

A simple spectrophotometric method for the determination of urea in blood and urine

T. K. WITH, TOVE DREYER PETERSEN, AND BIRGIT PETERSEN *From the Central Laboratory, Svendborg County Hospital, Denmark*

Several methods for determining urea in blood and urine are in use in clinical laboratories, but none is ideal in mass routine analysis. The spectrophotometric method based on a modified Ehrlich reagent proposed by Watt and Chrisp (1954) for pure solutions is therefore of interest because it can be modified for use in mass clinical analyses of blood and urine. Brown (1959) has modified the method for use on blood, but his procedure involves treatment with urease as well as spectrophotometric measurement and is hardly simple enough for routine clinical pathology laboratories. In this laboratory a modified Watt and Chrisp method has been used as a routine procedure for a year, and proved simple and reliable except in specimens from patients receiving sulphonamides or para-aminosalicylic acid, when it is unsatisfactory. Such specimens are easily recognized because of the atypical colour reaction (bright citrus colour) with the modified Ehrlich reagent. Other laboratories might therefore be interested in a detailed description of the technique.

REAGENTS

(1) SOMOGYI'S ZINC SULPHATE SOLUTION Crystalline zinc sulphate, 12.5 g., dissolved in 125 ml. 0.25 N sulphuric acid and diluted to 1,000 ml. with distilled water.

(2) 0.75 N SODIUM HYDROXIDE

(3) ALCOHOLIC EHRLICH REAGENT p-Dimethylaminobenzaldehyde (analytic), 20 g., dissolved into 1,000 ml. 96% alcohol. To this solution is added 100 ml. concentrated hydrochloric acid (analytic) and mixed.

(4) STANDARD SOLUTION Urea, 100 mg., is dissolved in distilled water, 100 ml., in a measuring flask.

PROCEDURE FOR BLOOD

Twenty-four millilitres of reagent (1) are run from a burette into a 100 ml. Erlenmeyer bottle. To this is added 3 ml. whole blood (stabilized by oxalate), the bottle is shaken to obtain complete haemolysis, and 3 ml. of reagent (2) added and the mixture shaken again. It is then filtered through a special filter (Schleicher and Schull No. 589³, Blauband) to remove the precipitated proteins. (It is important to use a suitable filter paper because the

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usual laboratory filters may give falsely increased values. Of the clear and colourless filtrate 10 ml. is pipetted into a 25 ml. measuring flask and 10 ml. reagent (3) added. After mixing dilute with distilled water to the mark and renew mixing. After 10 minutes the mixture is ready for reading. The colour is stable, according to Watt and Chrisp, for 11 days.

READING

The reactions are read in a spectrophotometer at 420 nm with a blank prepared in the same way as the reaction mixture but with 3 ml. of distilled water instead of 3 ml. of blood. The urea standard (reagent 4) is treated in the same way as the blood. Cuvettes of 1 cm. thickness are suitable.

If p, s, and b are the extinctions of the sample, the standard, and the blank respectively, the urea concentration is

$$100(p-b)/(s-b) \text{ mg./100 ml.}$$

A Hilger Uvispec photoelectric spectrophotometer was used in this laboratory, as attempts to use a Bausch and Lomb Spectronic were unsuccessful. This is in good agreement with the finding of Brown (1959) that a Coleman Junior spectrophotometer was not suitable for reading the Watt and Chrisp reaction.

