CCLI. THE MICRO-DETERMINATION OF UREA IN BLOOD AND OTHER FLUIDS

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(Received 24 September 1937)

A KNOWLEDGE of the level of urea in the blood is of considerable practical importance in various diseases and numerous methods have been suggested for its estimation [Van Slyke & Cullen, 1916; Folin & Wu, 1919; Mirkin, 1922; Taylor & Blair, 1932; Kay & Sheehan, 1934; Peskett, 1934; Taylor & Adams, 1935]. Within recent years micro-methods, which can be applied to 0.2 ml. of blood, have been devised. Several of these depend upon the hydrolysis of the urea with urease and the subsequent estimation of the ammonia [Archer & Robb, 1925; Conway, 1933; Barrett, 1935], or of the carbon dioxide [Peters & Van Slyke, 1932]. Other methods are based upon the fact that urea forms, with xanthydrol, a water-insoluble compound, dixanthylurea [Beattie, 1928; Yoshimatsu, 1929; Allen & Luck, 1929; Cuny & Robert, 1932]. This compound dissolves in sulphuric acid to give a yellow solution. Under standard conditions the precipitation is quantitative, and the colour produced with sulphuric acid is proportional to the amount of dixanthylurea present. This colour may be matched directly against that produced from a standard urea solution treated in the same way [Beattie, 1928], or Folin's phenol reagent may be added to the sulphuric acid solution and the blue solution obtained determined colorimetrically [Yoshimatsu, 1929]. Allen & Luck [1929] and Cuny & Robert [1932] recommend the oxidation of the dixanthylurea precipitate with potassium dichromate and titration of excess dichromate with thiosulphate.

A MODIFICATION OF THE XANTHYDROL METHOD

During the course of an investigation on human salt deficiency, which was being carried out in this laboratory [McCance, 1936], accurate estimations of the urea in blood and also in urine were required. Since xanthydrol is said to be a specific precipitant for urea, it was decided to make use of this reaction for the purpose. A technique was finally devised, which is a modification of Beattie's [1928] method, and which has been found satisfactory for all purposes. Blood ureas varying from 10 to 500 mg. per 100 ml. may be determined accurately without changing the conditions.

Reagents.

10% sodium tungstate.

2/3 N sulphuric acid.

Glacial acetic acid.

5% solution of xanthydrol in methyl alcohol. This solution should be filtered to remove any insoluble material and should be kept in a dark bottle.

Methyl alcohol saturated with dixanthylurea. (Solution A, *v. infra*.)

Methyl alcohol-water mixture (3 : 1) saturated with dixanthylurea. (Solution B, *v. infra*.)

50% sulphuric acid (by volume).

Standard urea solution, 6 mg. per 100 ml. This is prepared from a stock standard solution of 0.6 % urea. Both urea solutions should be kept under toluene.

Preparation of solutions A and B. In each of four 50 ml. centrifuge tubes are placed 5 ml. of a solution of urea containing about 12 mg. of urea per 100 ml., 5 ml. of glacial acetic acid are added and 1 ml. of a 5 % solution of xanthydrol in methyl alcohol. The mixture is stirred and allowed to stand for 30 min. 20 ml. of methyl alcohol are then added and, after stirring, the tube is centrifuged and the supernatant liquid poured off. The precipitate is washed with 20 ml. of methyl alcohol, which is again removed by decantation after centrifuging. The precipitate obtained should be sufficient to saturate 1000 ml. of both solutions. It should be allowed to stand in contact with the methyl alcohol or methyl alcohol-water mixture overnight and then filtered.

