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AN OUTLINE

FOR THE

QUANTITATIVE ANALYSIS OF URINE.

BY

HENRY ERNI, A. M., M. D.,

PROFESSOR OF MEDICAL CHEMISTRY AND MEDICAL JURISPRUDENCE IN SHELBY MEDICAL COLLEGE, NASHVILLE, TENN.

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QUANTITATIVE ANALYSIS OF URINE.

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HENRY ERNI, A. M., M. D.,
PROFESSOR OF MEDICAL CHEMISTRY AND MEDICAL JURISPRUDENCE IN SHELBY MEDICAL COLLEGE, NASHVILLE, TENN.

In a previous essay,[*] we endeavored to furnish medical practitioners with an accurate description of the properties and chemical composition of all such bodies as occur in normal as well as abnormal urine, to facilitate the task of tracing, by the aid of a microscope and a limited number of plain chemical reactions, the presence of those urinary constituents which of themselves allow us in many instances to draw conclusions in regard to the healthy or diseased functional performance of certain organs. By such simple means, requiring very little chemical knowledge, skill, or loss of time, every practitioner may be enabled to contribute something to the as yet very meagre store of facts pertaining to pathological chemistry.

As in many instances, however, it is of paramount importance to know the exact quantity of one or several ingredients discharged in the urine within a given time, we design, in this paper, to describe plainly the methods and manipulations necessary for the quantitative valuation of the more immediately important constituents of urine.

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[*] A Systematic Outline for the Qualitative Analysis of Urine, with two illustrative lithographic plates. Nashville; Berry & Co.

It cannot lie within our province to write for the experienced practical chemist. The fact is, that even where skill with all the necessary apparatus is combined, to enter into minute investigations of this kind, the results to be desired are often altogether impracticable, as they involve such acute chemical tact, expense, and sacrifice of time, as are not presumed to be possessed by practitioners of medicine. In an appendix we have arranged the necessary reagents, with their probable cost, suitable for our purpose, and also tables for the comparison of French weights and measures—now generally used in laboratories—with the English standards.

Determination of the urine voided.

§ 1. How to weigh (measure) the amount of urine discharged within a certain time; for instance, twenty-four hours.

Where any approach to accurate determination of the component parts of urine is desired, we have to procure an average sample for analysis. It needs hardly to be said, that owing to slight causes, the same patient might on one day pass urine scarcely heavier than distilled water, and on another a fluid equal in density to diabetic urine.

Thus, under equal circumstances, the night urine is always of greater specific gravity than that voided in the morning. Hence, where the medical attendant should not find it practicable to have all the urine collected which has been excreted within twenty-four hours, the average composition of these two will come sufficiently near the truth.

The urine for twenty-four hours, requires for storage a cylindrical vessel which can hold at least 2000 cub. cent., equal to 4 pints English. The whole measure is subdivided into 100 cc., or into still smaller divisions. The vessel containing the urine is covered with a glass plate, smeared over with tallow or better with wax, and must be kept in a cool place. Such vessels we can, for the sake of economy, prepare ourselves.

1 cc. of pure water weighs 1 grm.; hence we can readily graduate a suitable glass jar, by weighing repeatedly 100 grms. of pure water, and after pouring the liquid into the jar, marking its level with a file or a flint. The true weight of the whole or any part by measure of the urine, we find by multiplying the number of cc. by the specific gravity of the urine. Presuming that we have 1000 cc. of urine, of specific gravity 1,025, the whole quantity weighs

$$1000 \times 1{,}025 = 102{,}5 \text{ grms.}$$

Urine voided at shorter intervals, and designed for examining into transient effects upon the urinary secretion, may be collected in smaller graduated cylinders, which ought to be subdivided into single cub. cent.

§ 2. Modes of determining the total amount of solid matter and water in the urine.

Different processes may be resorted to for this purpose. more or less suitable according to our desire to obtain strictly accurate results, or only comparative estimates, to be ascertained quickly at the bedside of patients.

The amount of solid matter existing in the urine, is most accurately determined by the evaporation of a given quantity of urine to dryness; but its management requires much care and expenditure of time. Besides, this task can only be executed, without great inconvenience, in well ventilated and arranged laboratories, since the stench emitted by this operation can scarcely be imagined by one who never performed it. To obviate these difficulties, it has been proposed to calculate the quantity of solid matters present in urine from its specific gravity; but as the various elements composing this excretion do not always exist in the same proportions, and as each has its different density, we only attain an approximate valuation of the solids removed from the system in a given time; a result, nevertheless, answering all clinical purposes, and to be ascertained in a few minutes.

Density or Specific Gravity of Urine.

The same familiar modes of finding the specific gravity of liquids generally, apply to that of urine.

1. By hydrometers and gravimeters.

A special instrument has been constructed, for discovering the specific gravity of urine, and is called the *urinometer*. It consists of a graduated, cylindrical glass tube, enlarged at the bottom. The divisions are numbered downwards, commencing with "0," which is the water-mark, (i. e., the instrument, when immersed in pure water, sinks to that point; in urine, which is denser than water, it will of course not sink so far, but on the contrary rise,) extending to 49 degrees, or still higher.

Suppose the instrument, floating in urine, should mark 12; then, its specific gravity is equal to 1012: for we have in every instance to add the number of 1000 to that marked on the stem, which is on the same level with the fluid.

A vessel, then, which can hold 1000 grms, of distilled water, could contain 1012 grms. of the above-mentioned urine; as the density of water, being always marked equal to 1, that of the examined urine would be equal to 1,012.

All such hydrometers are, however, constructed for use at a certain temperature, such as 15 C, (about 60 F.) since the density of fluids decreases at a higher, and increases at a lower temperature. Provided the temperature ranges between 15 and 27 C (59 and 80.6 F.,) the errors committed are very nearly compensated by the expansion of the glass instrument.*

2. If an urinometer is not at hand, a small-stoppered vial may be substituted. The following example will explain the operations:

Glass vial exactly filled with distilled water, weighs	80 grms.
The empty vial weighs	30 "
" " contains	50 "
The same vial, filled with urine, weighs	81.2 grms.
Vial alone,	30.0 "
It can hold urine,	51.2 "

The specific gravity of water being equal to 1,000, we find the specific gravity of the urine by simple calculation:

$$50 : 51.2 :: \overset{\text{Spec. grav. water.}}{1{,}000} : \overset{\text{Spec. grav. urine.}}{x}$$

$$x = \frac{51.2 \times 1000}{50} = 1{,}024$$

The construction of tables, allowing us to estimate from the known specific gravity of urine, the quantity of solid matter present therein, is based upon the following operations. After the specific gravity of a great number of different urines has been ascertained, at a certain temperature, a given weight of each sample is evaporated, when by a simple calculation the proportionate quantities of solid matters contained in 1000 parts of any urine may pretty closely be determined. The following table gives the amount of solids and fluids in 1000 parts, by weight, (grammes or grains) of urine of different densities. It was calculated from Dr. Christison's formula:†

If d is equal to the difference between 1000 and the density of the urine, then the quantity of solids in 1000 grammes of urine is $= d \times 2.33$.

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* See Ackland's table of corrections, in G. Bird, p. 70.

† G. Bird, pp. 75, 76.

Specific gravity.	Solids.	Water.	Specific gravity.	Solids.	Water.
1001	2.33	997.67	1021	48.93	951.07
1002	4.66	995.34	1022	51.26	948.74
1003	6.99	993.01	1023	53.59	946.41
1004	9.32	990.68	1024	55.92	944.18
1005	11.65	988.35	1025	58.25	941.75
1006	13.98	986.02	1026	60.58	939.42
1007	16.31	983.69	1027	62.91	937.09
1008	18.64	981.36	1028	65.24	934.76
1009	20.97	979.03	1029	67.57	932.43
1010	23.30	976.70	1030	69.90	930.10
1011	25.63	974.37	1031	72.23	927.77
1012	27.96	972.04	1032	74.56	925.44
10 3	30.29	969.71	1033	76.89	923.11
1014	32.62	967.38	1034	79.22	920.78
1015	34.95	965.05	1035	81.55	918.45
1016	37.28	962.72	1036	83.88	916.12
1017	39.61	960.39	1037	86.21	913.79
1018	41.94	958.06	1038	88.54	911.46
1019	44.27	955.73	1039	90.87	909.13
1020	46.60	953.40	1040	93.20	906.80

The mode of using this table is obvious; for having ascertained the density of the urine passed in twenty-four hours, in the manner previously described, the table shows us at a glance, the proportion of solid matter and water in 1000 grms. of urine; then, by converting the whole measure (in cc.) of urine into weight, as already directed, the total weight of solids secreted by the kidneys, is calculated by a simple rule of proportion.

Ex. A patient passes, in twenty-four hours, 1½ litre, (equal to 1500 cc.) of urine, of the specific gravity of 1.020; 1000 grammes of this urine hold, dissolved according to our table, 46.6 grms. of solids.

$$1500 \text{ cc. weigh} = 1500 \times 1.020 = 1530 \text{ grms.}$$
$$1000 : 46.6 :: 1530 : x$$
$$x = 71.29$$

1530 grms. of the above-mentioned urine contain 71.29 grms. of solid matter.

Determination of solid matter, bydirect weighing.

10 to 15 grms. of urine (by weight or measure, to be ascertained) are brought into a porcelain capsule or crucible, provided with a cover, and evaporated to dryness in a water-bath heated by a small alcohol lamp. Great care is required in this management, especially when we approach a certain point of dryness; for the mass is apt to spatter. If we heat beyond a certain temperature, we sustain a loss by the decomposition of urea and extractive matter. The residue thus obtained, (not yet entirely deprived of moisture,) we bring into an air-bath* for about an hour; the thermometer con-

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* See T. F. Luhme & Co.'s Catalogue, fig. 341.

nected with it must mark about 110 C. The crucible is then suffered to cool over sulphuric acid, in a dessicator*, and the contents weighed, subtracting the amount of dry matter from the total weight of urine employed, we obtain the amount of water.

