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ON THE RELATION OF THE REDUCING POWER OF NORMAL URINE TO THE AMOUNT OF CERTAIN NITROGEN COMPOUNDS PRESENT.

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THE reducing power of normal urine is easily observed by the application of certain reagents and was at one time supposed to be largely or mainly due to the presence of dextrose or some similar body. In fact, based on the reducing power alone, estimates of the amount of carbohydrates in urine were frequently made and are still occasionally found in the text-books of urine analysis and physiological chemistry. Later, doubt was thrown on this conclusion and the presence of even traces of sugar in normal urine was disputed. Seegen[1] made many experiments in this direction and came to the conclusion that if any sugar at all is present it cannot be in amount above 0.006 per cent. The same result essentially was reached by several others, but in most cases the methods of examination employed were open to criticism as they did not provide for the actual separation and identification of a sugar, supposing it present.

Meanwhile E. Fischer[2] proposed the reaction for the separation of sugars by the phenylhydrazine test, and von Jaksch[3] and

[1] *Ztschr. physiol. Chem.*, 9, 332.

[2] *Ber. d. chem. Ges.*, 17, 579 and 20, 82.

[3] *Ztschr. anal. Chem.*, 24, 478.

others applied it to the identification of sugar in urine. Baumann[1] showed the ready production of benzoic esters of dextrose and this general method was soon applied in urine examination by Wedenski,[2] Roos,[3] Salkowski,[4] and others. The amount of the pentabenzoic ester found in this way was quite variable but always small. In Salkowski's test the range was from 1.22 grams to 3.36 grams in 1000 cc. of urine representing the whole day's excretion. In Wedenski's experiments the maximum amount was ten times the minimum found. Later Baisch made two important contributions on the subject of the nature and amount of the carbohydrates in the urine[5] and places the content of these bodies somewhat higher than Seegen, but still very low. His results give the average excretion of reducing carbohydrates as 0.100 gram to the liter. About the same time Allen published an interesting paper on the subject[6] and reached nearly the same result.

It is quite evident from the foregoing that while the existence of sugar in the urine may be looked upon as settled, the amount is very small and far from accounting for the total reduction. This has been reported by some authors as corresponding to 0.15 per cent. of dextrose while others place it as high as 0.4 per cent., in the mean.

NATURE OF THE REDUCING BODIES.

When speaking of reduction the behavior toward some metallic solution, generally toward copper oxide, is usually in mind. Among substances, other than sugar, which occur normally in urine the most important from the standpoint of this behavior are doubtless uric acid and creatinin. Glycuronic acid is often referred to as having an important action here, but the amount ordinarily present is too small to be practically considered in comparison with the others. The reducing power of uric acid has been long known and under certain definite conditions an equation may be written expressing the amount of oxygen absorbed in passing into several related bodies. The reducing

[1] *Ber. d. chem. Ges.*, 19, 3218.
[2] *Ztschr. physiol. Chem.*, 13, 122.
[3] *Ibid.*, 15, 513.
[4] *Ibid.*, 17, 229.
[5] *Ibid.*, 18, 193 and 19, 339.
[6] *Analyst*, 19, 178.

power of creatinin is fully as important as is that of uric acid, and besides it is more readily followed and measured.

It occurred to me, therefore, that some light could be thrown on the question of what part a sugar plays in the total reduction by determining as accurately as possible in a large number of normal urines the amount of uric acid and creatinin present, and calculating then the reducing power of these from their relations to the oxidizing solution previously determined. The difference between the total reduction and that due to these bodies would roughly measure the reducing power of the sugar present, provided no other substance has been overlooked which exhibits the same action. The possibility of the existence of such a substance or substances in the urine must, of course, be conceded, especially since the ratio of carbon to nitrogen present, as found by direct analysis, is much higher than that calculated from the sum of the determinable constituents present. The new substance recently separated from urine and called oxyproteic acid by Bondzynski and Gottlieb[1] is supposed by Pregl[2] to account for this ratio, and to be, after urea, the most important body in the urine. The analyses made by Pregl show it to contain about 30 per cent. of carbon and 8 per cent. of nitrogen, and to amount to 6 or 8 grams daily. He states, however, that the product, as separated by his methods of precipitation and purification, is quite devoid of any reducing action on alkaline copper solutions. Toepfer has also recognized this peculiar urinary acid[3] but claims that the amount found by Bondzynski and Gottlieb is too high, because the barium salt separated is always impure from presence of much coprecipitated matter. It is certainly remarkable that an acid as abundant as this is supposed to be, should have so long escaped detection, and this fact suggests caution in generalizing too much from the results of urine analyses, however carefully they may be made; but up to the present time no substances have been found in the urine in sufficient quantity to account for the reducing power save those mentioned, and it is from this, as a working basis, that the following investigation was undertaken.