Method. Duplicate samples of 0.2 ml. of blood are diluted with 1.4 ml. of water. 0.2 ml. of sodium tungstate and 0.2 ml. of 2/3 *N* sulphuric acid are added to precipitate the proteins, which are then removed by centrifuging. 1 ml. of the supernatant 1 in 10 blood filtrate is placed in a 15 ml. conical centrifuge tube together with 1 ml. of glacial acetic acid and the mixture stirred with a thin glass rod. Then 0.2 ml. of a 5 % solution of xanthydrol in methyl alcohol is added directly into the solution (not down the side of the tube), the mixture stirred and left to stand for about 10 min. A white precipitate forms slowly, which flocculates on further stirring. The tubes are left to stand overnight in the ice-chest to complete the precipitation. 4 ml. of solution A are added, the mixture stirred, centrifuged and the supernatant liquid poured off. The precipitate is washed by stirring with 4 ml. of solution B, the tube again centrifuged and the supernatant liquid poured off. It is essential that solutions A and B should be run in round the sides of the tube so that these are washed free from any xanthydrol that may inadvertently have been dropped there. Xanthydrol itself gives a yellow colour with sulphuric acid.

The precipitate is dissolved in a measured volume (2–10 ml.) of 50 % sulphuric acid, so that the colour produced corresponds closely with that obtained by treating 1 ml. of the standard urea solution (6 mg. per 100 ml.) with glacial acetic acid and xanthydrol exactly as described above and dissolving the washed precipitate in 8 ml. of 50 % sulphuric acid. For high blood ureas the unknown solutions need further dilution with 50 % sulphuric acid. The unknown is compared with the standard in a colorimeter.

Calculation.

$$\frac{\text{Standard reading}}{\text{Unknown reading}} \times 60 \times \frac{\text{Volume of acid added (ml.)}}{8} = \text{mg. urea per 100 ml. blood.}$$

Discussion of the xanthydrol method

Reason for the addition of methyl alcohol to the mixture before centrifuging. The precipitate of dioxanthylurea is very light and tends to float on the top of the liquid. It was found impossible to centrifuge it down to the bottom of the tube unless the specific gravity of the liquid was first reduced by the addition of methyl alcohol. Other workers with this method do not appear to have encountered this difficulty [Allen & Luck, 1929; Yoshimatsu, 1929].

Reason for the addition of water to solution B. It was found by experience that the addition of water to the methyl alcohol (solution B) greatly facilitated the centrifuging of the washed precipitate. The addition did not interfere in any way with the accuracy of the method (*vide infra*) and was therefore adopted.

Solubility of the precipitate in methyl alcohol. Dixanthylurea is slightly soluble in methyl alcohol, and this constitutes an important potential source of error. The solubility of the precipitate in methyl alcohol was demonstrated on standard solutions of urea containing 1–12 mg. per 100 ml. Precipitation was carried out as described above, but 4 ml. of methyl alcohol were added before the first centrifuging, and 4 ml. of methyl alcohol were used to wash the precipitate. In some cases the precipitate was washed once, in others twice and in others three times with 4 ml. of methyl alcohol on each occasion. The precipitate was dissolved in 50 % sulphuric acid, and the colour compared against that given by a standard urea solution (6 mg. per 100 ml.), treated as described on p. 2036. The results are shown in Fig. 1. It will be observed that for solutions containing

Fig. 1. Solubility of dixanthylurea in methyl alcohol.

6 mg. of urea per 100 ml. (corresponding to a blood urea of 60 mg. per 100 ml.) and above, 100 % recoveries were obtained when the precipitate was washed once with methyl alcohol. For all weaker solutions the recoveries were less, and with solutions containing 1 mg. per 100 ml. (corresponding to a blood urea of 10 mg. per 100 ml.) not more than 10 % could be recovered. Each washing caused a further loss of precipitate, indicating that significant amounts of the dixanthylurea were dissolving in the methyl alcohol.

In the method described on p. 2036 errors due to the solubility of the precipitate in methyl alcohol are avoided by the use of solutions A and B saturated with dixanthylurea.

