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Simplified Colorimetric Determination of Blood Urea Clearance.

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The urea contents of urine and blood are compared in a colorimeter in such a manner that a single reading gives directly the percentage of average normal renal function in terms of the blood urea clearance.[1] The urine is first diluted to such an extent that if the clearance, either standard or maximum, is the average for a normal subject, the urea concentrations in blood and diluted urine will be equal. The urea in both blood and urine is converted into ammonia with urease; proteins and other interfering substances are removed, and the ammonia contents of the 2 filtrates are compared colorimetrically.

No standard solutions are required, because the blood is compared directly with the urine.

The number of times the urine must be diluted for comparison with the blood is found by reference to the curve of Fig. 1, computed as follows: The average *maximum* clearance (for urine volumes over 2 cc. per minute) is 75. The maximum clearance is calculated as $C_m = \frac{U}{B}V$ where V is reckoned in cc. of urine per minute, B is blood urea concentration, and U is urine urea concentration. When C_m has the average normal value of 75, the ratio U/B is calculated therefore as $U/B = 75/V$. When V is expressed in cc. of urine per hour, the calculation changes to $U/B = 4500/V$. The ratio U/B is the number of times a portion of the urine must be diluted to bring its urea concentration down to that of the blood, when the clearance is average normal. The U/B values thus calculated are expressed by the higher part of the curve of Fig. 1.

[1] For a discussion of the significance of the blood urea clearance, see Møller, McIntosh and Van Slyke, *J. Clin. Inv.*, 1928, **6**, 427; or Peters and Van Slyke, *Quant. Clin. Chem.*, 1931, **1**, 345.

If the urine volume is below 2 cc. per minute, the formula which holds is that of the standard clearance, $C_s = \frac{U\sqrt{V}}{B}$, and the average normal value of C_s is 54. The number of times dilution required for urines excreted at rates less than 2 cc. per minute is therefore calculated as $U/B = 54/\sqrt{V}$ when V is expressed in cc. of urine per minute, or as $U/B = 54 \times \sqrt{60}/\sqrt{V} = 418\sqrt{V}$, when V *is* expressed in cc. of urine per hour The dilution ratios thus calculated are indicated by the lower part of the curve of Fig. 1. Plotting the curve logarithmically gives it a rectilinear form, with a break at the augmentation limit where the C_s formula is replaced by the C_m formula.

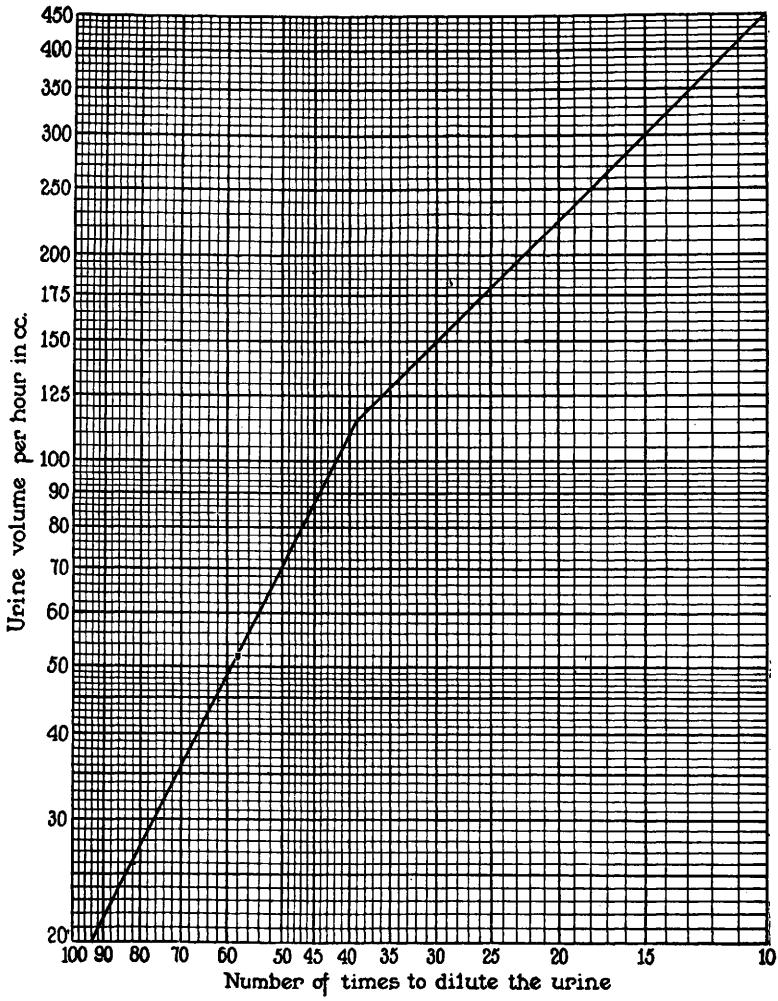

FIG. 1.