While many substances have been employed as reagents in

[1] *Centralblatt für Physiologie*, 11, 648.
[2] Pflüger's *Arch.*, 75, 87, 1899.
[3] *Centralblatt für Physiologie*, 11, 850.

this reduction test some form of copper solution has usually been found practically the most useful. A weak solution of methylene blue is reduced readily by sugar and creatinin in alkaline solution, and very slowly by uric acid, but as the reduced product is oxidized with extreme readiness in contact with air, with return of the blue color, the reagent is not suitable for quantitative measurements. A weak solution of safranine is also reduced by sugar in alkaline medium and apparently not at all by uric acid or creatinin, but the amount of sugar necessary to produce an appreciable effect is greater than that usually found in normal urine, so that this reagent is not practically available. Measurement of reduction by aid of bismuth or mercury salts is also possible, but, as is well known, the methods are lacking in delicacy.

Among the various copper solutions used in sugar analysis there are several which may be employed with very weak solutions corresponding to normal urine. The best of these appears to be some form of the ammonia-copper solution recommended by Dr. Pavy. This was first made by diluting 120 cc. of Fehling's solution with 300 cc. of strong ammonia and water enough to make 1 liter. Pavy assumed that each cubic centimeter of this solution oxidizes 0.5 mg. of dextrose, and has, therefore, one-tenth the strength of the ordinary Fehling solution, which is approximately true. Several modifications of the solution have been proposed with variations in the amounts of copper sulphate, ammonia, and fixed alkali. Finally, the Loewe solution containing glycerol has been made the basis of the dilution, instead of the Fehling solution containing a tartrate; the Purdy solution is made in this way.

As the oxidizing power of the solution changes with these variations, it is important to recognize the extent of this alteration and allow for it if necessary. The composition of several of the best known of the modifications is here given, the original Pavy solution being added for comparison. The volume is one liter in each case.

	Copper sulphate. Grams.	Sodium hydroxide. Grams.	Rochelle salt. Grams.	Glycerol. cc.	Ammonia. 0.90. cc.
Pavy	4.158	(7.20)	20.76	..	370
Hehner	4.502	15.6—19.5	22.49	..	370
Purdy	4.752	23.5 KOH (= 16.8 NaOH)	....	38	350
Peska	6.927	10.00	34.50	..	135

Variations in the oxidizing power of each solution follow mainly from alterations in the amount of fixed alkali or ammonia used, and this is shown in some tests to be now given. I made solutions as follows:

A.

Copper sulphate, cryst	5 grams.
Caustic soda (100 per cent.)	18 "
Glycerol	35 cc.
Water, to make	250 "

B.

Ammonia water	0.90 sp. gr.

These solutions were mixed in the proportions given below and used in the titration of a 0.2 per cent. dextrose solution in the usual manner. The results are shown in the following table:

TABLE I.—REDUCTION WITH AMMONIA VARIABLE.

Vol. of Sol. A. cc.	Vol. of Sol. B. cc.	Vol. of water. cc.	Sugar solution required. cc.	Mg. of $CuSO_4.5H_2O$ to 1 mg of $C_6H_{12}O_6$.	Mols. of CuO to 1 mol. $C_6H_{12}O_6$.
25	75	00	28.5	8.772	6.32
25	60	15	29.5	8.474	6.11
25	50	25	30.3	8.251	5.95
25	40	35	30.8	8.117	5.85
25	30	45	31.4	7.962	5.74

The oxidizing power varies greatly with the amount of ammonia present and is decreased with increase in the latter. The change amounts to about 10 per cent. of the whole in the limits given.

The effect of adding an excess of sodium or potassium hydroxide is even more marked as was found by experiments given below. The following solutions were made:

C.

Copper sulphate, cryst	5 grams.
Glycerol	35 cc.
Water, to make	250 "

D.

Strong ammonia water	0.90 sp. gr.

E.

Sodium hydroxide, 100 per cent	50 grams.
Water, to make	250 cc.

These solutions were mixed in different proportions and were used to test a dextrose solution of about 0.2 per cent. strength. The results obtained were as follows :

TABLE II.—REDUCTION WITH FIXED ALKALI VARIABLE.