Completeness of precipitation. Effects of temperature and time of standing. Standard urea solutions of different concentrations (1–12 mg. per 100 ml.) were estimated after allowing precipitation to continue for varying lengths of time, both at room temperature and in the ice-chest (5°), the special solutions A and B

being used throughout. All were matched against a standard urea solution (6 mg. per 100 ml.) as before. The results, which are the average of duplicate determinations, are shown in Table I. It is clear that for solutions containing 5 mg. of urea per 100 ml. and above (corresponding to blood ureas of 50 mg. per 100 ml. and above) 2 hr. at room temperature will ensure complete precipitation. The % recovery at room temperature for low concentrations depended very much on the actual temperature. For solutions containing 3 mg. of urea per 100 ml. complete precipitation was obtained after 2 hr. in the ice-chest. For solutions containing 2 mg. per 100 ml. it was necessary to allow precipitation to continue overnight in the refrigerator, while for solutions containing 1 mg. per 100 ml. 20 hr. or more were required.

Table I. *Effects of temperature and time of standing on the precipitation of urea by xanthydrol*

Urea solution* used mg. per 100 ml.	% recovered			
	Room temp. 2 hr.	Refrigerator 2 hr.	Refrigerator overnight	Refrigerator 20 hr.
1	7.5	57.4	80.4	102.0
2	63.8	90.0	103.8	99.5
3	95.3	100.5	103.5	102.0
4	96.9	—	101.1	—
4.8	99.1	—	99.9	—
6	99.6	99.5	100.0	101.2
9	101.2	—	100.0	—
12	103.5	—	101.3	—

* These concentrations correspond to blood urea levels ten times as great.

Table II. *Accuracy of the xanthydrol method at high concentrations*

Urea solution* used mg. per 100 ml.	% recovered
6	100.0
12	100.5
24	100.5
48	99.4
54	98.2

* See note, Table I.

Table III. *Effect of other blood constituents on the estimation of urea by xanthydrol*

Concentration of* urea in mixture mg. per 100 ml.	% recovered
2	106.3
3	99.2
4.5	100.0
6	99.5
9	101.4
12	100.6

* See note, Table I.

Since it is not often that blood ureas fall much below 20 mg. per 100 ml., precipitation overnight in the ice-chest has been adopted as the routine method. If accurate results are required at levels below this, it is necessary to increase the time allowed for precipitation. Prolonged standing does not appear to affect significantly the results at higher levels.

Range of the method. It has already been shown that urea solutions corresponding to blood ureas varying from 10 to 120 mg. per 100 ml. can be estimated satisfactorily by this method. Table II shows that stronger urea solutions, corresponding to blood urea levels up to 500 mg. per 100 ml. may also be determined with equal accuracy. No adjustment in the method was found to be necessary at these high concentrations, and one standard only is required. After dissolving the larger precipitates in 8 or 10 ml. of 50 % sulphuric acid, a known volume of this solution must be further diluted with sulphuric acid so that the final colour corresponds approximately with that of the standard solution.

Similar results were obtained with blood. A sample of blood containing more than 500 mg. urea per 100 ml. was estimated as described on p. 2036. A second sample was diluted 1-4 before estimation. Exactly the same results were obtained with or without preliminary dilution of the blood.

Permanence of standards. It was found that the solution of dioxanthylurea in 50 % H_2SO_4 would keep indefinitely without deterioration, provided that precautions were taken to avoid contamination with any substance which would be charred by the sulphuric acid, causing a brown coloration in the solution. If a cork or stopper is placed in the mouth of the tube it is difficult to be certain that none of the acid ever comes in contact with the stopper, especially if the contents of the tube are frequently poured into the colorimeter cup and back into the tube again.

In practice therefore it was found advisable to put up a fresh standard with each batch of determinations, but one or two old standards were generally kept as a precaution and check in case of emergency.