Ex. Suppose the urine collected within twenty-four hours to be 1000 cc., and its specific gravity equal to 1,024; 10 cc. of urine are evaporated to dryness, and the residue dried at 110 C.

Weight of crucible, with residue =	24,580 grms.
Crucible alone weighs	24,350 "
Amount of dry residue,	0,230 "

These 0,230 grms. residue correspond to 10 cc. of urine; hence, 1000 cc. of urine contain 23.0 grms. of dry matter.

The total weight of 1000 cc. of urine, of 1,024 specific gravity, is equal to	1024.0 grms.
Subtracting from it the amount of residue,	23.0 "
we ascertain the quantity of water,	1001 grms;

i. e., 1024 pts. (by weight) of urine, contain 1001 pts. of water,
23 " dry matter;
1024

whence the respective quantities in 100 or 1000 pts. of urine are readily deduced.

The evaporation of urine may be still more safely accomplished under the receiver of an air-pump, over sulphuric acid.

§ 3. Determination of the fixed salts, not decomposable at a red heat.

The dried and weighed residue is moistened with a few drops of strong nitric acid, to facilitate the oxydation of organic matter, and the crucible gradually heated to a red heat, when the whole is incinerated, and looks gray. We weigh after cooling (in a desiccator,) quickly.

Ex. Weight of crucible, with ashes of 10 cc. of urine, equal to	24,406 grms.
Crucible alone,	24,350 "
Weight of ashes,	0,056 "

Hence, 1000 cc. of urine contain 5,60 grms. of fixed salts. The quickest and best method for ascertaining the amount of solids, organic as well as inorganic, is that advised by Rose, which is as follows:

From 20 to 30 grms. (by weight or measure) of urine, are evaporated upon the water-bath, in a porcelain or platinum dish whose

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* Ibid., fig. 346.

weight has previously been determined. Into the nearly dry mass we stir, by means of a platinum wire, a known weight (one to two grms.) of finely ground platinum sponge. We evaporate to full dryness, and after bringing the capsule for some time into the air-bath, we weigh. Deducting from the gross weight that of the crucible and platinum sponge, we find the whole amount of solids. To learn the quantity of fixed salts, we ignite the mass over a spirit-lamp, until all the carbon is burnt off—an operation greatly promoted by the porous platinum sponge. Deducting now from the gross weight obtained, that of the capsule and platinum, we obtain the amount of fixed salts—ashes.

If a more minute analysis becomes desirable, we can from this mass easily separate,

1st, The amount of salts soluble in water, (potassa and soda, united with sulphuric, chlorohydric, and phosphoric acids,) from,

2dly, Those soluble in chlorohydric acid, such as lime and magnesia, combined with phosphoric acid.

We treat the mass first with boiling water, and after that extract with the above acid.*

The platinum sponge remains behind after extracting the ashes with both fluids, and may again and again be made use of. From a trace of silicic acid found in urine, it is freed by potassa.

For information in regard to the quantitative determination of each single inorganic constituent, we refer to the works of Rose and Fresenius.

The solid matter varies in quantity much less in urine discharged within twenty-four hours, than the watery contents. Hence, the variable density of urine must be attributed to the latter. For instance, the same amount of solids may at one time be dissolved in 900 grms. of water; at another, in 1300 grms. The causes which much contribute to the augmentation of water, are various drinks, polydipsie, mental anxiety, hysteric affections, diabetes. Those which diminish it, are fever, heart-disease, abundant sweats, agony. This diminution may vary between 700 and 250 grammes—Becquerel.

From a large number of observations, Becquerel gives the [physiological] average amount of solids in the urine of twenty-four hours; for a man 39 grms, for a woman 34 grms. According to Bird, the excretion of solids by the kidneys, in the adults, within the same time, oscillates between 40 and 45 grms. [650 to 700 grains.] The causes which affect the diminution of solid matter

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* Pharmaceut. Centralb, 1850. p. 534.

are fear, debilitating diseases, anemia, chlorosis—the quantity varying between 24 and 5 grammes. A minimum of solids is observed in long, tedious diseases.—Becquerel.

§ 4. Gaseous constituents of urine.

Professor Planer found in fresh urine, free carbonic acid, nitrogen, and traces of oxygen.* The urine was boiled in vacuo, and the gases collected in the eudiometer. The carbonic acid absorbed by a strong solution of caustic potassa, an excess of hydrogen added, and the mixture exploded by an electric spark. From the diminished volume, the amount of oxygen was calculated, and the nitrogen left determined, as usual, by measure.

After the free gases were expelled from the urine, Planer determined the amount of carbonic acid combined in urine, by adding some crystals of tartaric acid, and continuing to boil the fluid.

	Specific gravity.	Amount of Urea.	1000 cc., of Urine contain at 0°c and 0,76 mm., pressure.				Nitrogen.	Oxygen.	100 parts of the free gas contains		
			Gas.		Carbonic acid.				Carbon. acid.	Nitrogen.	Oxygen.
			Free	Combined.	Free.	Combined.					
1. Morning Urine.	1,01 5	1,54	54,7	20,78	45,4	20,7	8,7	0,6	83,0	15,8	1,1
2. " Urine, after 14 hours Fasting.	1,0113	1,37	52,4	18,8	44,1	18,8	8,0	0,2	84,2	15,2	0,5
3. Afternoon Urine.	1,0213	2,43	108	52,5	99,6	52,5	7,8	0,5	92,8	7,2	0,5

The author gives a list of interesting results from his analysis of urine in disease.

For example, in fevers we find both the free and combined carbonic acid, greatly increased even where the patient rested without food, and the gas could not possibly have been derived from the medicine prescribed. Hence we must conclude that in febrile diseases, either an accumulation of carbonic acid gas takes place in the blood, or the increase is due to the more intensified oxydation going on in the tissues.

§ 5. Determination of the coloring matter.

A highly ingenious and valuable method of ascertaining quantitatively the amount of coloring matter in urine, has been invented by Vogel.†

He succeeded, by a great number of comparative observations, in preparing a scale of colors comprising all the various shadings belonging to healthy and diseased urine, and which he imitated by

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* Prof. J. Planer Ueber die Gase des Harnes and der Transsudate. Zeitschrift der Gesellschaft der Aerzte zu Wien, 1859. No. 30 p. 466–475.

† Arcniv zur Foerderung wissenschafll Heilkunde, 1855, and in Days contributions to urology, and Neubauer and Vogel's Analyse des Harns.

mixing together different quantities of the following paints: gamboge, carmine, and Prussian blue.

A. *Yellowish urines.*

1. Pale yellow, like a weak solution of gamboge.
2. Bright yellow, like a medium solution of gamboge.
3. Yellow, like a very strong solution of gamboge.

B. *Reddish urines.*

4. Reddish yellow, like gamboge with a little carmine.
5. Yellowish red, like gamboge with more carmine.
6. Red, like carmine with a trace of gamboge.

C. *Brown* [dark] *urines.*

7 Brownish red.
8. Reddish brown.
9. Brownish black.

These nine shadings stand in certain relations to the quantity of coloring matter in the urine. It has recently been ascertained, that by the addition of water to a higher numbered specimen of urine, all the lower proceeding numbers can be prepared. In other words, all the variously colored urine may be looked upon as being derived from one and the same coloring ingredient, existing in different degrees of dilution. We must, however, except bile pigments and others, derived from food and medicines accidentally present. Quantitative experiments proved that, if a certain number of urine is diluted with an equal bulk of water, we obtain the next lower one.

Ex. Take of number five, which is yellowish red, 200 cc., and dilute it with 200 cc. of water, and the mixture will correspond to urine number four, reddish yellow, etc. The above scale of urines may therefore serve us for a quantitative determination of pigment in urine, to which purpose Vogel arranged the following table:

I.	II.	III.	IV	V.	VI	VII.	VIII.	IX		
1	2	2	8	16	32	64	128	256	Pale Yellow,	I.
	1	4	4	8	16	32	64	128	Bright Yellow,	II.
		1	2	4	8	16	32	64	Yellow,	III.
			1	2	4	8	16	32	Redish Yellow,	IV.
				1	2	4	8	16	Yellowish Red,	V.
					1	2	4	8	Red,	VI.
						1	2	4	Brownish Red,	VII
							1	2	Redish Brown,	VIII.
								1	Brownish Black,	IX.

To be able to express in numbers the relative proportions of pigment present in different samples of urine, Vogel takes that quantity of pigment as unit [1] which is contained in 1000 cc. of pale yellow urine. The same volume [1000 cc.] of yellowish red urine [V] contains 16 parts of pigment, of red urine 32 parts, of brownish black urine 250 parts.

Suppose a person discharges in twenty-four hours' time 1000 cc. of yellow urine, and another, within the same space of time, 4000 cc. of pale yellow urine, then both secreted an equal quantity of pigment.

To attain unity in results, and render these comparable, the urine when examined must be clear, i. e., must in most cases be filtered; and further, equal layers of fluid must be examined by transmitted light, as of course thinner layers of the same urine appear paler.

Vogel uses cylindrical glasses of 4 to 5 inches in diameter, and which may contain 800 to 1000 cc. of urine.

Ex. The urine voided in twenty-four hours, amounts to 1800 cc., and is of a yellow color. 1000 cc. pale yellow urine, = 1 pt. pigment [being the unit] yellow urine contains, according to our table, four times as much; whence we have the following proportion:

$$1000 : 4 :: 1800 : x$$

$$x = 7.2:$$

i. e., the amount of pigment in 1800 cc. of yellow urine, is equal to 7.2, when that quantity of pigment found in 1000 cc. of pale yellow urine is marked equal to 1.

Separate determination of the constituents of urine.

A Organic constituents.

§ 6. Urea.