Vol. of Sol. C. cc.	Vol. of Sol. D. cc.	Vol. of Sol. E. cc.	Vol. of water. cc.	NaOH in grams per liter. Grams.	Sugar solution required. cc.	Mols. of CuO to 1 mol. $C_6H_{12}O_6$.
25	35	25	15	50	34.8	5.18
25	35	20	20	40	33.3	5.41
25	35	15	25	30	32.0	5.63
25	35	10	30	20	30.4	5.93
25	35	5	35	10	28.7	6.28
25	35	2.5	37.5	5	28.0	6.44

We have here an extreme variation of about 25 per cent. in the oxidizing value of the copper solution. As the fixed alkali itself possesses marked oxidizing power, as shown, in fact, in our ordinary Moore's test for sugar in urine, the increase in alkali must add to the oxidizing power of the finished solution. Hehner has shown that the solution can be made and will oxidize perfectly without the addition of any fixed alkali, but the action is then very slow and the oxidation ratio still lower.[1]

These experiments indicate that to secure anything like uniformity in the results obtained by the Pavy solution or its modifications, care must be taken to employ definite and constant amounts of fixed alkali and ammonia. The statement of Allen ("Chemistry of Urine," p. 67, 1895) that considerable variation in the amount of caustic alkali and ammonia may be made in the Pavy solution without altering appreciably its oxidizing value, does not appear to be correct. Of the two cheap fixed alkali hydroxides, caustic soda is preferable to caustic potash and is much more commonly used.

Variations in the amount of glycerol or tartrate are of less importance, but still have an appreciable influence, as I have found in several trials. As the great excess of ammonia present is sufficient to hold the copper hydroxide in solution, it is not necessary to use a large amount of either.

In the Pavy and Purdy liquids the oxidizing power is apparently assumed to be independent of the strength of the sugar solution added. Pavy and Purdy assume the factor 8.316 grams of

[1] *Analyst*, 6, 219.

$CuSO_4.5H_2O$ to 1 gram of $C_6H_{12}O_6$. But Peska has shown that the factor varies over 2 per cent. with solutions ranging from 0.1 to 1 per cent. in strength.[1]

As in practical work it is desirable to employ a solution, 1 cc. of which oxidizes some simple unit amount of sugar. I have used one in the work below with a value of 1 cc. for each milligram of sugar oxidized in 0.2 per cent. solutions. It is made with the following amounts per liter:

Copper sulphate, cryst	8.166 grams.
Sodium hydroxide (100 per cent.)	15.000 "
Glycerol	25.000 cc.
Ammonia water (sp. gr. 0.9)	350.000 "
Water, to make	1,000.000 "

The value of this solution in copper oxide, CuO, is 2.6042 grams per liter, and 1 molecule of sugar = 5.88 molecules of CuO, as it is employed.

Of the solution, I use 50 cc. and dilute with water to 100 cc. To prevent too rapid an escape of ammonia and avoid reoxidation to some extent, I add to the mixture, while warming, enough pure white solid paraffin to make a layer of 3 or 4 mm. in thickness when melted. The burette tip for discharging the sugar solution or urine is made long enough to pass down the neck of the flask and below this paraffin. By boiling gently and adding the weak saccharine liquid slowly, very close and constant results may be obtained. At the end of the titration the paraffin is solidified by inclining the flask and immersing it in cold water, or by flowing cold water over it. The reduced liquid is then poured out and the cake of paraffin is thoroughly washed for the next test. A flask so prepared may be used for a hundred titrations. The solid paraffin is much preferable to the oil recommended by Allen and Peska. To prevent bumping and facilitate easy and uniform boiling, I add a few very small fragments of pumice-stone.

A solution made as above is not too strong in copper for accurate work, but the volume of ammonia necessary to hold a much larger amount of the reduced oxide in solution would render the process very inconvenient. The sugar employed in fixing the value of the above standard solutions was a very fine sample of

[1] *Zischr. anal. Chem.*, 35, 94.

pure crystal dextrose made for me by Dr. Gudemann, of the Chicago Sugar Refining Co. It was further purified by crystallizing from hot alcohol. After careful drying at 80° C., it was examined by a very accurate polarimeter and found to have a degree of purity not less than 99.9 per cent.

An ammoniacal solution made in this way and used with the layer of paraffin is preferable to the usual Fehling liquid in the titration of weak dextrose solutions. It cannot be conveniently used for strong solutions, however, because of the large volume of standard required to oxidize a small volume of the saccharine liquid.

The behavior of this solution with the weak sugar solutions being established, it remains to show how it acts with creatinin and uric acid.

CREATININ AND COPPER SOLUTIONS.

The importance of creatinin as a reducing body is commonly overlooked although referred to in Neubauer and Vogel's "Urine Analysis," and in other large works. This is partly due to the fact that the amount present is generally underestimated, as shown by the figures given in several of our best known handbooks of urine analysis.[1] The reducing effect of creatinin on alkaline copper solutions has been observed by Worm Müller,[2] Johnson,[3] and others. Müller places the reducing power low, 1 molecule of creatinin to not over 0.75 molecule of copper oxide.