Effect of other blood constituents. Samples of blood, diluted with water, were incubated with urease to remove the urea. The proteins were then precipitated with sodium tungstate and sulphuric acid and were removed by centrifuging. The dilution of the blood at this stage was 1 in 5 instead of the usual 1 in 10. Known volumes of these urea-free blood filtrates were mixed in duplicate with equal volumes of standard urea solutions, so that the final dilution of the blood was the same as that normally employed (1 in 10). 1 ml. samples of the mixture were then estimated as already described. The results, shown in Table III, indicate that the addition of blood filtrate does not interfere in any way with the accuracy of the method. Hence, it may be concluded that blood constituents other than urea have no effect on the xanthidrol method.

The urea added to these blood filtrates and the urea in standard urea solutions could be estimated to within $\pm 3\%$. The error lies almost entirely in the colorimeter readings.

Application of the xanthidrol method to the estimation of urea in urine

Throughout the work on NaCl metabolism, to which reference has already been made, the urea in both bloods and urines has been determined by the procedure described above. The dilution required for urines depends of course upon the concentration of the urine, but for normal urines the following table may be found useful.

Urine dilutions

Volumes ml	Dilution ml.
Less than 1	0.5 $\rightarrow$ 200
1- 2	1 $\rightarrow$ 200
2- 4	1 $\rightarrow$ 100
4- 8	2 $\rightarrow$ 100
8-16	4 $\rightarrow$ 100

1 ml. samples of the diluted urine are treated with glacial acetic acid and xanthidrol in the same manner as for blood filtrates.

A COMPARISON OF THE XANTHYDROL PROCEDURE WITH VARIOUS UREASE
METHODS FOR THE MICRO-ESTIMATION OF BLOOD UREA

The hydrolysis of urea to $(\text{NH}_4)_2\text{CO}_3$ by the action of urease has been applied extensively to the quantitative micro-estimation of blood urea. Since the xanthydrol method had proved to be satisfactory for the determination of small amounts of urea, it was decided to compare it with two urease methods which have been in clinical use in this laboratory, and with another method involving urease for which a high degree of accuracy has been claimed.

Urease-Nessler methods

In these methods, a sample of blood is incubated with urease. After dilution of the blood the proteins are precipitated with tungstic acid, the precipitate removed and the ammonia in the filtrate estimated colorimetrically after nesslerization. A standard solution of an ammonium salt is used for comparison.

Methods based on this procedure have been shown by various workers to be full of pitfalls, though in actual practice a combination of compensating errors appears to give results which are sufficiently accurate for clinical purposes.

Probably the chief difficulty in the original method [Archer & Robb, 1925] lies in the fact that a few minutes after nesslerization the unknown solutions become turbid, and this turbidity gradually increases. The cloudiness appears to be much greater with some blood filtrates than with others, and exactly the same phenomenon occurs with standard urea solutions which have been incubated with urease and treated with tungstic acid. Both urease and tungstic acid appear to be contributory causes. The standard ammonium solution, which has no reagents other than the Nessler's solution added to it, does not become turbid, and if colorimeter readings are made after the turbidity has developed the unknown solutions appear to be far too strong. Various workers have devised means to overcome this difficulty. Barrett [1935] recommends the addition of citrate before nesslerization, and this is in fact quite effective in preventing any precipitation. Taylor & Blair [1932] used 1 drop of a specially prepared concentrated urease extract, which was said to be completely removed with the protein precipitate. Shapiro [1934] tried various other protein precipitants but found none so satisfactory as tungstic acid. He found that if the urease can be completely removed, as in Taylor & Blair's method, the formation of the turbidity is delayed, but he could discover no method of doing this when commercial urease was used. He concluded that the best plan was to carry out the colorimeter readings as rapidly as possible after nesslerization.

A second difficulty in the direct nesslerization of blood filtrates has been pointed out by Barrett who states that other substances in the blood such as glucose and creatinine also give a yellow colour with Nessler's solution. To eliminate this error he recommends the addition of hypochlorite solution to the Nessler reagent.