The quantitative determination of urea is of special import, owing to the great physiological result, thus secured of measuring i. e. expressing in numbers, the average amount of decomposition or metamorphosis of tissue carried on in the human organism within a certain period.* These numbers constituting nothing less than correct exponents of functional lesion, are hence important guides in the treatment of many diseases.

Direct Determination of Urea.

1. Lecanu's process.

It is founded upon the fact that nitrate of urea is nearly insoluble in water containing nitric acid, and has an invariable composition corresponding to the formula $Ur, NO_5 + HO$.

100 parts of this salt contain hence

Nitric acid,	46.93
Urea,	53.07
	100.00

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* For the importance attached to, and the indications of functional disturbances afforded by a knowledge of the amount of solids and urea in particular, consult the XIV. (last) chapter in G. Bird.

500 grms. (about half a litre) of the urine, are evaporated to about 40 grms. in a porcelain or better platinum capsula. To the concentrated (syrupy) liquid we add while yet hot, about thrice its weight of alcohol of 36° Baume, we agitate, and when the mass grows cold, filter; the residue on the filter consisting of uric acid urates, and the fixed salts (also coagulated albumen ect. if the urine was albuminous) is washed with alcohol. All the urea passes thus into alcoholic solution, we evaporate the latter in a water-bath to about 40 grms. the vessel containing it is then placed in cold water to hasten the cooling process. We now add by degrees and under constant stirring an equal weight (40 grms.) of pure nitric acid, the mass becomes crystalline, it is thrown upon a piece of clean linen, and strongly pressed. After having ascertained that the mother liquor is no longer rendered turbid by nitric acid, we carefully remove the nitrate of urea from the cloth into a small capsule, then dry in the water-bath, weigh, and calculate the amount of urea from the previously mentioned formula of nitrate of urea.

This method is laborious but simple, and admits of a direct determination of urea.

Ex. The nitrate of urea yielded by 500 grms. of urine weighs 10 grms.

$$\overset{\text{Nitrate of Urea.}}{100} : \overset{\text{Ur}}{53.07} :: \overset{\text{Nitrate of Urea.}}{10} : \overset{\text{Ur.}}{x}$$

$x = 5.307$ hence

$$\overset{\text{Urine.}}{500} : \overset{\text{Ur.}}{5.307} :: \overset{\text{Urine.}}{100} : \overset{\text{Ur.}}{x}$$

$x = 1.061$

i. e the urine examined contains a trifle over 1 per cent of urea.

2. Millon's process:

It is based upon the following decomposition:

A solution of urea when heated to the boiling point in contact with nitrous acid or nitrite of suboxide of mercury, takes up two equivalents of water and is resolved into Carbonic acid, and Nitrogen gas.

The Carbonic acid is collected in a weighed bulb apparatus* filled with a concentrated solution of caustic potassa. The increase in weight of this apparatus at the end of the operation gives us, when previously multiplied with 1,371, the weight of the Urea.

Millon proceeds thus:

He prepares the yellow nitrite of mercury by a gentle heating of the crystallized Nitrate of suboxide of mercury; this product he dissolves in Nitric acid of medium strength, and then adding some

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* This same apparatus is employed in organic analysis for the determination of carbonic acid.

of the mixture to the solution of urea contained in a suitable vessel. To obtain the carbonic acid in a dry state a suitable tube filled with Chloride of calcium ought to be connected with the bulb apparatus. The experiment is completed when no more bubles arise from the mixture; it requires but a few grms. of urine and is very accurate since according to Millon, the error in the determination of urea does not exceed 1-1000 of its weight. None of the other components of urine whether normal or abnormal disengage carbonic acid under the same circumstances. Many such determinations which consume about half an hours time, can be executed at the same time.*

§ 3. Liebig's volumetric method.

This ingenious method is now almost exclusively employed for physiological purposes, by hospital and private physicians in Europe. It is accurate, simple and quickly executed; and dispenses with a balance altogether.

The following principles upon which it is based, will readily explain its practical execution. When a dilute solution of urea, such as urine, is gradually mixed with a dilute solution of nitrate of peroxide of mercury, and we neutralize from time to time by means of carbonate of soda the nitric acid which is set free, we obtain a floculent white precipitate, perfectly insoluble in water, and having invariably the same composition $= U + 4\ Hg\ O$ i.e. it contains 1 equiv. of Urea and 4 equiv. of Peroxide of mercury or for every 10 pts of the former, 77 parts of the latter.

If we add thus alternately nitrate of mercury and carbonate of soda, we reach a point where upon the further addition of the last named reagent (NaO, CO_2.,) no longer a white, but a yellow precipitate falls, consisting of hydrated oxide of mercury (HgO, HO). This marks the time when all the urea has been thrown down from its aqueous solution.

Now if we know previously the strength of a given measure of our mercurial solution, and further mark exactly the volume of it consumed, in the complete precipitation of urea (indicated by a yellow coloring upon the addition of a drop of soda) we have all the means necessary for calculating the amount of urea present in any solution. Suppose we prepare a solution of urea containing exactly 100 milligrammes of urea, and we ascertain what volume of our graduated mercurial solution was required for the complete precipitation of the urea; then the same volume will of course be

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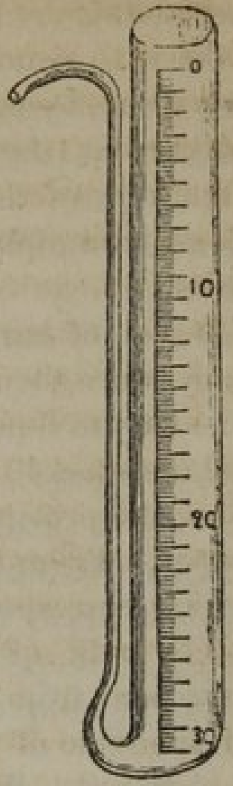
* Dr. Leconte employed more recently, for the oxidation of urea hypochlorites, for instance hypochlorite of soda whence carbonic acid, water and nitrogen gas are formed. Note sur un nouveau procede de dosage de l'uree. Lue dans la seance du 15 juin 1858.

necessary to throw down 100 milligrammes of urea from any solution of unknown strength.

Fig. 1.

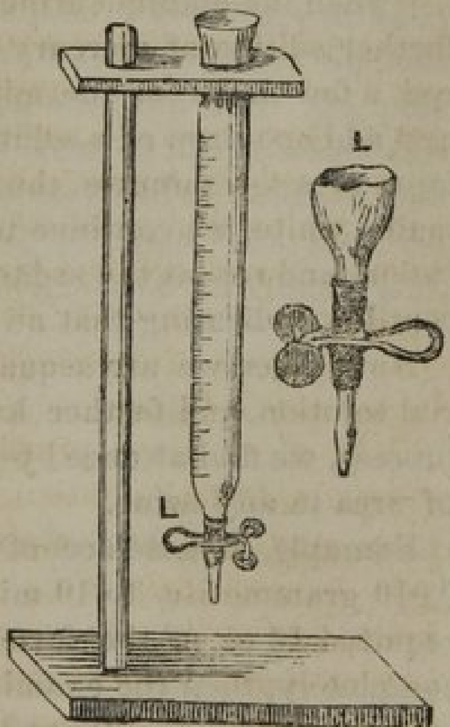


The apparatus necessary for the execution of the above mentioned method are,

1. A cylinder glass, divided into cubic centimeters which is to contain the urine to be examined.

2. A so called burette, fig. 1, which is to receive the graduated test liquors; the smaller tube of this instrument permits us to pour out the liquid drop by drop. To bring it into a vertical position necessary for reading off the quantity of liquor discharged from it, we hold it towards the glass-pane of a window.

Fig. 2.



3. Mohr's graduated pipette fig. 2, is preferable to the last mentioned instrument; because it is not so easily broken, and may be readily secured, (or several pipettes at the same time) in a vertical position. One end of this tube is drawn out to a point, to which is attached, by means of a narrow vulcanized caoutchouc tube, a short glass tube, likewise drawn out to a point. (see fig. 2, L.) There is a small space (about ¼ inch) between the two tubes, upon which is fixed a metallic clamp, which shuts off the connection between the graduated cylinder and the small glass tube. But by pressing with the fingers upon the ends of this clamp it opens and allows the liquid to flow out in any desirable quantity.

The instrument is divided into 40 cc. or more, and each of these is further subdivided into 10 parts, hence we can actually measure 1-10 cc. of the test liquor.

In proceeding now to the practical execution of this mode of ascertaining the amount of urea in urine, the first step we have to take is to remove the phosphoric and sulphuric acids, contained in the urine, by a saturated solutions of caustic baryta, and of nitrate of baryta, (the latter serves the purpose of preventing too great an alkalinity of the resulting fluid.) In preparing these baryta solutions, we simply saturate a certain amount of water with the dry salts.

2. vol. of baryta water, and 1 vol. of the solution of nitrate of baryta are then mixed and stored up for use, one vol. of this alkaline baryta liquor (i. e. about 20 cc) is mixed together with two volumes (= 40 cc.) of urine in a glass-flask, and after some agitation, the precipate formed is removed by filtering through (dry) paper. From the filtrate we measure of 15 cc. [for every analysis] which correspond exactly to 10 cc. of urine [for, as already stated 2-3 by bulk of the mixture is urine.] To this alkaline fluid we now pour from a pipette or burette, a gradulated solution of nitrate of suboxide of mercury, the mixture being constantly stirred with a glass-rod. When we begin to approach the limits of precipitation i. e. when the alkaline urine does not at once become cloudy upon further adition of mercury solution, we bring by means of a glass-rod, a few drops of the mixture, turbid as it is, into a watch-glass, and add one drop of a solution of carbonate of soda. If after the lapse of a few minutes, the mixture does not turn yellow, but remains white, we continue to add a few drops more of mercurial solution, and repeat the soda test until a distinct yellow color is perceptible, indicating that all the urea has been precipitated.