Reagents.

Permutit;[2] *Urease.* 10% solution of Squibb's urease;[3] *phosphate buffer*, 5 gm. of KH_2PO_4, and either 5 gm. of $Na_2HPO_4.12H_2O$ or 2 gm. of anhydrous Na_2HPO_4, per 100 cc. *Somogyi's acid zinc sulfate solution* for precipitating proteins.[4] 12.5 gm. of $ZnSO_4.7H_2O$ and 125 cc. of 0.25 *N* sulfuric acid diluted to 1 liter. 0.75 *N sodium hydroxide. Nessler's solution.*[5] *Sodium citrate,* pulverized.

Conditions for collecting blood and urine. Urine is collected during 2 successive periods of one hour each, and the volume in each hour is accurately measured. Blood is drawn at about the middle of the 2-hour period. The blood is collected with citrate, if possible, rather than oxalate as anticoagulant, because oxalate tends to produce turbidity in the blood filtrate when the latter is nesslerized.

Treatment of Urine.

Removal of preformed ammonia. Of the urine collected during each hour, place 10 cc. in a small Erlenmeyer flask with 2 gm. of permutit and rotate for 5 minutes to absorb ammonia. Filter through a dry filter paper.

Treatment with urease. Place in a 100 cc. cylinder a volume of permutit-treated urine, 1, 2, or more cc., such that when later diluted according to Fig. 1, the resultant volume will be between 50 and 100 cc. To the undiluted urine in the cylinder add 5 cc. of the phosphate buffer solution and 1 cc. of the 10% urease. Let stand 10 minutes, preferably not much longer, in order not to give ammonia opportunity to volatize.

Dilution and removal of protein and other interfering substances. After the urease has acted 10 minutes 3 cc. of the zinc sulfate solution are added. It reacts with the phosphate buffer to produce a precipitate which clears the urine. The mixture is then diluted up to the volume calculated from Fig. 1. After standing a few minutes, the solution is passed through a dry filter. A slight cloudiness may be present in the filtrate, but the turbidity disappears when the filtrate is nesslerized. A portion of the filtrate, 2 or 5 cc., is pipetted into a volumetric flask and diluted 10 times, in order to parallel the 10-fold dilution which the blood undergoes.

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[2] Folin, O., and Bell, R. D., *J. Biol. Chem.*, 1917, **29**, 329.

[3] Prepared from jack beans by the method of Van Slyke and Cullen, *J. Biol. Chem.*, 1914, **19**, 211.

[4] Somogyi, M., *J. Biol. Chem.*, 1930, **86**, 655.

[5] Koch, F. C., and McMeekin, T. L., *J. Am. Chem. Soc.*, 1924, **46**, 2066.

If the subject is a child, or deviates greatly from usual adult size, a correction for body size is introduced by multiplying the observed volume of the hour's urine by the proper factor from Fig. 1 of McIntosh, Møller, and Van Slyke,[5] and using the urine volume thus multiplied as the ordinate for Fig. 1 of this paper. For example, if the urine volume per hour is 80 cc., Fig. 1 indicates that the urine should be diluted 47 times. This holds for any adult within usual size limits. If, however, the subject is a child of 1.40 meters height, the 80 cc. observed hour's volume is multiplied by the factor 1.49 from McIntosh, Møller, and Van Slyke.[6] The corrected volume, 119 cc., is then interpolated on the curve of Fig. 1, and indicates that the urine should be diluted only 37.5 times.

Treatment of Blood.

Digestion with urease. The volume of blood sample required depends upon the volume of solution necessary for a reading in the colorimeter used. If 5 cc. of solution suffice for the colorimeter, one cc. of blood will do, unless an unexpectedly low clearance value necessitates the use of a fresh portion of filtrate for supplementary dilution. In general, a 2 cc. sample is adequate. The sample is mixed with 0.1 its volume of the 10% solution of urease, and let stand 10 or 15 minutes.

Removal of blood proteins. For each cc. of blood sample, 7.9 cc. of Somogyi's acid zinc sulfate solution are added, then 1 cc. of 0.75 *N* sodium hydroxide. After standing for some minutes the fluid is passed through a dry filter.

Nesslerization and Comparison of Blood and Urine Filtrates.