A third source of error is that alcoholic urease, which is used in some methods, produces a decrease in colour intensity after nesslerization. Taylor & Blair recommend that alcoholic urease shall be added to the standard solution as well as to the unknown. Even so, the decrease in colour intensity may not be the same in the two solutions.

A fourth difficulty was observed by Behre [1923], who showed that when whole blood was incubated with soya bean urease, the amount of urea estimated was partly determined by the amount of urease added, and that the "extra urea"

was derived from the interaction of some substance in the blood corpuscles with the urease. Anderson & Tompsett [1936] confirmed this observation, and considered that arginase present in the corpuscles is responsible for producing ammonia from arginine in the urease preparations. These authors concluded that "when an accurate estimation is necessary, the variable error introduced by the action of blood arginase may be far greater than any experimental error".

In consideration of all these observations it is not surprising that the present investigation on the urease-Nessler technique sometimes gave results which were difficult to interpret. The method, however, is still frequently used for clinical work [Harrison, 1937], and it therefore seemed desirable to have some idea of its limitations.

Archer & Robb's method

The particular method chosen for study was that devised by Archer & Robb [1925] with the following modifications. 0.3 ml. of urease suspension (instead of 0.2 ml.) was added to the diluted blood and the mixture incubated in an oven at 55° for 30 min. Archer & Robb recommended incubation in a water-bath at 55° for 15 min.

Standard ammonium sulphate solutions, standard urea solutions, and samples of blood with known amounts of added urea were estimated in duplicate after incubation with urease and treatment with tungstic acid.

The formation of the turbidity after nesslerization was confirmed, and the method adopted to overcome this was as follows. The standard solution was nesslerized immediately before matching. Actually, a slight error is introduced in this way, for the colour is not fully developed during the first few minutes, i.e. when the reading is made. This error, however, is of the order of 3 %, whereas the turbidity may cause an error of 50 % or more. It was found that a degree of turbidity which could not be detected without careful inspection of the tubes caused considerable error in the colorimeter readings.

It has been suggested that this turbidity is technical in origin and may be due to the presence of traces of acetone or other interfering substances on the glassware. Contamination of the blood with acetone certainly causes increased turbidity, but small amounts of acetone added to filtrates after nesslerizing do not produce a precipitate, but decolorize the solution. Every precaution has been taken to avoid contamination, and it is certain that the cloudiness must have some other explanation. It seems more likely that the Nessler's solution may be the chief cause of the trouble, for it was found that more turbidity was produced with some preparations than with others. In no case however was it entirely absent.

A further source of error was found to be due to the fact that with bloods of low urea concentration, the tint of the unknown solution after nesslerization is different from that of the standard. With more concentrated urea solutions the greater intensity of the colour appears to mask the difference in shade.

Estimations of standard solutions of ammonia and urea by Archer & Robb's method gave variable and contradictory results, even when precautions were taken to avoid turbidity in the solutions when the colorimeter readings were made. The amounts of sodium tungstate and sulphuric acid added to ammonia and urea solutions after incubation were 1/10 of the amounts employed for blood samples, since there were no proteins present to be precipitated, and an excess of tungstic acid caused an enormous increase in turbidity after nesslerization.

It was found quite impossible, under the conditions employed, to recover quantitatively urea or even ammonium salts added to blood (see Table IV).

Table IV. *Archer & Robb's method. The addition of standard solutions to blood*

Sample of blood used	Material added		% of added material recovered
	Urea or ammonia	Amount mg. per 100 ml.	
1	(NH ₄) ₂ SO ₄	25	92.8
1	"	50	80.3
1	"	100	83.4
2	"	25	91.5
2	"	50	86.0
2	"	100	85.3
3	Urea	40	83.8
4	"	20	88.0
5	"	25	80.0
6	"	40	86.3

Table V. *Incomplete hydrolysis of urea by urease at high concentrations*

	Urea found mg. per 100 ml.
Blood estimated by the xanthydrol method	535
Same blood estimated by Archer & Robb's method	364
Archer & Robb filtrate from above estimated by the xanthydrol method	180
Total recovered	544

Some workers, using slightly different techniques, appear to have obtained satisfactory recoveries of urea and ammonia [Taylor & Blair, 1932; Eveleth, 1934]. No explanation of this can be offered, but the result was consistently obtained.