Now, since we are acquainted with the strength of the mercurial solution, and further know how much of it was spent in this process, we find at once by the simplest calculation, the percentage of urea in any urine.

Example, Let each cc. of the mercurial test-liquor correspond to 0.010 gramme i. e. to 10 milligrammes of urea, and suppose that it required 16 cc. of the former solution to precipitate all the urea completely, then the examined urine contains $16 \times 10 = 160$ milligrammes of urea. Hence 10 cc of urine contain 0.160 grammes, [160 mgr.] of urea, and consequently 100 cc. of urine contain 1,6 gramme.

CC. Urine.		Urea.		CC. Urine.		Urea.
10	:	0.160	: :	100	:	x

$x = 1.6$ grammes.

whence 1000 cc. of urine must contain 16 grammes of urea.

If considerable quantities of chloride of sodium are present in urine i. e. from 1 to 1½ per cent. the accuracy of Liebig's method is somewhat affected, hence, where it becomes desirable to ascertain the absolute quantity of urea contained in urine, this chloride has first to be removed by nitrate of silver solution.

A silver solution of known strength is readily prepared, and with it we may in a few minute's time determine the amount of kitchen salt in urine.

The silver solution is graduated by dissolving 11,601 grammes of previously fused nitrate of silver in water, and diluting it further, until we have 400 cc. when 1 cc. of it corresponds to 10 milligrammes of kitchen salt.

Practical execution.

Of the alkaline urine, freed of sulphates and phosphates by baryta solution as described, we take for example, 30 cc. which, it will be remembered, contain 20 cc. of urine, and having acidulated with a drop or two of nitric acid [so as to turn blue litmus paper feebly, but distinctly red] we pour slowly from a burette or pipette, a graduated solution of the nitrate of silver as long as a precipitate is formed.

In order to be safely guided, we make from time to time a separate trial in a watch-glass, into which we pour a few drops of each liquid. After the reaction is complete, we learn from the amount of test-liquor left in the burette how much has been used. Suppose to precipitate all the chlorine in 30 cc. of alkaline liquor which corresponds to 20 cc. of urine, it required 35 cc. of silver solution how much kitchen salt was present in 100 cc. of urine. As each cc. silver solution indicates exactly 10 milligramme of kitchen salt, then 35 cc. contain 350 mgr.

whence the simple proportion.

CC. Urine.		Grm. salt.		CC. Urine.	Salt.
20	:	0·350	: :	100	x

$x = 1.75$

i. e. 100 cc. [nearly 100 grm.] of urine contain 1.75 grm. [per cent.] salt.

To determine now, further the amount of urea in the above sample of urine, we filter off the chloride of silver, and having wasted some of the fluid in the watch-glass [originally we had 30 cc. liquor corresponding to 20 cc. of urine, and in addition 35 cc. of silver solution making together 65 cc.] we take but one half of its former bulk =32½ cc. corresponding to 10 cc. of urine and separate the urea contained therein.

To that end we add from a burette the graduated nitrate of peroxide of mercury as formerly directed, and ascertain the limit by a conc. solution of carbonate of soda.

But Liebig's method has further this great advantage, that with the urea we can also, simultaneously determine the amount of chlorine [kitchen salt] in the urine. The reason why the presence of kitchen salt interferes with the precipitation of urea, is simply this: Solutions of nitrate of peroxide of mercury, and chloride of sodium brought together, form by double elective affinity, nitrate of soda, and per chloride of mercury, or our common corrosive sublimate. This last named body however, does not precipitate urea from a dilute, and slightly acidulous solution.

In order therefore to find the amount of chlorine, a definite volume of urine is treated with the solution of baryta; the urine which is filtered from the precipitate should then be completely neutralized with small quantities of nitric acid, and the solution of nitrate of mercury poured upon it, until the precipitate no longer dissolves on being stirred, that is to say as long as chloride of mercury is formed. The quantity of the chloride of mercury, or of the chlorine contained in the urine, may be calculated from the volume of the solution of mercury which has been consumed.

Ex. Suppose we had measured off into a tumbler 15 cc. of a filtered mixture of baryta solution, and of urine [corresponding as usual to 10 cc. of the latter] we neutralize carefully with nitric acid. From Mohr's pipette [or a burette] we suffer to flow slowly the solution of nitrate of mercury [each cc. corresponding to 10 mil. gr. = 0.010 grms. kitchen salt or 6.056 mgr chlorine] until an additional drop produces a lasting turbidity upon continued stirring, indicating that all the kitchen salt has been decomposed; suppose also it had required 15½ cc. mercurial solution, then 10cc. of the urine contain 15½ × 10 = 155 milligramme of salt; and hence 100 cc. of urine = 1.55 grms. of salt.

To determine next the quantity of urea of the same sample of urine, we measure out 30 cc. of filtered urine and baryta solution, and having ascertained by our previous proceeding that this mixture contains 155 milligr. of kitchen salt, we can at once remove this body by adding 2 × 15½ cc. = 31 cc. of our graduated silver solution. The total volume of our mixture is = 30 cc. + 31 cc. = 61 cc. we filter off the precipitated chloride of silver, and take of the filtrate one-half = 30½ cc. corresponding to 10 cc. of urine, and precipitate the urea with nitrate of mercury as previously described; in calculating the amount, we must of course always take into account the dilution caused by adding silver-solution.

Should the urine contain albumen, it becomes necessary to remove it, before resorting to Liebig's mode of determining the urea and chloride of sodium by means of nitrate of mercury, for the latter in common with all other soluble salts of mercury precipitates the albumen.

The following modifications in this process have to be made when the urine proves albuminous—50 cc of the albuminous urine are acidulated with a drop or two of acetic acid—unless the urine tests already distinctly acid—and the albumen coagulated by boiling the fluid. The coagulum is filtered off through a loosely folded paper, previously moistened with water. It is washed, and after the wash-water, collected separately, has been mixed with the filtered liquid, we measure the whole amount.

Ex. The joint liquor measures = 100 cc. which correspond to 50 cc. of urine. We next add 25 cc. of the already described baryta solution, the precipitate [phosphates, sulphate of lime] is filtered off; of the filtered liquor = 125 cc. we employ 25 cc. [corresponding to 10 cc. of urine] for the determination of urea, taking always as usual into account the increased bulk, occasioned by the addition of wash-water i.e. 125 cc. of the whole fluid contain 50 urine, and 1-5th of its bulk or = 25 cc. corresponds to 10 cc. urine.

In another, 25 cc. of the mixture first cautiously acidulated with nitric acid, we determine the chlorine with our graduated mercurial solution.

Finally we remark that Limpricht has found that allantoine is likewise precipitated by nitrate of mercury, but allantoine has as yet never been detected in normal or morbid human urine, though Prof. Staedeler has found it in the urine of dogs, in cases where respiration was impeded.

According to Becquerel,* the following numbers represent the average quantities of urea discharged in 24 hours in a state of health.

Men. = 18 grms.
Women. = 15 "

The quantities of urea secreted within 24 hours, by the same individuals placed in different circumstances of existence may, according to Lecanu, vary between 12 and 33 grms. whilst the quantities closely approach in individuals placed under analogous conditions of age, sex and food. He found the amount of urea voided considerably more in man at the age of manhood than in women; the quantities of urea being much diminished in old age [1-3,] and during early infancy [1-7.]

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* Hottot Lanalyse de l'urine, Paris 1856.

Berzelius and Lehmann found in 1000 pts. by weight of urine, 30 pts of urea [3 per cent;] when much urine is discharged, 1000 pts. of it contains 12 and 15 pts [1.2 and 1.5 per ct.] of urea, [Becquerel and Simon.]

In pathological conditions, Becquerel found that the urea does not exceed the physiological limits as often as might be expected.

Bouchardat observed in a case of polyurie, that the quantity of urea discharged in 24 hours amounted to 134 grms.

It appears to be a law, that in those diseases capable of altering the products of urinary secretion, this alteration consists of a diminution of the urea.

In febrile and liver diseasés, where the urine appeared dense and colored, the amount of urea discharged within 24 hours was 9 grms. Individuals much weakened by loss of blood, or long disease, voided in that time only about 7 grms.

§ 7. Hippuric acid.

Rarely found in a free state in urine, but mostly in the form of hippurates.

Free hippuric acid was traced in fevers [Lehmann] in urinary sediment of a drunkard [Bird] and in urine of a man of sedentary habits with a highly nutritious diet.

In a pathological view, this distinction seems immaterial.

The hippurates are soluble in water, the concentrated solution deposits, when treated with chloro-hydric acid, yellowish crystals of hippuric acid.

This process answers well to obtain it from urine of horses, which contains an abundance of this substance, but is insufficient to separate mere traces as found in human urine.

Liebig's method.

A certain quantity of urine, for instance 40—50 grms. are treated with a few drops of chloro-hydric acid, and evaporated to syrup consistency. The mass is now agitated with an equal volume of ether and the mixture set aside for an hour; should the ether not become clear, which is often the case, we add 1-20th of its volume of alcohol by which this result is at once accomplished.

We now separate the upper etherial layer, containing besides, hippuric acid traces of urea, by means of a pipette, and shaking it subsequently with a small quantity of water, which dissolves the alcohol and urea, whilst the hippuric acid remains dissolved in ether. Evaporating this latter, we obtain the hippuric acid in the form of crystals, the quantity of which is determined as directed under uric acid.

To remove the coloring matter, a process always attended with loss, we saturate the acid with lime water, and digest with a little animal charcoal, then add some chloro-hydric acid, and filter: upon separation of the filtrate, the hippuric acid is obtained in colorless crystals.

§ 8. Uric acid.

Although this body exists but in small quantities in human urine, still its quantitative determination deserves our attention, from the fact that its presence indicates, at times, important pathological conditions.