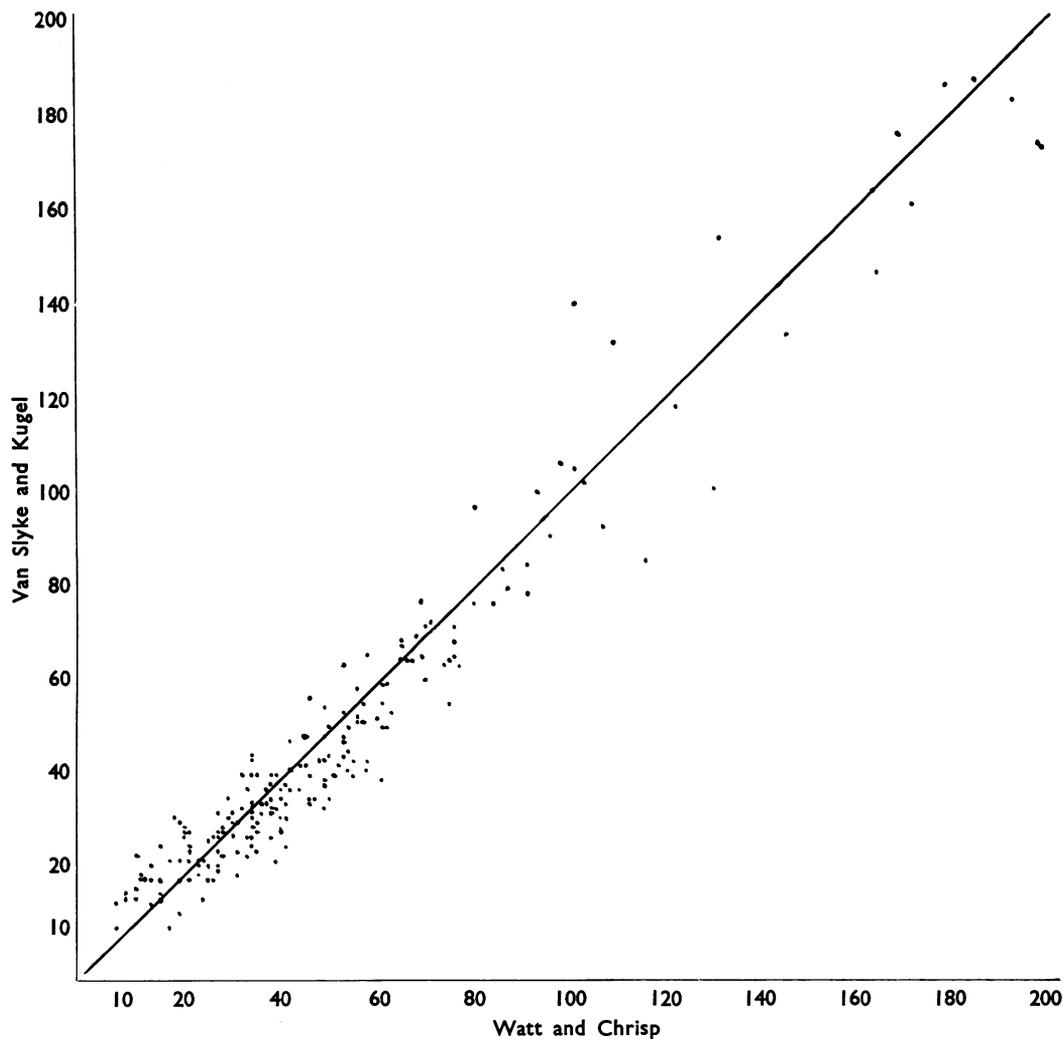
DETERMINATION IN URINE

The procedure is exactly the same as for blood, the difference being that if the concentration is above 1,000 mg./100 ml. the reaction has to be repeated on a diluted sample because Beer's law is not valid for concentrations above this limit. The urine filtrate is quite colourless with the technique described.

COMMENT

The blank extinction is high compared with that of the reaction mixture and may vary somewhat from day to day for the same Ehrlich reagent. For different batches of Ehrlich reagent the blank extinction may vary between 0.070 and 0.170. According to our experience Ehrlich reagents giving a blank extinction below 0.100 are unsuitable. The high blank value is a drawback, but the method is, in spite of this, sufficiently accurate for clinical purposes.

To test the clinical reliability of the method 200 duplicate analyses were performed, one with the modified Watt and Chrisp method and one with the hypobromite method of Van Slyke and Kugel (1933). In the diagram the analyses with the Watt and Chrisp method are plotted along the abscissa, those with the Van Slyke and Kugel method along the ordinate. It is easily seen that the points are scattered evenly on both sides of the identity line. All the analyses shown in this diagram were routine clinical analyses. The diagram shows that the deviations between the two methods are too small to be of any clinical significance. Serum creatinine determinations have been made on all samples taken for blood urea for a year, ranging between 10 and 20 samples every day, and there has in all



cases been reasonable agreement between the creatinine determination and the blood urea determined by Watt and Chrisp's method. All pathological values were controlled by double analysis with the Van Slyke and Kugel method and no clinically significant disagreement was encountered. The only exceptions were patients receiving sulphonamides or P.A.S.