Another disadvantage of Archer & Robb's method as used in this investigation is the danger that for blood, or urea solutions containing more than about 150 mg. of urea per 100 ml. the amount of urease added may be insufficient within the time of incubation for the complete conversion of the urea into ammonia. Thus, low results were usually obtained at these high levels.

A sample of blood containing over 500 mg. of urea per 100 ml. was estimated by both the xanthydrol and Archer & Robb's methods. A portion of the Archer & Robb filtrate, after incubation with urease and precipitation of the proteins, was treated with acetic acid and xanthydrol. A precipitate of dixanthylurea formed and this was estimated quantitatively. It will be seen from Table V that incubation of this blood with urease resulted in incomplete hydrolysis of the urea, and that the unhydrolysed urea was quantitatively recovered with xanthydrol.

Barrett's modification of Archer & Robb's method

Estimations were also carried out by Barrett's [1935] modification of Archer & Robb's technique. This method has the great advantage of preventing turbidity in the nesslerized solutions, and on these grounds alone it is recommended to all those using urease-Nessler methods.

Estimations on standard ammonia and urea solutions gave results which were consistently 90–95% of the theoretical value. These low values are possibly explained by a fading of colour caused by the alcoholic urease as suggested by Taylor & Blair [1932]. Recoveries of urea added to blood were usually of the order of 75–85%. Turbidity in the nesslerized solution, or the presence of reducing substances in the blood will not account, therefore, for the low recoveries noted in Archer & Robb's method.

Conway's method

Conway's method [Conway & Byrne, 1933] for the micro-determination of ammonia can be used for the estimation of urea after incubation with urease [Conway, 1933]. This method was accordingly applied to standard ammonia solutions, standard urea solutions and blood with standard urea solutions added.

Once the technique of the special units had been acquired, the method gave good duplicates, quantitative analyses of standard solutions of both ammonia and urea and quantitative recovery of urea added to blood. This confirms Conway's observations. For weak solutions the titration error, which is constant, becomes considerably more significant, and the results are less reliable. The

Table VI. *Accuracy of Conway's method*

Material used	Concentration of urea or ammonia used mg. per 100 ml.	% recovered
Standard $(\text{NH}_4)_2\text{SO}_4$	50	99.5
Standard urea	20	102.0
Standard urea	90	98.6
Blood (a) + urea	100	99.6
Blood (b) + urea	100	101.2

"blank" which was found to vary somewhat with the age of the urease was determined with each batch of estimations. It was also found advisable to make a fresh dilution of the indicator fairly frequently since it deteriorated on standing. The chief disadvantage of Conway's method for clinical purposes is that for blood urea levels higher than 100 mg. per 100 ml. a preliminary dilution of the blood must be made. If therefore a blood is not suspected to be of high urea concentration a repetition of the estimation on a diluted sample may be necessary. Typical results obtained by this method are shown in Table VI. The figures given are the means of duplicate or triplicate estimations.

ESTIMATIONS OF UREA IN A SERIES OF BLOOD SAMPLES BY EACH OF THE METHODS DESCRIBED

Table VII shows the results obtained upon a series of 18 different blood samples of varying urea concentration when analysed by each of the methods investigated. Plasmas corresponding to three of the bloods were also determined.