Johnson states that 4 molecules of ordinary creatinin have the same reducing action on copper salts as 2 molecules of grape-sugar, and that this reduction plus that due to the uric acid will account for the whole of the reducing action found in normal urine. The presence of sugar is disputed. The results of Müller and Johnson, are widely divergent, which is doubtless due to essential differences in the methods of observation. The reducing power of creatinin is shown only after long warming in the ordinary Fehling titration, and at the outset the cuprous oxide formed is held in solution, which is a disturbing element in making the test. But with the weak ammoniacal solution

[1] See on this point, Allen. *loc. cit.*

[2] *Ztschr. anal. Chem.*, **21**, 610.

[3] *Chem. News*, **55**, 304.

used for the sugar the behavior is quite different. The reduction appears to proceed as regularly and normally as with sugar itself. I have made a number of tests with solutions of different strengths, but all weak, and find that about 92 mg. of creatinin are required for 50 cc. of the standard copper solution containing 2.604 grams of CuO to the liter. This corresponds almost exactly to the proportion, 1 molecule $C_4H_7N_3O$: 2 molecules CuO, which is about one-third the reducing power of dextrose under the same conditions. The result is much larger than that of Worm Müller, but lower than the figures given by Johnson. The actual reducing values are given in the table below. The creatinin used was prepared in the laboratory and was practically pure.

TABLE III.—REDUCING POWER OF CREATININ.

Creatinin in 100 cc. Mg.	Copper solution taken and diluted to 100 cc. cc.	CuO equivalent. Mg.	Creatinin solution used. cc.	Creatinin to 130.2 mg CuO. Mg.	Mols. CuO to 1 mol. $C_4H_7N_3O$.
50	25	65.1	92.5	92.5	1.998
50	25	65.1	94.0	94.0	1.967
120	50	130.2	76.0	91.2	2.026
120	50	130.2	77.0	92.4	2.000
				Mean,	1.998

The oxidation of creatinin by alkaline copper solutions is usually explained on the assumption that oxalic acid and methyl guanidin are formed, the creatinin being first converted into creatin by the alkaline solution,

$$HN{=}C{=}NH_2.NCH_3.CH_2CO_2H + O_2 = C_2H_2O_4 + HN{=}C{=}NH_2.NHCH_3.$$

In the above experiments, however, the amount of oxygen absorbed corresponds with only half that necessary for this equation, and under the condition none can come from the air. The reaction must therefore take place in a different manner, which appears all the more probable in view of recent work by E. Wörner,[1] and Toppelius and Pommerehne,[2] who found that the reduction is variable with time of boiling with strong Fehling solution. With weak solutions the reduction is slow, but on boiling 5 cc. of a weak creatinin solution an hour with 60 cc. of strong Feh-

[1] *Ztschr. physiol. Chem.*, **27**, 1.
[2] *Archiv. der Pharmacie*, **234**, 380.

ling solution a much greater effect is observed. Under such conditions 1 molecule of creatinin appears to reduce over 4 molecules of CuO. It is quite possible that the oxidation of creatinin is preceded in weak solution by hydrolysis with formation of ammonia and methyl hydantoin and that this body is afterwards oxidized in several stages. The reduction of 4 molecules of CuO would correspond to the oxidation of the acetic acid group in creatin to oxalic acid, as required by the equation written above, but in the ordinary application of the copper tests in urine analysis no such degree of oxidation is likely.

URIC ACID AND COPPER SOLUTIONS.

The behavior of uric acid with alkaline copper solutions has been described by several chemists and notably by Riegler[1] who studied the reaction with Fehling solution. He found, as a mean result of a number of experiments, that 1 gram of uric acid yields cuprous oxide corresponding to 0.800 gram of copper. On the assumption that 1 molecule of uric acid reduces 2 molecules of copper oxide the reduced copper obtained should amount to 0.7556 gram ; the reduction therefore goes a little further than this theoretical relation.

The reducing action seems to be much more readily followed in the ammoniacal solution, however, and several tests were made to establish the relation under such conditions. The following are the details in tabular form :

TABLE IV.—REDUCING POWER OF URIC ACID.