Modern experimenters, such as Robin and Verdeil, have shown that uric acid rarely occurs free in urine, but is generally united with soda, lime, and magnesia. Since, however, in a medical point of view, this distinction appears to us as of little importance, we give in the method below, the plan best adapted for determining both forms jointly.

About 100 cc. of urine are brought into a tumbler, and 10 grms. of pure chlorohydric acid added, under constant stirring of the mixture with a glass rod. The vessel is then covered with a glass plate, and set aside for 36 to 48 hours. After the lapse of a short time, the uric acid will be found separated in colored crystals, which have subsided to the bottom, whilst other portions may float on the surface of the liquid, or adhere to the walls of the vessel. By stirring the fluid violently, most of the crystals may be gathered in the form of sediment. The supernatant liquor is carefully decanted, and the uric acid collected upon a filter of known weight; the still adhering portion is loosened by the aid of a feather. The whole, thus united, is well washed, dried in an air-bath, and weighed; which latter operations are best executed by enclosing the wet filter and precipitate in two large watch glasses of equal size, the edges of which have been ground smooth; both may then be tightly secured by a brass clamp.

For clinical purposes, the precipitated uric acid may be simply washed into a watch glass; after drying, the increase in weight furnishes the quantity of this body.

The quantity of uric acid discharged by the urine passed within twenty-four hours, under different circumstances of age, sex, and food, varies between 0.089 and 1,575 grms. [Lecanu.] Becquerel found the average quantity in perfect health, 0.465 grms. for men; 0.557 grms. for women. The difference in favor of females is, however, not constant, and the average lies between 0.4 and 0.6, or 0.5 grm.

Should the urine contain albumen, a portion of it would also be precipitated by chlorohydric acid; [unless the mixture be very dilute;] hence it becomes necessary, in that case, to get previously rid of the albumen, and subsequently to precipitate the uric acid in the liquid, concentrated by evaporation, if necessary. And since this process serves at the same time as a guide for the quantitative determination of albumen, we will in this connection turn our attention next to that body.

§ 9. Albumen.

To determine this quantitatively, we proceed thus: 50 cc. of fresh urine, filtered if sedimentary, are poured into a flask holding at least double that volume, and then heated over an alcohol lamp. As soon as a coagulum is formed, [70° C.] we add, by means of a glass rod, a drop or two of acetic acid, and continue heating until the albumen collects in flakes. It is filtered off upon a filter of known weight, well washed, dried at 110 to 115° C, and weighed, making use likewise of two watch glasses, previously described.

We would caution the inexperienced against a surplus of acid, keeping a portion of albumen in solution. Free alkali, on the other hand, has the same effect. The urine might equally well be acidulated before boiling; but in that case a small excess of acid might altogether prevent the coagulation of albumen, and thus cause us to overlook this body.

In the filtered liquid, together with the wash-water, [first evaporated if necessary,] we determine the uric acid as already described above.

§ 10. Sugar.

Urine containing sugar, is generally endowed with peculiar physical properties, which may enable us at once to suspect its presence.

Diabetic urine is usually colorless, and emits no odor, or but a faint one. It tastes mild or sweet, and putrifies very slowly.

The specific gravity of sugary urine is high, oscillating between 1,020 and 1,075. It exhibits mostly an acid reaction, attributed to the presence of lactic acid. The quantity of urine voided by diabetic patients within 48 hours, [varying, however, with the quantity of ingested water,] amounts, on an average to 4–8 litres,* increasing in some cases to 18, 20, or 24 litres. Occasionally, the quantity discharged is normal, when it presents the aspect of a clear syrup. The sugar contained in urine forms the 1-30th to 1-7th of the total weight of this fluid.

Quantitative determination of sugar.

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*1. Litre, equal to two wine pints or 1000 grms.

1. Fehling's method depends upon the property of grape sugar, at an elevated temperature, and in the presence of free alkali, to deprive oxide of copper of one half of its oxygen, thus converting it into suboxide characterized by a brownish red color.

Preparation of the graduated test liquid.*

40 grammes of pure crystalized sulphate of copper are dissolved in about 160 grms. of distilled water. We next prepare a concentrated aqueous solution of neutral tartrate of potassa, and mix with this 600 to 700 grms. of a solution of caustic soda or potassa, having a specific gravity of 1.12. We then pour the copper solution into this alkaline liquid, by small quantities at a time. The whole is finally diluted with distilled water, until it measures 1154.4 cc. at 15° C., [60° F.] 10 cc. of this clear, violet blue copper liquor, requires exactly 0.050 grammes [i. e. 50 milligrammes] of sugar, for its decomposition, [discoloration,] and will keep unchanged for years.

Suppose we fill a burette, graduated into cubic centimeters, with the urine to be tested, adding it gradually to the proper amount, [i. e. 10 cc.] of copper liquor, till it loses its blue color; what is wanting to make up the original measured quantity of urine, corresponds to 50 milligrammes of sugar.

To obtain accurate results, it becomes necessary to suffer very dilute solutions to react upon each other.

Practical example. We bring 10 cc. of copper solution into a porcelain dish, and after diluting it with 30 to 40 cc. of distilled water, we heat over an alcohol lamp, nearly to boiling. About 20 cc. of fresh urine are carefully mixed with ten or twenty times their bulk of distilled water,† and by the drop added to the copper liquor, until complete reduction has taken place—i. e., until the supernatant liquor is colorless. To reach this point accurately, requires some practice; it is therefore advisable to remove the dish from the fire, as soon as the precipitate [at first yellow] turns intensely red, and suffer the same to settle, when the slightest blue tint of the clear liquid is strongly contrasted with the walls of the porcelain dish. Should we still have our doubts, whether to add more urine or not, we pour a little of the clear liquid into a test tube, add a drop of urine, and apply heat, when, if there is any undecomposed copper salt left, a red cloud appears. In that case, the tube is emptied into the dish, and more urine supplied.

In order to be absolutely certain whether we accomplished the

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* This test liquor may also be purchased as mentioned in our list of volumetric reagents. p. 33.

† The urine ought to contain only about 1 per cent. of sugar, which we can previously ascertain by a separate experiment.

end in view, i. e. to know whether the mutual decomposition has been completed, if not overreached, we filter a very small quantity of the copper liquor* into a test tube, divide it into two parts, and after adding a drop of Chlorohydric acid to each, we test with sulphide of hydrogen and ferro-cyanide of potassium for copper, the former reagent, throwing down black sulphide of copper, the latter reddish brown ferro-cyanide of copper.

To ascertain finally if no excess of saccharine urine has been added, we make use of the deportment of caustic alkalies with grape sugar, which, as is well known, darken its solutions; hence if the filtrate after finishing the experiment is yellow or brown, we conclude that there was free sugar present, which, acted upon by the alkali of the copper liquor, gave rise to this alteration, in which case it is best to repeat the analysis; the first result serving us nevertheless to control and rectify the second.

Calculation of the result.

Of course that quantity by volume of the urine poured out of the burette into the copper solution, which it decomposes, contains exactly 0.05 grms. of sugar. Now it is evident that the less urine was required the more sugar is contained; in other words the amount of sugar stands in an inverse ratio to the volume of urine consumed.

If m. [quantity consumed] cubic cent, of urine contain 0.05 grms. of sugar, how much do 100 cc. of urine contain?

m ; 100 :; 0.05 : x; (x quantity of sugar in 100 cc.)

whence

$$x = \frac{100 \times 0.05}{\text{m.}} = \frac{5}{\text{m.}} = \frac{[\text{5 grms. of sugar.}]}{[\text{number of cc. of urine.}]}$$

It follows then that we obtain the per centage of sugar in the urine analyzed, by dividing 5 by the number of cc. of urine necessary for the complete reduction of the test copper liquor.

This result refers to urine which has not been diluted with water, but where this was the case, we have of course to bring it into account. Suppose the urine had been diluted with 20 times its volume of water, thence we have to divide $20 \times 5 = 100$ by the number of cc. of urine used.

Assumed it required 10 cc of original urine, and that this was likewise mixed with 20 times its bulk of water, then we have

$$\frac{5 \times 20}{10} = \frac{100}{10} = 10 \text{ per cent of sugar.}$$

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* It is to be remembered that the suboxide of copper is easily reoxidized, thus becoming soluble. The mass must rapidly be filtered whilst hot, after the experiment has been finished for by suffering is to get cold, the supernatant liquid will always have a blue color, derived from reproduced free oxide of copper.

2 method by fermentation.

The principle involved in the process of fermentation is that yeast cells brought into contact with a solution of sugar, decompose the latter into alcohol and carbonic acid gas.

1. equiv. of grape sugar $= C_{12}H_{12}O_{12}$ yields:
2. equiv. of alcohol, $= C_8H_{12}O_4$
4. equiv. of carbonic acid, $= C_4 \quad O_8$

$$\overline{C_{12}H_{12}O_{12}}$$

Hence if we determine the weight of carbonic acid resulting from the decomposition of diabetic urine, we can readily calculate the amount of sugar contained therein.

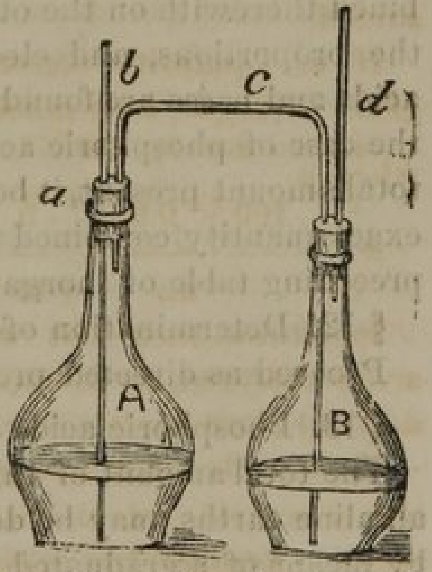
4. equiv. = 88 pts. of carbonic acid correspond to
1 equiv. = 180 pts. of grape sugar.