Equal volumes of the blood filtrate, 5, 10, or 20 cc., as required by the colorimeter, and of the 10-fold diluted urine filtrate are pipetted into dry vessels, and a trace of sodium citrate powder is added from the point of a knife to each and allowed to dissolve. The citrate retards development of turbidity after nesslerization To each measured portion of filtrate one-tenth its volume of Nessler's solution is added, and the solutions are at once compared in a colorimeter. The nesslerization may be done in the colorimeter cups if they are dried before the filtrates are measured in. The reading must be taken quickly after nesslerization, or turbidity may develop in the blood filtrate, making the blood urea content appear too high, and the clearance too low.

[5] McIntosh, J. F., Møller, E., and Van Slyke, D. D., *J. Clin. Invest.*, 1928, **6**, 467.

The use of the urease method for comparing contents of urea in the blood filtrate and diluted urine is not obligatory. Any other procedure which will give direct comparison with sufficient accuracy may be substituted.

Calculation. If the colors of the 2 filtrates are such that the higher scale reading does not exceed twice the lower, the clearance is calculated directly from the colorimeter scale readings. *% of average normal clearance* $= \frac{100\ (B)}{(U)}$. (B) represents the reading of the blood filtrate, and (U) the reading of the urine filtrate. (The readings are inversely proportional to the concentrations, hence the inversion of the usual concentration formula.)

If the clearance is less than half average normal, the blood filtrate when nesslerized will be more than twice as deep in color as the urine filtrate. In such a case the preliminary $(B)/(U)$ reading is made in the above manner. Then a fresh portion of the blood filtrate is diluted 2, 3, 5, or 10 times, as indicated by the preliminary reading in order to bring the $(B)/(U)$ ratio into the neighborhood of unity. A portion of the diluted blood filtrate is then nesslerized and compared with a fresh portion of the urine filtrate simultaneously nesslerized. The calculation then becomes: *% of average normal clearance* $= \frac{100\ (B)}{D\ (U)}$. D represents the number of times the blood filtrate has been diluted before the final nesslerization, in cases where a low clearance necessitates such dilution. If the subject is known to have a low clearance, the time required for the preliminary reading may be saved by diluting the blood filtrate to the extent probably necessary before the first reading is made.

TABLE I.

Subject No.	% of Average Normal Clearance		Deviation of Colorimetric from Gasometric in Percentage of Gasometric Clearance
	Colorimetric	Gasometric	
1	153.9	145.7	+5
2	141.0	131.8	+7
3	84.2	80.0	+5
4	56.9	59.3	—4
5	49.6	48.0	+3
6	46.2	49.2	—7
7	40.2	37.6	+6.5
8	40.1	44.7	—11
9	38.4	39.9	—4
10	38.1	35.8	+7
11	37.3	38.7	—4
12	36.5	33.7	+8
13	34.8	32.1	+8
14	32.1	35.1	—6
15	28.0	28.6	—2

Results. Urea clearances in normal subjects and nephritic patients have been determined by the above method, and compared with clearances calculated from urea determinations made on the same blood and urine by the exact gasometric urease method.[7] Table I gives a representative series of results. The first 3 determinations were on a hypertension patient with normal renal function; the other determinations were on nephritics with diminished renal function.

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Is Erythropoietic Action of Copper Dependent upon Presence of Adequate Supply of Iron in Diet?*

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In studying the effect of evaporated milk upon the development of nutritional anemia in the albino rat, it was observed that the erythrocytes were maintained at a normal level over a period of 8 weeks in spite of the fact that the hemoglobin fell slowly. This was in sharp contrast to the findings on rats which were fed raw milk, where both the erythrocyte count and the hemoglobin showed a marked decrease in 7½ weeks.

In the majority of evaporated milk plants the milk comes in close contact with copper during the manufacturing process. Since according to Rice and Miscall[1] copper is dissolved by milk under such conditions, an investigation has been undertaken to determine if this metal is the causative factor in the maintenance of the red cell count when evaporated milk is fed.

Albino rats, 21 days of age, were fed raw milk supplemented by 0.025 mg. of copper (as $CuSO_4$) daily over a period of 8 weeks. Erythrocyte counts and hemoglobin determinations were made at the beginning of the experiment and at intervals of 2 weeks thereafter. The experimental data obtained, with those from rats on raw milk alone and on evaporated milk without supplement, are given in Table I.

[7] Van Slyke, D. D., *J. Biol. Chem.*, 1927, **73**, 695.

* This work was aided by a grant from the Evaporated Milk Association.

[1] Rice, F. E., and Miscall, J., *J. Dairy Sci.*, 1923, **4**, 261.