Uric acid in 100 cc. Mg.	Copper oxide taken.	Uric acid solution used. cc.	Uric acid to 130.2 mg CuO. Mg.	Mols. CuO to 1 mol. $C_5H_4N_4O_3$.
80	39.8	36	94.2	2.92
80	65.1	58	92.8	2.96
120	130.2	76.5	91.8	2.99
120	65.1	37.8	90.8	3.03
			Mean,	2.98

The mean result of 1 molecule of uric acid to 2.98 molecules of copper oxide is essentially in the relation of 1 : 3, or 1 molecule of the acid to 1.5 atoms of oxygen absorbed. This is a larger amount of oxygen than corresponds to the reactions usually given for the oxidation of uric acid which is generally

[1] *Ztschr. anal. Chem.*, 35, 31.

represented as taking place primarily in one of two directions, leading either to allantoin or to alloxan and urea. These reactions require each one atom of oxygen as follows :

$$C_5H_4N_4O_3 + O + H_2O = CO_2 + C_4H_6N_4O_3$$
$$C_5H_4N_4O_3 + O + H_2O = C_4H_2N_2O_4 + CON_2H_4.$$

Secondary reactions, however, doubtless take place resulting in the further oxidation of one or the other of these products and using more oxygen. Thus, from alloxan parabanic acid and other substances may be derived, and possibly are derived in the oxidation in question. A reaction leading to parabanic acid from the partial oxidation of the alloxan in the above case may be represented in this way :

$$2C_5H_4N_4O_3 + 3O + 2H_2O =$$
$$2CON_2H_4 + C_4H_2N_2O_4 + C_3H_2N_2O_3 + CO_2.$$

Whether this reaction represents the course of the oxidation or not it remains true that the uric acid takes up more oxygen than is required by the simpler reactions given above. This is true also of a reaction in sulphuric acid solution referred to below.

Having described the three important substances in urine which exert a reducing action on the ammoniacal copper solution, it remains to explain how the last two mentioned, along with urea and ammonia, the other important products of nitrogenous excretion, were practically determined in the investigation in hand. The amounts of these last substances are of interest in connection with the actual reducing compounds.

DETERMINATION OF CREATININ.

This body is most accurately obtained by precipitation from the prepared urine in the form of zinc chloride double salt. This is the method of Neubauer modified by Salkowski, but it is desirable to use as large a volume of urine as possible. In the work below I used, when available, 480 cc. which was treated with barium hydroxide to faint alkaline reaction and precipitated with barium nitrate in the cold. In a few cases milk of lime and calcium chloride were used in this preliminary treatment. The volume was then made up to 600 cc., filtered after half an hour, and of the filtrate 500 cc., representing 400 cc. of the original urine, was taken for the further work. Care was observed to

secure and maintain a nearly neutral reaction in this liquid so as to avoid, in the following evaporation, the conversion of creatinin into creatin. The final precipitate of the zinc chloride salt was collected on a Gooch crucible, dried at 100°, and weighed. From this weight that of the pure creatinin was calculated and this is given in the table below.

DETERMINATION OF URIC ACID.

Of all the methods now available, the Fokker-Hopkins process seems to give the most regular and trustworthy results. 100 cc. of urine are precipitated with about 30 grams of pure ammonium chloride, or enough to produce a saturated solution. The beaker containing the precipitate is allowed to stand in a cool place over night. Then the precipitate is collected on a filter and washed thoroughly with a saturated solution of ammonium sulphate to remove all chlorine. The filter is perforated and by aid of a jet of hot water the precipitate is washed into a flask. About 100 cc. of the water should be used. To this turbid liquid 20 cc. of pure strong sulphuric acid is added, and then, without delay, twentieth-normal permanganate solution from a burette until a faint pink tinge is secured which is permanent several seconds. For this stage of the reaction Hopkins gives 1 cc. of the twentieth-normal permanganate as corresponding to 3.75 mg. of uric acid. By waiting and adding more permanganate from time to time, as the color fades, a considerably larger volume may be used, for which no factor has been worked out. For the direct oxidation, using 1 atom of oxygen to 1 molecule of uric acid, each cubic centimeter of the twentieth-normal permanganate solution should correspond to 4.2 mg. of the acid instead of 3.75 mg. The results obtained by taking a later reading correspond more nearly to an oxidation with 1.5 atoms of oxygen to each molecule of acid, but they are not definite enough for calculation. The Hopkins factor has therefore been used, and, it is believed, with a considerable degree of accuracy, since numerous blank experiments were carried out with pure uric acid to fix the point for observation of the end color.

DETERMINATION OF UREA.