CO2. Sugar. Carbonic acid. Sugar.
88 : 180 :: 100 : x
$x = 204.54$ i. e.

100 pts. of carbonic acid correspond to 204,54 pts of sugar.

Execution.

The collecting and weighing of the gas is readily accomplished by means of Fresenius & Will's simple apparatus,* Fig 3.



Into the flask A we measure 30 to 40 cc. of urine, add to it a little well washed Brewer's yeast, together with a trace of tartaric acid. A quantity of strong sulphuric acid is put in the flask B; The tubes are then affixed as shown in the figure where all parts are represented in relative proportions. The opening on the top of the straight tube *a* which dips into the flask A, is closed by a little ball of wax and the whole apparatus weighed. It is then brought into a warm place [15—25°C.=59-70° F.] where fermentation speedliy sets in. The bubbles of carbonic acid gas pass through the tube C. into the flask B. filled with sulphuric acid, and after being thus completely dried, the gas finds exit through the tube d.

§ 11. Kreatine and kreatinine may be isolated and determined, if desirable according to the directions given in our qualitative analysis of urine, pg. 8.

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* This same apparatus is not only well suited for the analysis of carbonates, but is also adopted for the determination of nitric acid, Peroxide [Binoxide] of manganese ect. see Luhmes descriptive catalogue.

B. Separate determination of the inorganic constituents of urine.

In a physiological state, the urine contains the following salts:*

Sulphate of Potassa.
" Soda.
" Lime.
Phosphate of Lime [basic.]
" " [acid.]
" of Magnesia.
" Potassa.
" Soda [neutral.]
" " [acid.]
Chloride of Sodium.
" " Potassium.

or presented in a simpler form, we find therein

$$\left.\begin{array}{l}\text{Chlorine combined with,}\\ \\ \text{Sulphuric acid " "}\\ \text{Phosphoric acid " "}\end{array}\right\}\quad\begin{array}{l}\left\{\begin{array}{l}\text{soda}\\ \text{potassa.}\end{array}\right.\\ \text{lime.}\\ \text{magnesia.}\end{array}$$

Chemists who occupied themselves with the analysis of the inorganic ingredients of urine, have limited this task to chlorine, sulphuric and phosphoric acids on the one hand, and to the bases combined therewith on the other, without having regard generally to the proportions, and elective affinities according to which both acids and bases are found united to one another. For instance, in the case of phosphoric acid, it most frequently suffices to know the total amount present, it being unnecessary to ascertain likewise the exact quantity combined with each and every base mentioned in the preceding table of inorganic salts.

§ 12. Determination of Chlorine.

Proceed as directed previously in connection with urea, pg. 15.

§ 13. Phosphoric acid.

The total amount of this acid combined as it is with alkalies and alkaline earths, may be determined by weighing or volumetrically by means of a graduated solution of sesquichloride of iron.

The principle involved is the following:

If we mix with a solution of phosphates containing but a very slight excess of nitric or chlorohydric acids, a solution of acetate of soda, containing free acetic acid—and then add sesquichloride of iron,† we obtain a yellowish bulky precipitate of basic phosphate of iron, having the composition $=Fe_2\,O_3\,PO_5$.

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* Robin & Verdeil's "Traite de Chemie Anatomique et Physiologique.'

† It must be free of proto chloride of iron.

To discover whether all the phosphoric acid has been thrown down, i. e. to trace a slight excess of sesquichloride of iron, we place a piece of filtering paper previously drenched with a solution of ferrocyanide of potassium [yellow prussiate of potash] upon a white plate or porcelain dish, and press with a glass rod, on which a drop of the mixture hangs, a double layer of filtering paper against it. In a few seconds time a blue spot [prussian blue] will appear, if a surplus of iron solution was present.

Upon trial it has been found that for every 10 or 15 cc. of the graduated chloride of iron solution, 1 grm. of acetate of soda is required. To that end we prepare an aqueous solution of 20 grm. crystalized acetate of soda, dilute it until it measures 160 cc. adding subsequently to it 40 cc. acetic acid [acetum concentratum] so that the whole mixture amounts to 200 cc. 15 cc., of this solution contain 1 grm. of [dry] acetate of soda and 2 grms. free acetic acid.[*]

Example: a determination of the total amount of phosphoric acid found in urine.

By means of a pipette we measure 50 cc. of urine into a beeker glass and add to it 10 cc. of acetate of soda solution. The graduated iron solution is next brought under constant stirring into the mixture and only drop by drop from a pipette. To meet the point of complete saturation, we test frequently a drop of the joint mixture with the prepared filtering paper previously described. It is necessary to test immediately upon the addition of the iron liquor,[†] and much the safest to do so after each ⅛ cc. of test liquor escaped from the pipette.

The sesquichloride of iron solution is usually so graduated that each cc. corresponds to 10 milligrammes of phosphoric acid.

In alkaline urine a portion of the phosphates will occur as a sediment which has first to be brought into solution by a drop or two of chlorohydric acid. According to the amount necessary of the latter, it will require from 10—20 cc. of acetate of soda for the 50 cc. of urine to be examined.

b. separate determination of the phosphoric acid combined with the alkaline metals.

In that case, we take the same amount of urine [50 cc.] and first remove the earthy phosphates by means of a few drops of ammonia. The precipitate consisting of phosphate of lime, and of phosphate of ammonia and magnesia is filtered off and washed

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[*] It may be purchased ready prepared as mentioned in our appended list of volumetric reagents.

[†] After a short time this reaction for free oxide of iron does not take place. The phosphate of iron mainly decomposes the chloride of iron solution added in excess, and taking up more oxide of iron, it passes into a still more basic phosphate. The first distinct coloring produced by ferrocyanide of potassium (although disappearing again) must terminate the experiment

with water, containing a little ammonia.[*] The filtered portion is then again neutralized with acetic acid, mixed with 10 cc. of acetate of soda solution, and tested with sesquichloride of iron; from the number of cc. thus spent, we learn the amount of phosphoric acid combined with alkalies, and by subtracting that quantity from the total amount of phosphates determined in *a.* the difference indicates the quantity of phosphoric acid united with the metals of the earth's.

§ 14. Sulphuric acid.

This acid is determined in the usual way by a solution of chloride of barium, when the resulting sulphate of baryta is weighed, or volumetrically by employing a solution of chloride of barium, previously graduated, and as in the latter case, the final reaction cannot be easily ascertained and may deceive, we shall describe briefly both:

A. Determination by weighing.

50 cc. of filtered urine we bring by means of a pipette into a small porcelain dish and heat to ebullition, then after acidulating with a little chlorohydric acid, we precipitate with a solution of chloride of barium. At this elevated temperature, the sulphate of baryta thrown down being more compact and subsiding quickly, the supernatant liquid soon turns clear and may readily be filtered, whilst otherwise the liquor passing through the filter is turbid.

The precipitate is collected upon a small filter, the amount of its ashes [1—1½ milligramme] having previously and in a separate experiment been determined, and washed with hot water.[†] The precipitate on the filter is now dried in an airbath, and then ignited in a platinum or porcelain crucible until the filter is completely incinerated [forming a little white ashes.] The crucible is now suffered to cool over sulphuric acid [desiccatur] and weighed, from the total weight thus obtained, we deduct the weight of the crucible and that of the ashes of the filter, and calculate the amount of sulphuric acid.

116.59 parts by weight [1 equivalent] of sulphate of baryta correspond to 40 parts [1 equivalent] of anhydrous sulphuric acid.

B. Volumetric determination.

For that purpose, we choose two differently graduated solutions of chloride of barium, A. concentrated solution 1. cc. of which cor-

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[*] These phosphates are insoluble in water charged with some ammonia.

[†] To ascertain generally whether all soluble matters have been removed by washing, we collect a drop of the filtering liquor upon a platinum spatula or a slip of glass and evaporate it, if nothing remains behind the washing process is finished. In this special case we can by a drop of sulphuric acid added to some of the washwater, learn whether all the surplus chloride of baryum is removed.

responds to 10 mgrm. of sulphuric acid, B. dilute solution 1. cc. = 1 mgrm. Sulphuric acid.

Example. Into a long necked flask,* (bolt head) we measure in the usual manner 50 cc. of urine, treat it with 20—30 drops of chlorohydric acid and heat to boiling; from a pipette or burette, we add 2—4 cc. of chloride of barium solution and suffer the sulphate of baryta to subside completely. If the supernatant liquor (or at least the uppermost layers) is clear, we add another cc. of the reagent, and heat again and filter some 10 drops through a very small paper filter (not larger than a thimble) into a test tube of the smallest size, and try whether a drop of chloride of barium (of course not to be taken from our measured test solution) produces cloudiness indicating yet the presence of sulphuric acid; if this is not the case, we empty the test tube into the flask, wash both filter and test tube with a little water, and join this too.

If we have thus far consumed 5 cc. of chloride of barium solution (A) we may add another cc. then filter off again a few drops, thus prolonging this process until chloride of barium no longer indicates sulphuric acid.

Suppose it took for that purpose 8 cc. of reagent A and that a solution of sulphate of potassa or sulphuric acid now indicated in a freshly filtered portion of liquid an excess of chloride of barium, then we know that the proper limit of the reaction lies between 7 and 8 cc., in other words we learn that 50 cc. contain between 70 and 80 mgrm. of Sulphuric acid. To attain greater accuracy we make a second experiment.

To 50 cc. of urine are added 20 drops of chlorohydric acid, and next 7 cc. of test-liquor (A) we apply heat, and after the precipitate has settled, we finish the process by employing chloride of barium solution B. of which as already stated, each cc. corresponds to 1 mgrm. of sulphuric acid. The further proceeding is the same exactly as described, i. e. we filter off occasionally a few drops and test as directed, whence we may approach closely to truth.

The whole operation requires hardly half an hour's time.

