10	14.0640	14.1164	.0524	.0073	
10	15.5410	15.5950	.0540	.0075	
	Average New Method ----------				.0074

Table II.

Check on Accuracy of Manipulation.

Benedict's Method.

Urine cc.	Crucible Weighed(1).	Crucible Weighed(2).	$BaSO_4$	Sulphur
10	16.5883	16.6008	.0125	.0017
10	16.2199	16.2332	.0133	.0017
10	14.4747	14.4872	.0125	.0017
10	14.7264	14.7385	.0121	.0016
10	14.7639	14.7754	.0115	.0015
10	15.5478	15.5611	.0133	.0018
10	15.3967	15.4088	.0121	.0016
10	15.5850	15.5977	.0127	.0017
10	15.5661	15.5793	.0132	.0018
10	15.7820	15.7945	.0125	.0017
10	15.9057	15.9180	.0123	.0017
10	14.0900	14.1029	.0129	.0017

Subject W. Table III.[a]

Sulphur Excretion.

Lab. No.	Urine cc.	Gooch weighed before.	Gooch weighed after.	SO_3	Total vol. per day. cc.	SO_3 per day.	Average SO_3 per day.
			Preliminary Period.				
7112a	9.984	16.6056	16.6835	0.0266	888	2.366	
7112b	9.984	16.2325	16.3101	0.0266	888	2.366	
7112c	9.984	14.4867	14.5641	0.0264	888	2.350	2.366
7113a	9.984	14.7382	14.8106	0.0248	945	2.350	
7113b	9.984	14.7750	14.8477	0.0248	945	2.350	
7113c	9.984	15.5608	15.6342	0.0251	945	2.379	2.350
Average							2.360
			Water Period.				
7114a	9.984	15.4084	15.4302	0.0074	3110	2.304	
7114b	9.984	15.5974	15.6187	0.0072	3110	2.241	
7114c	9.984	15.5788	15.6007	0.0075	3110	2.340	2.295
7115a	9.984	15.7943	15.8099	0.0053	4570	2.427	
7115b	9.984	15.9175	15.9370	0.0065	4570	2.979	
7115c	9.984	14.1025	14.1179	0.0053	4570	2.427	2.427
7116a	9.984	14.6914	14.7088	0.0059	4230	2.702	
7116b	9.984	16.5696	16.5867	0.0058	4230	2.659	
7116c	9.984	15.7225	15.7405	0.0061	4230	2.797	2.719
7117a	9.984	16.0757	16.0950	0.0066	3810	2.520	
7117b	9.984	16.2368	16.2560	0.0065	3810	2.480	
7117c	9.984	15.6062	15.6253	0.0065	3810	2.480	2.480
7118a	9.984	15.8612	15.8813	0.0068	3300	2.249	
7118b	9.984	16.4980	16.5185	0.0070	3300	2.318	
7118c	9.984	15.6028	15.6223	0.0065	3300	2.160	2.242
Average							2.433
			Final Period.				
7119a	9.984	15.8085	15.8667	0.0199	1100	2.198	
7119b	9.984	15.9324	15.9934	0.0208	1100	2.294	
7119c	9.984	16.5566	16.6174	0.0208	1100	2.294	2.294
7120a	9.984	15.3803	15.4492	0.0235	930	2.194	
7120b	9.984	17.3244	17.3943	0.0239	930	2.230	
7120c	9.984	15.7082	15.7780	0.0239	930	2.230	2.230
7121a	9.984	16.7243	16.7955	0.0243	965	2.355	
7121b	9.984	15.7400	15.8105	0.0241	965	2.333	
7121c	9.984	16.2556	16.3263	0.0242	965	2.342	2.343
7122a	9.984	16.5181	16.5903	0.0247	965	2.392	
7122b	9.984	16.6165	16.6888	0.0247	965	2.392	
7122c	9.984	16.2107	16.2822	0.0243	965	2.352	2.392
7123a	9.984	16.4958	16.5698	0.0253	920	2.339	
7123b	9.984	16.5898	16.6650	0.0257	920	2.374	
7123c	9.984	14.8091	14.8833	0.0254	920	2.343	2.352
Average							2.322

Subject H. Table 111 b.

Sulphur Excretion.