This has been carried out by the two common methods, the Liebig process by titration with mercuric nitrate, giving properly

a rough measure of the total nitrogen rather than the urea itself, and by the Knop-Hüfner hypobromite or hypochlorite process. The results of the first method are always too high if calculated as urea, while those of the latter are too low. As the last process was carried out it was assumed that 95 per cent. of the urea present was decomposed normally according to the equation

$$CON_2H_4 + 3O = CO_2 + N_2 + 2H_2O,$$

the calculations being made accordingly from the experimental results. The observations were made in a Lunge nitrometer, the gas volumes obtained being always reduced to standard conditions. Even with the various corrections the results were considerably lower than those by the Liebig process because of the inherent positive errors in the latter. Something will be said about this below when the experimental results are discussed.

DETERMINATION OF AMMONIA.

Of all the processes recommended for the determination of the small amounts of ammonia found in urine the most reliable is the old Schloesing method of liberation by milk of lime and absorption by standard acid, under a bell-jar. The urine, about 50 cc., is measured into a glass crystallizing dish and mixed with an excess of milk of lime. Over this dish a glass evaporating dish containing a small known volume of standard sulphuric acid is supported on a triangle. The whole is covered without delay with a clean glass bell-jar, resting on a glass plate and is allowed to stand three days for the liberation of the ammonia, and its absorption by the titrated acid. At the end of the time the bell-jar is rinsed out with a little water which is added then to the dish with the standard acid. This is finally titrated and the loss in acidity measures the ammonia absorbed; while the process is very slow little or no risk is incurred of decomposing urea or other substance containing nitrogen and thus liberating an excess of ammonia. It is, of course, necessary to operate on fresh urine, as the object is to measure that normally present, and not that which may be made by the alkaline fermentation of urea. The results are always a little low, because a small portion of the ammonia escapes either liberation or absorption. To determine the probable error here a number of experiments were made with weak ammonium chloride solutions, corresponding in ammoniacal

strength to normal urine. These experiments gave very concordant results from which it appeared that after three days' time, at the temperature of the tests, 90 per cent. of the ammonia present was liberated and absorbed by the acid. In all the experiments below the results obtained were corrected by the aid of this factor.

Table V, following, gives the result of examinations of a number of normal urines, embracing determinations of the above-described constituents. These urines were collected so as to secure the whole day's excretion and were kept cold until the full twenty-four hour sample was obtained. The analysis was then begun immediately, and finished as speedily as possible. Fifty cc. of the standard copper solution were always employed in the reduction test, and the following table gives the volume of urine required to reduce this under the conditions described above. The reaction of the mixed day's urine was always acid and all samples, except No. 7, were of the usual normal yellow color. This sample was dark, probably because of its concentrated condition. It will be noted that the average total volume is smaller than is usually assumed as the mean daily excretion.

TABLE V.—RESULTS OF URINE ANALYSES.

No. of sample.	Excretion in twenty-four hours.	Specific gravity at 20°.	Urine required to reduce 50 cc. of copper solution.	Ammonia from 1000 cc. of urine.	Uric acid from 1000 cc. of urine.	Creatinin from 1000 cc. of urine.	Urea from 1000 cc. of urine by the Liebig process.	Urea from 1000 cc. of urine by the Knop-Hüfner process.
			cc.	Mg.	Mg.	Mg.	Grams.	Grams.
1	1230	1.031	21.0	645.0	840.0	1930	30.50	....
2	1450	1.035	21.2	522.0	743.0	1876	25.60	....
3	845	1.027	23.6	654.8	832.5	1268	38.80	....
4	1920	1.019	22.5	427.0	646.0	851	25.75	....
5	1950	1.023	20.2	465.0	678.0	1681	24.74	23.72
6	1200	1.025	20.5	593.1	619.0	1195	29.69	27.66
7	765	1.024	18.9	526.6	637.5	961	31.10	27.46
8	1775	1.020	58.0	227.7	....	653	12.90	....
9	1020	1.030	14.9	858.8	971.2	1750	39.18	37.27
10	1280	1.018	27.3	408.0	423.7	1016	17.57	14.83
11	1090	1.024	23.3	631.0	510.0	1374	25.04	24.54
12	1190	1.025	25.6	431.7	611.0	1381	32.72	29.72
13	915	1.025	21.1	711.7	630.0	1604	28.17	25.56