§ 15. Lime and magnesia.

Those ingredients are best determined according to Vogel's method.†

In two equal portions of urine of 50 cc. each the earthy phosphates are precipitated by ammonia and separately filtered off, and thoroughly washed with water containing ¼ its volume of ammonia.

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* See fig. 609, Luhme's catalogue.

† Consult Neubauer & Vogel on the various analytical methods employed.

In the phosphates collected upon the one, filter A. and corresponding to 50 cc. of urine, we determine the phosphoric acid by sesquichloride of iron as explained fully § 13, the precipitate being first brought into solution by as little chlorohydric acid as possible.[*]

The second portion of phosphates upon filter (B) is dissolved in acetic acid, and the lime thrown down by oxalate of ammonia. The precipitated oxalate of lime is collected upon a filter, well washed with water and afterwards dissolved in water, into which we pour as many drops of chlorohydric acid as are necessary to accomplish the desired solution. To the gently heated clear liqour we add next a solution of permanganate of potassa (Chameleon mineral) of previously determined strength, until an additional quantity is no longer discolored.[†]

From the quantity of oxalic acid thus ascertained, we calculate the corresponding amount of lime, from that quantity of lime we can learn by calculation with how much phosphoric acid this earth is united in precipitate A. (ante) and subtracting this portion of phosphoric acid from the total amount of the same acid separately determined in precipitate A. the difference obtained will be the proportionate amount of phosphoric combined with magnesia, and this (phosphoric acid) again enables us to calculate the corresponding quantity of magnesia, without having isolated it chemically at all.

Example. Portions A and B of urine measuring each 50 cc. are treated with ammonia, and thus the earthy[‡] phosphates secured.

Suppose it required to throw down all the phosphoric acid in part A. 15 cc. of our graduated iron solution (1 cc. = 10 mgrm. see §13,) these correspond to 0.150 grm. i. e. 150 mgrm. of phosphoric acid. The oxalate of lime dissolved in chlorohydric acid, and obtained of the second precipitate, B had discolored 14 cc. permanganate of potassa solution (whereof 1 cc. indicates 10 mgrm. of oxalic acid) then this salt contained 0.140 grm. oxalic acid.

1 equiv. Oxalic acid = 36 corresponds to 1 equiv. of lime = 28 then from the equation.

$$36 \quad : \quad 28 \quad :: \quad 0.140 \quad : \quad x = \frac{28 \times 0.140}{36} = 0{,}1088$$

it follows that 0.140 grm. of oxalic acid are proportionate to 0.1088 grm. of lime.

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[*] This may be done on the filter by water acidulated with a few drops of chlorohydric acid; or the filter with precipitate is removed carefully from the funnel into a suitable glass vessel and treated with acid water; the paper filter is removed with a glass rod, repeatedly washed, and the wash-water added to the main solution

[†] It will be remembered that a solution of this salt is of an intense purple color, and the slightest excess may be recognized by imparting a rosy tint which is more readily seen by placing the glass vessel with the fluid upon white paper.

[‡] That portion combined with alkalies is of course not effected but remains in solution.

3 equiv. of lime $= 3 \times 28 = 84$ parts require 1 equiv. of phosphoric acid $= 71{,}36$ parts; 0.1088 grm. of lime in precipitate A, were united to 0,0924 grm. of phosphoric acid, for we have

$$84 \ : \ 71{,}46 \ :: \ 0.1088 \ : \ x \ : \ x = 0.0924$$

The total amount of phosphoric acid found in portion, A was 0.150 grm. from which subtracting that proportion of phosphoric acid 0.0924 grm. united to lime; the difference $= 0.0576$ grm. shows the amount of phosphoric acid combined with magnesia.

$$\begin{array}{r} 0.1500 \\ 0.0924 \\ \hline 0.0576 \end{array}$$

2 equiv. of magnesia $= 40$ parts require 1 equiv. of phosphoric acid, $= 71.36$ parts; thence 0.0576 grm. of phosphoric acid, must be combined with 0.0323 grm. of magnesia, for

$$71.36 \ : \ 40 \ :: \ 0.0576 \ : \ x \ ; \ x = 0{,}0323$$

In 50 cc. of urine, we found 0.1088 grm. of lime, and 0.0323 grm. of magnesia whence 100 cc. contain

Lime, $= 0.2176$ grm.
Magnesia, $= 0.0646$ "
Phosphoric acid, $= 0.3000$ "

Total $= 0.5822$ grm. earthy phosphates.

§ 16. The quantitative determination of iron, potassa and ammonia in urine might only in rare cases be of any practical interest, and we refer therefore to larger works, such as Neubauer & Vogel's "Analyse des Harne's" etc., etc.

§ 17. Approximative analysis of urine.

In conclusion it may be proper to say a few words on the approximative valuation of certain urinary constituents which—though the recently introduced volumetric determinations leave little to be desired in most cases—is still highly useful at the bedside, where the mere aspect of urine suffices often times to judge of the quantity of certain important ingredients.

Thus, 1, the density of urine, collected during 24 hours if possible, will indicate the amount of water and solid matters discharged in 24 hours; and a still denser urine permits of suspecting abumen, sugar.

2. The coloring of urine ought not to be neglected, for when in rapport with the density it shows a large amount of solids and most frequently fevers.

3. If the urine is dense but pale, we may look for albumen sugar.

4. If of small density and pale, we might suspect anemia; nervous condition etc.

5. An examination of urine, whether it be acid, neutral or alkaline, enables us to draw important conclusions.

6. We consider the transparency of urine, a turbid urine when at the same time acid would indicate the presence of mucus, pus, blood, fat and uric acid, if alkaline exhibit phosphates, carbonates.

7. Treatment of the urine with nitric acid may [in luxurious diet] throw down at once the nitrate of urea, and further indicate albumen, also an excess of uric acid, the kind of coloring matter [bile.]

8. Heating of urine [by itself if acid and acidulated previously if reacting alkaline] exhibits albumen; it causes the solution of deposited urates; it promotes the separation of phosphates and carbonates in alkaline urine. 9. The use of the microscope,* on the bedside, allows us to recognize at once different sorts, ingredients such as mucus, pus, and blood globules, yeast cells [indicating sugar] etc. and further the nature of certain sediments.

The quantity of many of the mentioned components of abnormal urine may upon proper ocular inspection merely, be nearly correctly guessed at.

As a standard example and scheme how to proceed in other cases we annex the approximative determination of earthy phosphates and of oxalate of lime in urine, by Dr. Benecke.†

The earthy phosphates in urine are kept in solution by free acid, and separate as soon as the urine turns alkaline. Therefore by saturating the free acid by any alkali, all the earthy phosphates are at once precipitated if present in the urine, and from the mere turbidity [cloudiness] produced, or the amount of precipitate which falls, we may draw approximative conclusions as to quantity.

Benecke makes use of glass tubes of the same diameter, each holding up to a certain mark, from 17—20 cc. of urine.

For the quantitative valuation Benecke distinguishes 3 degrees of opacity and precipitation, and having once and for all accurately determined the quantity of phosphates proportionate to each degree, this method yields comparatively very good results.

Benecke marks.

1st. With O urine, which, when boiled together with 5,10—15 drops of caustic soda solution, [1 pt. soda and 12 pts. water] in the test glasses, remains clear.

2nd. With ½ urine, which, treated in the same manner, shows a slight opalescense.

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* Of the effects of reagents upon animal structures, when viewed under the microscope, consult L.Beal's application of the microscope to clinical medicine, pg. 54—258.

† Zur Physiologie and Pathologie des phosphorsauren and oxalsauren Kalks, Gottingen 1850.

3d. With 1 urine exhibiting strong opalescense, in such a degree that objects behind the tube, for instance the bars of a window may yet be recognized.

4. With 1½ urine, which with soda solution becomes so cloudy though yet somewhat opalescent, that objects behind the glass tube are nearly invisible.

5. With 2 urine, which becomes strongly turbid and exhibits no opalescence.

6. With 2½ urine, which in a few seconds after the soda addition, forms a considerable precipitate.

7. With 3 urine yielding immediately a large precipitate.

8. With 3—4 urine with a still more abundant, and the largest observed precipitate of phosphates. It is obvious, that upon frequently repeated observations of that kind, we may readily rank a new urine into the proper scale, in varying cases not suitable to those here described, we mark the specimen with ¼, ¾, 1¼, 1½, ect. as the case may be.

In alkaline urine already sedimentary with earthy phosphates, we divide the latter uniformly by agitation, boil a portion of this urine, and add according to its alkalinity little or no soda solution. Should the urine contain albumen, we coagulate it by boiling, filter off and test the filtrate for phosphates as directed.

For the 8 numbered scale mentioned, Benecke determined the real quantitative value by accurate analysis, and found the following numbers for one ounce of urine.

1	Urine	marked	O	contains	0,100—0,150	grains	earthy	phosphates.
2	“	“	½	“	0,250—0,300	“	“	“
3	“	“	1	“	0,400—0,450	“	“	“
4	“	“	1½	“	0,550—0,600	“	“	“
5	“	“	2	“	0,700—0,750	“	“	“
6	“	“	2½	“	0,850—0,900	“	“	“
7	“	“	3	“	1,000—1,050	“	“	“
8	“	“	3-4	“	1,000—1,300	“	“	“

From these data we readily calculate the approximative amount of phosphates of the earth discharged within 24 hours by the urine.

Estimation of Oxalate of lime.

To examine urine for its contents of Oxalate of lime, Benecke proceeds thus:

A portion of the urine is poured into a test glass where it remains at rest 24 hours. If a sediment is produced we decant the clear liquid, and examine some of the last drops microscopically, a process not to be neglected even when no distinct deposit can be

Should the sediments include urates, we heat a drop upon an object glass whence the latter are dissolved; phosphate of lime is removed by a drop of acetic acid* whilst the oxalate of lime, if present, remains behind.