Lab. No.	Urine cc.	Gooch weighed before.	Gooch weighed after.	SO_3	Total vol. per day. cc.	SO_3 per day.	Average SO_3 per day.
			Preliminary Period.				
8112a	9.984	15.4481	15.5096	0.0210	1130	2.379	
8112b	9.984	17.3937	17.4544	0.0207	1130	2.342	
8112c	9.984	15.7770	15.8385	0.0210	1130	2.379	2.379
8113a	9.984	16.7945	16.8370	0.0145	1580	2.297	
8113b	9.984	15.8096	15.8523	0.0146	1580	2.311	
8113c	9.984	16.3254	16.3677	0.0144	1580	2.281	2.296
Average							2.337
			Water Period.				
8114a	9.984	16.5900	16.6065	0.0056	4237	2.380	
8114b	9.984	16.6883	16.7050	0.0057	4237	2.421	
8114c	9.984	16.2817	16.2978	0.0055	4237	2.339	2.380
8115a	9.984	16.5695	16.5878	0.0048	4700	2.260	
8115b	9.984	16.6643	16.6789	0.0049	4700	2.303	
8115c	9.984	14.8826	14.8948	0.0042	4700	1.978	2.180
8116a	9.984	15.5096	15.5264	0.0057	3955	2.258	
8116b	9.984	17.4544	17.4718	0.0059	3955	2.338	
8116c	9.984	15.8385	15.8557	0.0058	3955	2.297	2.297
8117a	9.984	16.8370	16.8536	0.0056	4000	2.243	
8117b	9.984	15.8523	15.8688	0.0056	4000	2.243	
8117c	9.984	16.3677	16.3846	0.0057	4000	2.282	2.243
8118a	9.984	16.6065	16.6225	0.0054	4010	2.177	
8118b	9.984	16.7050	16.7208	0.0054	4010	2.177	
8118c	9.984	16.2978	16.3146	0.0057	4010	2.296	2.177
Average							2.255
			Final Period.				
8119a	9.984	15.5264	15.5827	0.0192	1085	2.085	
8119b	9.984	17.4718	17.5287	0.0194	1085	2.107	
8119c	9.984	15.8557	15.9119	0.0192	1085	2.085	2.085
8120a	9.984	16.8536	16.8956	0.0143	1480	2.120	
8120b	9.984	15.8688	15.9100	0.0141	1480	2.091	
8120c	9.984	16.3846	16.4261	0.0142	1480	2.105	2.105
8121a	9.984	16.6225	16.6938	0.0244	920	2.250	
8121b	9.984	16.7208	16.7921	0.0244	920	2.250	
8121c	9.984	16.3146	16.3859	0.0244	920	2.250	2.250
Average							2.147

Table IV a.

Subject W.

Comparison of Sulphur Output to Nitrogen.

Day	Urine vol.	Nitrogen[1]	Sulphur $AsSO_3$	Fecal Nitrogen[2]
		Preliminary Period.		
12	888	14.535	2.366	4.447
13	945	14.533	2.355	2.708
		Water Period.		
14	3110	15.378	2.295	1.387
15	4570	15.071	2.427	1.720
16	4230	14.518	2.719	1.433
17	3810	14.216	2.480	1.593
18	3300	13.547	2.242	2.411
		Final Period.		
19	1100	12.042	2.294	1.459
20	930	13.381	2.230	1.896
21	965	14.145	2.343	0.431
22	965	14.130	2.392	1.499
23	920	13.497	2.352	

[1] Nitrogen values taken from unpublished results of Wills and Hawk.

[2] Values taken from unpublished results of Matill and Hawk.

Table lV b.

Subject H.

Comparison of Sulphur Output to Nitrogen.

Day	Urine vol.	Nitrogen[1]	Sulphur $AsSO_3$	Fecal Nitrogen[2]
		Preliminary Period.		
12	1130	14.047	2.379	2.170
13	1580	14.024	2.296	4.289
		Water Period.		
14	4237	14.160	2.380	0.537
15	4700	13.684	2.180	1.249
16	3955	12.829	2.297	0.761
17	4000	12.392	2.243	3.602
18	4010	13.760	2.177	1.788
		Final Period.		
19	1085	11.434	2.085	1.892
20	1480	12.584	2.105	1.763
21	920	12.882	2.250	2.028