No. of sample.	Excretion in twenty-four hours.	Specific gravity at 20°.	Urine required to reduce 50 cc. of copper solution.	Ammonia from 1000 cc. of urine.	Uric acid from 1000 cc. of urine.	Creatinin from 1000 cc. of urine.	Urea from 1000 cc. of urine by the Liebig process.	Urea from 1000 cc. of urine by the Knop-Hüfner process.
			cc.	Mg.	Mg.	Mg.	Grams.	Grams.
14	1440	1.015	27.8	522.0	491.0	868	17.67	15.85
15	1220	1.029	21.2	716.0	671.0	757	26.46	20.94
16	1200	1.025	19.7	711.6	712.5	1735	32.11	31.23
17	800	1.030	17.9	669.0	693.8	1681	34.23	30.29
18	900	1.022	23.7	488.7	581.3	1239	21.91	17.83
19	1100	1.027	18.1	507.7	581.2	1898	26.32	21.73
20	835	1.032	17.1	1001.0	1020.0	1417	39.10	34.68
21	900	1.023	17.4	569.4	626.0	2224	26.63	23.66
22	970	1.026	23.4	374.8	521.0	976	18.61	16.15
23	1600	1.018	28.0	601.6	416.2	926	19.30	17.60
24	1120	1.027	20.7	811.3	701.2	1477	24.55	22.47
Means,	1167	1.025	23.0	602.0[1]	658.7	1392[1]	27.68[1]	24.37

The analytical results as obtained are given in Table V. These, as might be expected, are quite variable, and at first glance do not reveal any relationship that appears characteristic. For better comparison I have stated the amounts of urea, uric acid, ammonia, and creatinin found in grams or milligrams per 1000 cc. rather than for the excretion of twenty-four hours. In Table VI, I have calculated the total reducing power in terms of CuO for 1000 cc. of each urine, and have then done the same for the uric acid and creatinin, basing the calculation on the assumption that under the conditions of the experiments each molecule of uric acid reduces 3 molecules of copper oxide and each molecule of creatinin reduces 2 molecules of the oxide. The sum of these two reductions is given in a separate column, and finally the ratio of this to the total reduction is given in the last column.

[1] No. 8 not included.

TABLE VI.—RELATION OF REDUCING POWERS.

No. of sample.	Total reducing power of 1000 cc. of urine in grams of CuO.	Reducing power of the uric acid in 1000 cc. of urine in grams of CuO.	Reducing power of the creatinin in 1000 cc. of urine in grams of CuO.	Sum of the uric acid and creatinin reductions.	Relation of the uric acid and creatinin reductions to total reduction.
1	6.200	1.194	2.719	3.913	0.631
2	6.141	1.056	2.643	3.699	0.602
3	5.517	1.184	1.786	2.970	0.538
4	5.787	0.911	1.199	2.110	0.365
5	6.446	0.964	2.368	3.332	0.517
6	6.351	0.880	1.684	2.564	0.404
7	6.889	0.906	1.354	2.260	0.328
8	2.245	....	0.920	....	....
9	8.738	1.381	2.370	3.751	0.429
10	4.769	0.602	1.432	2.034	0.427
11	5.588	0.725	1.936	2.661	0.476
12	5.086	0.869	1.946	2.815	0.554
13	6.171	0.896	2.260	3.156	0.511
14	4.684	0.698	1.223	1.921	0.410
15	6.141	0.954	1.066	2.020	0.329
16	6.609	1.013	2.444	3.457	0.523
17	7.291	0.986	2.369	3.355	0.460
18	5.494	0.826	1.746	2.572	0.468
19	7.192	0.825	2.674	3.499	0.487
20	7.614	1.417	1.997	3.414	0.448
21	7.483	0.890	3.133	4.023	0.538
22	5.564	0.741	1.375	2.116	0.380
23	4.650	0.592	1.305	1.897	0.408
24	6.290	0.997	2.081	3.078	0.489
Means,	6.204	0.935[1]	1.961[1]	2.896	0.466

From the mean values given at the foot of the last table several interesting relations may be established. As in the ammoniacal solution 1 molecule of dextrose reduces 5.88 molecules of copper oxide, the above average reduction corresponds to 2.836 grams of sugar per liter, or about 0.28 per cent. The uric acid amounts to 0.658 gram per liter, or 0.065 per cent. The creatinin is equivalent to 1.392 grams per liter or 0.136 per cent. The data of the last column show that a large portion of the total reduction is due to the action of these latter products.

[1] No. 8 not in averages.

This, in the mean, amounts to 46.6 per cent., which calculated as sugar is equivalent to 1.322 grams per liter, leaving 1.514 grams as the amount of sugar possibly present. This, however, represents a maximum value, as there are unquestionably traces of other bodies present which, like the uric acid and creatinin, exert a reducing action. If the effect of these could be estimated it is likely that the reducing power, in the mean, would be found to be pretty evenly distributed between the saccharine and non-saccharine products.