REFERENCES

- Brown, H. H. (1959). *Analyt. Chem.*, 31, 1844.
 Van Slyke, D. D., and Kugel, V. H. (1933). *J. biol. Chem.*, 102, 489.
 Watt, G. W., and Chrisp, J. D. (1954). *Analyt. Chem.*, 26, 452.

ADDENDUM

Since this paper has been printed we have learned that W. Daubenmerkl (personal communication) has elabor-

ated a modification of our method. He read the extinction at 435 nm. instead of 420 nm. By reading at this wavelength instead of at the maximum (420 nm), the extinction of the blank value decreases to about 1/7 while the specific extinction (extinction of test solution minus extinction of blank) decreased by only about 40%. Thus it is possible to read the extinction with the test solution against the blank instead of reading both with water as blank, a modification which saves time. Also, this modification makes it possible to read the reaction in a simpler and cheaper type of instrument, e.g., the Bausch and Lomb Spectronic, whereas the reading with our original technique had to be performed in an instrument with a quartz prism and quartz cells. The accuracy of the method becomes much better at the lower range by reading at 435 nm. instead of 420 nm.

The considerable advantages of Daubenmerkl's modification is illustrated by the following table:

CONCENTRATION (MG./100 ML.)

Sample No.	Van Slyke	Watt and Chrisp Reading at 420 nm.	Watt and Chrisp Reading at 435 nm.	Sample No.	Uvispec Reading at 435 nm.	Spectronic Reading at 435 nm.
1	19	30	14	1	28	29
2	41	73	38	2	28	30
3	97	126	102	3	21	24
4	32	50	28	4	29	31
5	97	137	107	5	21	36
6	72	103	70	6	146	141
7	29	38	21	7	126	116
8				8	54	55
9	116	155	133	9	185	178
10	23	26	18	10	312	275
11	27	17	23			
12	48	47	47			
13	36	55	29			

With this valuable modification the method of Watt and Chrisp comes within the reach of laboratories with modest equipment and attains the same grade of accuracy as the other routine laboratory procedures for blood urea besides being much more suitable for routine clinical pathology than the other available methods.

It must, however, be emphasized that extreme care is necessary in preparing the reagents, that high quality distilled water is required, and that fresh reagent (4) has to be used every week.

A method for the collection of saliva

P. J. COUSINS¹ *From the Hospital for Sick Children, Great Ormond Street, London*

The collection of saliva from normal adults and children—unless they are very young—presents no difficulty, but where the patient is unable to use (or, as in one case, considered it rude to spit for any reason) a sputum-receiver the collection presents a problem.

During a study of saliva from children with phenylketonuria (Cousins, Philips, Stroud, Wise, and Woolf, 1960), a simple method of collection was adopted. A pasteur pipette with a bulb was cut short and a piece of polythene tubing of 2 mm. bore affixed. The end of the polythene tube was warmed and pushed on to the pipette tip, this having been similarly warmed; on cooling the joint was found to be very firm. The tubing was cut, so that its length was 6 cm., the cut being diagonal across the tube. This end was warmed until pliable and then squashed against a glass plate, thus forming a thickened and wide orifice approximately 4 mm. × 2 mm.

The patient was in one of two positions depending on the degree of cooperation:

(1) The lateral position of the head while lying flat; assistance in holding the head is necessary in cases of poor cooperation.

(2) Normal sitting position; assistance is necessary only in the case of the very young.

The pipette is introduced (a) to the cheek wall, and (b) under the tongue. Saliva can then be collected by normal pipetteage. Amounts of the order of 2 to 3 ml can be collected in a comparatively short time and transferred to a stoppered test-tube (Exelo 10 cm. × 1 cm most useful).

The best times for collection are just before a feed or at least two hours afterwards, there being no contamination by food residues at these times.

The advantages of such a pipette are:

(1) Should the patient bite down on the pipette no harm is done, the portion in the mouth being polythene.

(2) No trauma occurs using polythene.

(3) Stimulation of salivary glands is easily effected.

(4) Polythene is easily cleaned and the pipette is easily and quickly cleaned in water.

(5) Such a pipette has a longer life than an ordinary bulb pasteur pipette.

REFERENCE

- Cousins, P. J., Phillips, S. J., Stroud, C. E., Wise, J., and Woolf, C. L. (1960). *Amino Acids of Sweat and Saliva in Phenylketonuria*. In preparation.

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¹Present address: Royal National Orthopaedic Hospital, Brockley Hill, Stanmore, Middlesex.