Operating thus with a single drop of the sediment, covered with a thin glass plate, we are enabled to draw conclusions as to the approximate amount of oxalate in the urine.

For the better comparison, Benecke adopted the following scheme.

1	Urine marked with	O,	is destitute	of Oxalate of lime.
2	" " "	½	contains traces,	" " "
3	" " "	1	" little,	" " "
4	" " "	1½	" a moderate amount	" "
5	" " "	2	" a good deal	" "
6	" " "	2½	" much,	" "
7	" " "	3	" very much,	" "
8	" " "	3–4	" extreme quantities,	" "

It is self-evident, that the physician may choose similar ways for the approximate determination of albumen, uric and sulphuric acids, etc.

TABLE I.

Indicating the average composition of urine in man and woman, and the means deduced from the addition of both by Becquerel and taken from L'analyse de l'urine par E. Hottot, Paris, 1856.

	Man.		Woman.		Means.	
	Urine of 24 hours.	Compos. of 1000 pts.	Urine of 24 hours.	Compos. of 1000 pts.	Urine of 24 hours.	Compos. of 1000 pts
Chemical Constituents of the Urine.						
Quantity of Urine	1267,3	1000.	1371,7	1000.	1319,8	1000
Specific gravity	1018,900		1015,120		1017 010	
Water	1227,779	968,815	1337,489	975,052	1282,634	971,935
Total amount of solid matter by direct evaporation	39,521	31,185	34,211	24,948	36,866	28,066
Urea	17.537	13,838	15,582	10,366	16,555	12,102
Uric Acid	0,495	0,391	0,557	0,406	0,525	0,398
Fixed salts not decomposable at a red heat.						
Chlorides of Soda	9,751	7,695	8,426	6,143	*9,089	**6,919
of Potassa						
Sulphates of Lime						
Phosphates of Magnesia						
Organic matter not isolated.						
Lactic Acid	11,738	9,261	9,65	8,033	10,696	8.647
Lactate of Ammonia						
Coloring Matter						
Extractive Matter						

Composition of the fized salts secreted in 24 hours, and also in 1000 parts of urine.

*Urine of 24 hours.		**1000 pts. of Urine contain.	
Total sum	9.089	Total sum	6.919
Chlorine	0.659	Chlorine	0.502
Sulphuric Acid	1.123	Sulphuric Acid	0.855
Phosphoric Acid	0.417	Phosphoric Acid	0.317
Potassa	1.708	Potassa	1.300
Alkaline and Earthy Bases.		Alkaline and Earthy Bases.	
Soda	5.181	Soda	3.944
Lime		Lime	
Magnesia.		Magnesia.	

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* Carbonate of lime will be dissolved with effervescence. This salt as also carbonate of soda and potassa, are met with in normal urine, during early infancy—Robin & verdeil.

NOTICE.

The apparatus and chemical reagents, mentioned in our treatise on the analysis of urine,* both qualitative and quantitative, may be obtained at fixed prices of J. F. Luhme & Co., 556 Broadway, New York. We give below a complete list of the graduated solutions, etc., employed in the volumetric analysis of urine.

	Price per litre, or two pounds.
Solution of Pernitrate of mercury, 1 cub. cent.=10 mgrm. of chloride of sodium,	$0 75
" " " " 1 cc.=10 mgrm. urea	1 00
" " nitrate of silver, 1 cc.=10 mgrm. chloride of sodium,	2 00
" " chloride of barium, 1 cc.=10 mgrm. sulphuric acid,	0 50
" " " " 1 cc.=1 mgrm. " "	0 40
" " sodium, 1 cc.=1 mgrm silver,	0 40
" " phosphate of soda, 1cc.=10 mgrm. phosphoric acid,	0 50
" " oxalic acid, 1 cc.=10 mgrm. oxalic acid,	0 50
" " hydrate of soda. 1cc.=10 " " "	0 50
" " permanganate of potassa 1cc.=10 mgrm. oxalic acid,	1 00
" " " " " 1cc.=2.800 grm.=1 eq. of iron,	1 50
" " ferrocyanide of potassium for do.	0 60
" " sesquichloride of iron. 1 cc.=10 mgrm. phosph. acid,	0 75
" " hydrate of soda for the ammonia test,	0 50
" " " " " the determination of lime,	0 40
Alkaline solution of sulphate of copper, 10 mgrm.=5 mgrm. sugar,	1 00
Mixture of acetate of soda and acetic acid,	0 75
Sulphuric acid for the ammonia test,	0 30
Mixture of 1 vol. of saturated solution of nitrate of baryta and 2 vol. of caustic baryta,	1 00
Hydrochloric acid for the determination of lime,	0 40
Urea pure, cryst.	3 00

Messrs. Luhme & Co. send descriptive catalogues of chemical apparatus and lists of chemical preparations and reagents, upon application, gratuitously.

Some of the above mentioned reagents serve merely for determining the relative strength of the real test solutions. For their mode of preparation we refer such practitioners as have time and means of manipulation at their disposal, to "Manual of Zoochemical Analysis," by Gorup-Besanez, translated by J W. Slater; "Analyse des Harnes," by Neubauer & Vogel; ' L'analyse de L'urine," par Ernest Hottot; safe guides, which we have freely made use of throughout our little treatise. It cannot be denied that medical practitioners, in making physiological and pathological chemistry, aided by the microscope, more and more an essential branch of their study, will be vastly benefitted and guided in forming a correct diagnosis of disease and in clearing up its mysteries. And should we have succeeded in at least arresting the attention of practitioners, or awakening some interest in the perusal of an important subject, greatly neglected as yet, we shall feel that we have not labored quite in vain.

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* For sale at Messrs. W. T. Berry & Co., Nashville, Tenn., who will forward each part separate at 30 cents, or both for $1; mailed free of charge.

TABLE II.

For converting French Decimal Measures and Weights into English Measures and Weights.

A. Measures of Length.

	1	2	3	4	5	6	7	8	9
Metre.									
English Yards........	1.09363	2 18727	3.28090	4.47453	5.36816	6.56180	7.65543	8.74906	9.84270
" Feet.........	3.28090	6 56180	9 84270	13.12360	16.40450	19.68539	22.96629	26.24719	29.52809
" Inches.......	39.37080	78 74158	118.11236	157.48315	176.85394	236.22473	275.59552	314.96630	354.33709
Decimetre									
Feet.......................	0.32809	0.65618	0.98427	1.31236	1.64045	1.96854	2.29663	2.62472	2.95281
Inches	3.93708	7.87416	11,81124	15.74832	19.68539	23.62247	27.55955	31.49663	35.43371
Centimetre									
Inches	0.39371	0.78742	1.18112	1.57483	1.96845	2.36225	2.75596	3.14966	3 54337
Millimetre.									
Inches.........	0.03937	0.07874	0.11811	0.15748	0.19685	0.23623	0.27560	0.31497	0.35434

B. Measures of Capacity.

		2	3	4	5	6	7	8	9
Cubic Centimetre									
Cubic Inch............	0.06103	0.12205	0.18308	0.24411	0.30514	0.36616	0.42719	0.48822	0.54924
Litre.									
English Imp. Gal's..	0.22017	0.44033	0 66050	0.88066	1.10083	1.32100	1.54116	1 76133	1.98149
" " Quarts.	0.88066	1 76133	2.64199	3.52266	4.40332	5.28398	6.16465	7 04531	7.92598
" " Pints..	1 76133	2.52266	5.28399	7.04231	8.80664	10.56797	12.32930	14.09062	15.85195
Litre.									
Wine Gallon, (231 c i.)	0.26459	0.52918	0.79378	1.05837	1.32296	1.58755	1.85213	2.11673	2 38132
Stere.									
Gallons.......	220,16643	440.33287	660.40930	880.66574	1100.83217	1320.99860	1541.16504	1761.33147	1981.49791

TABLE II—*Continued.*

C. WEIGHTS.

	1	2	3	4	5	6	7	8	9
KILOGRAMME.									
Cwt	0.01970	0.03939	0.05909	0.07879	0.09348	0.11818	0.13788	0.15758	0.17727
lb. (avoirdupois.)....	2.20486	4.40971	6.61457	8.81943	11.02428	13.22914	15.43400	17.63886	19.84371
KILOGRAMME.									
pound (troy)..........	2.67951	5.35903	8.03854	10.71805	13.39757	16.07708	18.75659	21.43610	24.11562
GRAMME									
Grains.....................	15.44242	30.88184	46.32726	61.76968	77.21210	92.65352	108.09694	123.53936	138.98178
DECIGRAMME.									
Grains	1.54424	3.08848	4.63273	6.17697	7.72121	9.26535	10.80969	12.35394	13.89818
CENTIGRAMME.									
Grains	0.15442	0.30885	0.46327	0.61770	0.77212	0.92654	1.08097	1.23539	1.38982
MILLIGRAMME.									
Grains......................	0.01544	0.03089	0.04633	0.06177	0.07721	0.09265	0.10810	0.12354	0.13898

These tables are arranged on the same plan as the Analytical Tables in Rose's "*Analytical Chemistry*," and other works of similar character. One example may suffice to illustrate the mode of using them. Let it be required to find how many grains are equal to 87.435 grammes:—

By Column 8 line 4 of Table C, we find: 80 grammmes = 1235,3936 grains.
" 7 " 4 " " " 7 " = 108.0969 "
" 4 " 5 " " " ·4 " = 6.1770 "
" 3 " 6 " " " .03 " = 0.4633 "
" 5 " 7 " " " .005 " = 0.0772 "

87.435 grammes = 135.2080

ERRATA.

On page 634, second line from bottom, read, $1000 \times 1.025 = 1025$.

On page 636, first paragraph from top, ° marks omitted.

On page 637, in table, 13th number from top, read 1013.

On page 641, in table, sec. 3. for 2. 4. 1, read 4, 2, 1.