It is not possible to draw any very exact general conclusions from the figures of the above table connecting the numerical values with the character of the food of the individual or with any other factor. Most of the urines were obtained from young men, students or teachers, and all pretty well nourished. It may be noticed, however, that the urines showing the highest reduction ratio for uric acid, and creatinin, as compared with the total reduction, were from men with the strongest physique with a diet containing much meat. On the other hand the lowest uric acid, and creatinin reductions correspond to cases of slighter physique and lower nutrition. Urines Nos. 1 and 2 were from a man consuming a diet largely of meat. No. 15 was from the same individual some weeks later after a change of diet to bread and vegetables largely. But from most of the urines no characteristic relation is apparent; another series of investigations is in progress in which the question of food in relation to the reducing power is being more closely determined.

RATIO OF UREA TO URIC ACID.

This ratio is more important and more characteristic than is the absolute amount of the acid. In the older works on urine analysis or physiological chemistry it was always stated too high, and usually as 50 : 1 or 60 : 1. This is due to the fact that the amount of uric acid was generally underestimated, while that of urea was naturally overestimated if found by the Liebig process, and not corrected.

In making an examination of a fraction of the day's excretion, instead of on the mixed twenty-four hour sample, a false ratio is also usually found because the urea and uric acid are not eliminated at a constant rate following ingestion of food. The ratio

from the above table is 36.9 : 1 if we consider the urea as measured by the Knop-Hüfner process and 42 : 1 if we take the uncorrected values for urea as found by the Liebig process. The results here are in the mean about 10 per cent. high as may be shown when we take into consideration the effect of the other nitrogenous bodies, especially the ammonia, uric acid, and creatinin, on the mercuric nitrate solution. With such a correction subtracted the amount of urea approaches closely that found by the gas volume method and the ratio becomes again about 37:1; this result is in accord with the average normal ratio as found in experiments on the excretion of the twenty-four hours as made by Hopkins and others. See an interesting paper by Hopkins and Pope[1] in which variations in the excretion of urea and uric acid in relation to kind of food and time of meals is discussed.

THE AMOUNT OF AMMONIA.

The ammonia in 1000 cc. of urine, as shown by the above figures, is, in the mean, 602 mg., but the individual variations are between 228 and 1001 mg. These correspond roughly to the variations in the other nitrogen bodies. The average daily excretion is seen to be 703 mg. The daily extremes are 364 and 908 mg. The average correction to be applied to the Liebig urea titration on account of the ammonia present is about 1.2 cc. of the usual standard mercuric nitrate solution. This result was found in experiments carried out on the titration of urea solutions containing small amounts of added ammonia, and is somewhat lower than the factor given by Feder.[2] Experiments are now in progress to determine more definitely the behavior of uric acid and creatinin in this titration, the statement in Neubauer and Vogel's Harnanalyse[3] requiring, apparently, a slight correction.

THE DAILY AVERAGES.

The numbers above given are expressed, for convenience in comparison, in grams or milligrams per liter. The values for the daily excretion are often interesting and these will now be given as calculated from the volumes collected in the twenty-

[1] *Journal of Physiology*, 23, 271.
[2] Neubauer and Vogel's Harnanalyse, 9th German edition, p. 519.
[3] *Loc. cit.*

four hours as shown by the figures of the second column in Table V.

TABLE VII.—MEAN RESULTS FOR DAILY EXCRETION.

Volume excreted	1167.00	cc.
Total reduction, equivalent to	3.31	grams $C_6H_{12}O_6$.
Uric acid and creatinin reduction equivalent to	1.54	" "
Remaining reduction equivalent to	1.77	" "
Amount of ammonia	0.703	gram.
Amount of urea (mean of gas method and Liebig, corr.)	29.75	grams.
Amount of creatinin	1.624	"
Amount of uric acid	0.799	gram.

The urines examined in the above experiments were all normal and represent the excretion from average meat and vegetable diet. In a following paper I will give results obtained from urines of consistent vegetarians, noting the same relations. Most of the experimental work detailed above was done by Mr. Frank Wright, assistant in the laboratory of physiological chemistry, and Mr. Charles Ericson, to whom my thanks are due.

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AN EXAMINATION OF BROWN AND TAYLOR'S OFFICIAL METHOD OF IDENTIFYING BUTTER.

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THE recent great increase in the production of renovated butter, due probably to the improvements in the method of its manufacture and the prevailing high price of creamery butter, has necessitated, in several states, the enactment of laws regulating its sale. Since the enactment of these laws there has been some discussion as to reliable methods for its identification. The fact that the chemical properties and the proximate composition of samples examined are entirely within the range of normal butter, has directed attention to its physical properties more particularly its behavior with polarized light. As is already well-known, in the manufacture of renovated butter, the butter-fat is melted and then cooled rather rapidly in a stream of cold water. This melting and rapid cooling induces a semicrystallization of