As has already been shown, Archer & Robb's method has a number of possible sources of error, and most of these tend to give results which are too high. It is probable that, in the hands of workers who were unaware of its limitations and difficulties, results obtained by this method would have been much higher than those obtained in the present investigation. Even here, where precautions were taken to avoid turbidity in the nesslerized solutions, all samples containing 60 mg. of urea per 100 ml. or less give values by Archer & Robb's method which are higher than those obtained by the xanthidrol or Conway technique and in most cases higher than those by Barrett's method also. For blood ureas above 60 mg. per 100 ml. this tendency to high results by Archer & Robb's method is not so noticeable, and for blood ureas above 150 mg. per 100 ml. the incompleteness of hydrolysis of the urea by both Archer & Robb's and Barrett's methods becomes apparent and the results are too low.

The values obtained for high blood ureas by Conway's method are not too low because the blood samples were diluted till their urea contents were less than 100 mg. per 100 ml. before the addition of urease.

Table VII. *Estimations of urea in a series of blood samples by four different methods*

Blood no.	mg. per 100 ml.			
	Xanthydrol	Archer & Robb	Barrett	Conway
1	17.3	20.0	16.2	15.3
2	18.3	19.1	17.5	17.0
3	26.3	29.3	31.0	28.0
4	29.2	35.3	27.2	31.4
5	28.0	34.6	31.1	30.7
6	29.5	32.5	34.0	28.0
6 (plasma)	31.0	33.5	37.0	32.8
7	34.6	38.1	32.5	32.0
8	39.1	41.2	41.9	—
9	38.6	44.0	41.2	37.3
10	55.6	60.7	54.5	53.9
11	71.6	69.7	67.3	70.8
12	104	104	108	103
13	110	104	107	111
13 (plasma)	114	100	108	118
14	114	116	110	115
15	159	142	156	160
16	188	170	178	186
16 (plasma)	191	178	188	190
17	236	182	188	224
18	535	379	337	—

A comparison of the results obtained by the xanthydrol and Conway methods shows that generally speaking the agreement is fairly close. The percentage differences are much greater at low than at high values. This is probably due to the greater error in Conway's method at low levels.

In considering the relative advantages and disadvantages of these four methods, it is clear that Archer & Robb's, while giving satisfactory results under the conditions employed in the present investigation, is a dangerous method in that it may give very inaccurate values unless those working with it are fully alive to the necessity for matching the colours within a minute or two of nesslerizing the blood filtrate.

Barrett's modification is a great improvement on the original method, is rapid, relatively simple to operate, and gives more accurate results for bloods in spite of the low recoveries on standard solutions. It is the method to be recommended for clinical work.

Both these methods may give low results with very high blood ureas, and the figure at which they become inaccurate will vary with the conditions employed by the individual worker. In Conway's method there is no danger of obtaining falsely low results at high levels because it is obvious whenever the range has been exceeded. For clinical purposes, however, it is unsatisfactory to use a method with such a limited range, and requiring such special technique.

The xanthydrol method has the following points to recommend it:

- (1) It can be used for both bloods and urines.
- (2) The reaction is specific for urea, and moreover, ammonia does not interfere with the determination.
- (3) It can be used for blood containing fluoride.
- (4) The method has a very wide range, and one standard only is necessary.

For research purposes, especially where urea clearances are involved, the xanthydrol method is to be preferred; but it is slower than Barrett's method and is therefore less suitable for routine hospital work.

SUMMARY

1. A method is described for the estimation of urea in 0.2 ml. of blood. The urea is precipitated with xanthydrol and the dixanthyurea precipitate estimated colorimetrically.

2. Blood ureas varying from 10 to 500 mg. per 100 ml. may be estimated to within $\pm 3\%$ without altering the conditions. Urea in urine, suitably diluted, may be determined by exactly the same technique.

3. Three methods depending upon the use of urease have been studied, and their relative advantages and disadvantages discussed.

4. A series of 18 blood samples of varying urea concentration have been analysed by all four methods and the results compared.

The authors wish to express their thanks to Dr R. A. McCance for his helpful criticism and suggestions. They are indebted to the Medical Research Council for personal grants.

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