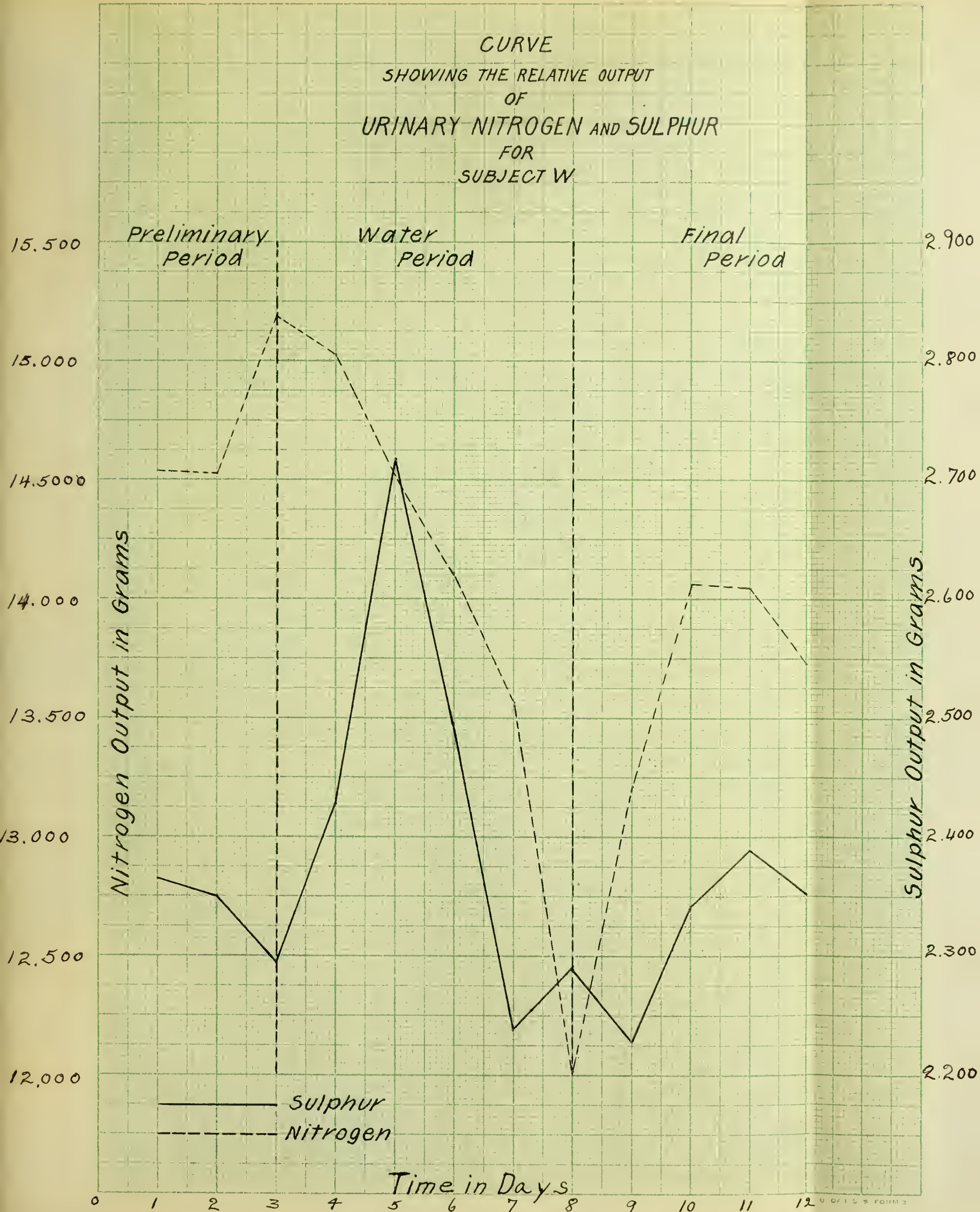
[1]Nitrogen values taken from unpublished results of Wills and Hawk.

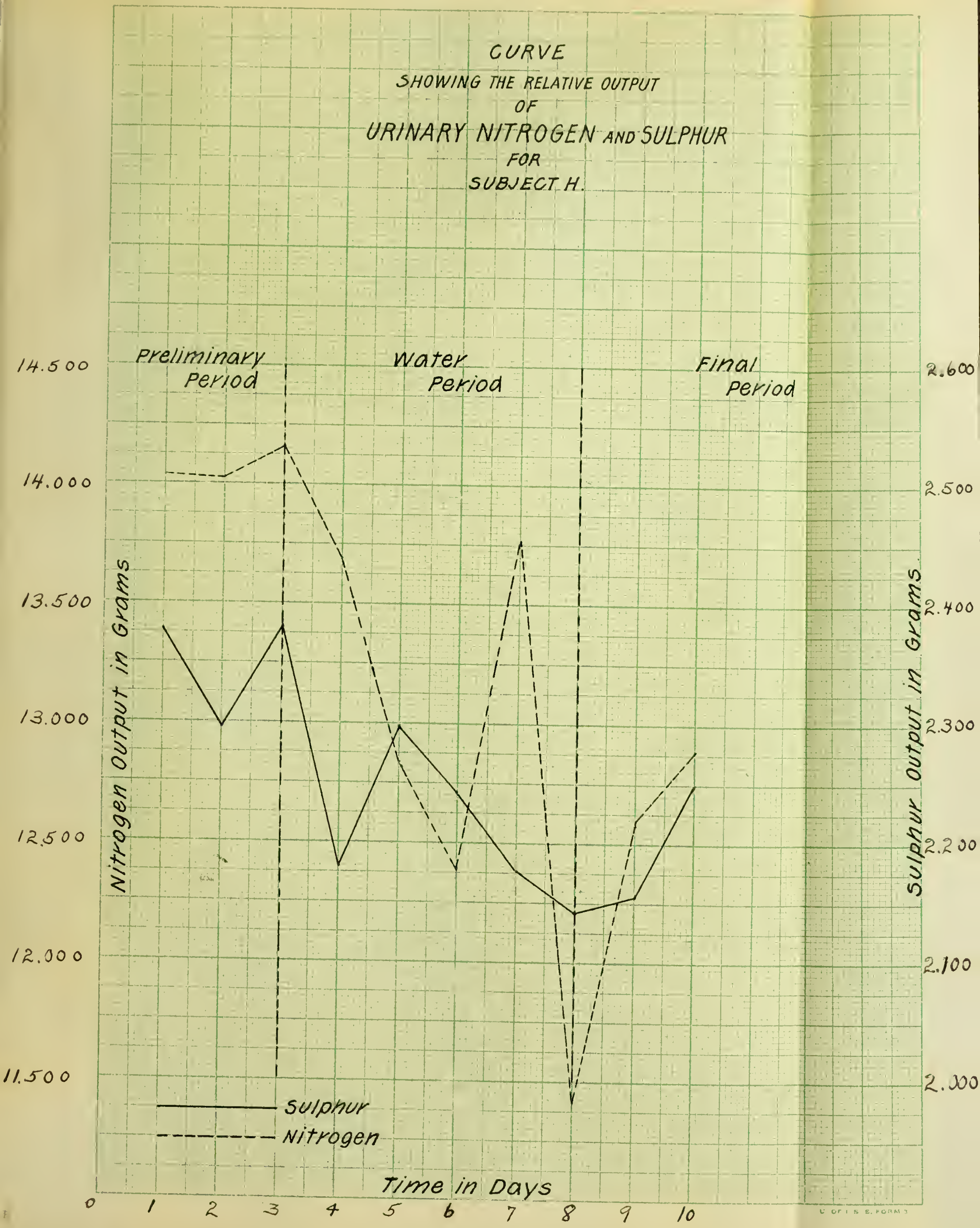
[2]Values taken from unpublished results of Matill and Hawk.

Table V.

Average Sulphur and Nitrogen Output for each Period.

Subject	Period	Urinary Nitrogen.	Urinary Sulphur.	Fecal Nitrogen.
W.	Prelim.	14.534	[illegible].73[illegible]	2.7[illegible]5
	Water.	14.546	2.4[illegible]	1.4[illegible]
	Final.	13.239	2.7[illegible]	1.7[illegible]
H.	Prelim.	14.035	2.[illegible]7	2.153
	Water.	13.365	2.253	1.5[illegible]7
	Final.	12.7[illegible]	[illegible].147	1.8[illegible]4


CURVE
SHOWING THE RELATIVE OUTPUT
OF
URINARY NITROGEN AND SULPHUR
FOR
SUBJECT W
Preliminary Period
Water Period
Final Period
15.500
15.000
14.5000
14.000
13.500
13.000
12.500
12.000
2.900
2.800
2.700
2.600
2.500
2.400
2.300
2.200
Nitrogen Output in Grams
Sulphur Output in Grams.
Sulphur
Nitrogen
Time in Days
0
1
2
3
4
5
6
7
8
9
10
11
12



CURVE
SHOWING THE RELATIVE OUTPUT
OF
URINARY NITROGEN AND SULPHUR
FOR
SUBJECT H.
Preliminary Period
Water Period
Final Period
14.500
14.000
13.500
13.000
12.500
12.000
11.500
2.600
2.500
2.400
2.300
2.200
2.100
2.000
Nitrogen Output in Grams
Sulphur Output in Grams
Sulphur
Nitrogen
Time in Days
0
1
2
3
4
5
6
7
8
9
10

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