CCXCVII. CONDUCTOMETRIC METHOD FOR THE MICRO-DETERMINATION OF UREA.

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(Received 16 June 1936.)

IN view of the great physiological importance of urea in protein metabolism, its diagnostic significance in diseases and its wide distribution in the tissues of organisms, numerous methods have been developed for its quantitative estimation in micro- and macro-quantities. Most of these methods involve its preliminary decomposition with urease and subsequent titrimetric or colorimetric estimation of the ammonia or the manometric determination of the CO_2 of the ammonium carbonate [Van Slyke, 1927]. A comprehensive survey of the methods available to the analyst is given by Peters & Van Slyke [1932].

Increasing importance is attached to micro-methods for elucidating physiological processes necessitating the study of reactions in single cells or in isolated regions of specific tissues. The micro-method developed by Linderstrøm-Lang [1933] for the estimation of ammonia represents an extreme refinement in technique, but in common with most other methods when applied to the determination of urea, involves a high degree of manipulative skill and is laborious and time-consuming. Colorimetric methods with an error of 2-3% are not sufficiently accurate; the manometric method with an accuracy of 1% and the micro-method of Conway [1933] are the two convenient methods available for the estimation of urea.

The applicability of conductometry to the study of the hydrolysis of urea, suggested by Bayliss [1925], was examined by Sastri & Sreenivasaya [1936] and it was shown that the progressive increase in electrical conductivity in the urea-urease system is a true measure of the hydrolysis of urea. It was further observed that the changes in conductance accompanying the hydrolysis were of considerable magnitude and could be employed when the quantity of urea was as low as 5×10^{-5} g. The present communication gives a critical study of the method and its application to a determination of urea in blood and other biological fluids.

EXPERIMENTAL.

The usual Kohlrausch bridge method was employed. A calibrated Kohlrausch slide wire—which can be read to 1 part in 100,000 when extension coils are in circuit—a 4-dial resistance box and an Arrhenius-Ostwald cell (cell constant 0.5879) formed the components of the circuit. With the cell employed about 5 ml. were required for immersing the electrodes. The cell was immersed in a thermostatic bath maintained at $30.0^\circ \pm 0.1^\circ$. The Audio-Oscillator (General Radio Co.) giving an alternating current of frequency 1000 was employed as the source of high frequency current and a telephone receiver was used for detecting the null-point. The capacitance of the cell was balanced by means of an air condenser connected in parallel with the resistance box.

2.5 ml. of the urea-containing solution were placed in the conductivity cell together with 1-3 ml. of *M*/20 phosphate buffer (*pH* 7.0) and sufficient conductivity water to make up the volume to 5.5 ml. and the contents of the cell well mixed. The cell was immersed in the thermostat, and after about half an hour the resistance was measured at 5-min. intervals till a constant value was obtained, indicating that the contents of the cell had reached the temperature of the thermostat. 0.5 ml. of a dialysed urease solution, raised to the temperature of the thermostat, was added and the cell contents well mixed. The addition of the enzyme was timed by a stopwatch; the first reading can be conveniently taken after an interval of 30 sec. Subsequent readings are taken at 1 min. intervals. The quantity of enzyme employed was such as to effect complete hydrolysis of the urea in 5-15 min. Table I gives the results obtained with 1.02 mg. and 0.10 mg. of urea.

Table I.

Time min.	1.02 mg. of urea		0.10 mg. of urea	
	Balancing resistance ohms	Bridge reading	Balancing resistance ohms	Bridge reading
Before addition of enzyme.				
0	160	560	440	540
30	160	540	440	520
35	160	542	440	521
40	160	541	440	520
After addition of enzyme.				
0.5	160	687	440	623
1	160	652	440	604
2	160	581	440	565
3	160	515	440	535
4	160	460	440	520
5	160	421	440	512
10	160	333	440	507
15	160	319	440	507
20	160	316	440	507
30	160	319	440	—

Fig. 1.

Table II. *Relation between change in conductivity and quantity of urea.*

Quantity of urea (mg.)	0.051	0.102	0.212	0.330	0.408	0.508	0.635	0.817	0.847	1.021
Change in conductivity × 10 ⁴	0.42	0.72	1.34	2.00	2.43	3.03	3.72	4.75	4.98	5.60

The readings are plotted on a graph, time against resistance and by extrapolation the resistance at 0 time can be read. The total change in resistance gives a measure of the electrolyte released during the reaction and hence the quantity of urea.

The change in conductivity accompanying the hydrolysis of varying quantities of urea (0.05–1.0 mg.) was determined similarly, and from the readings obtained a graph (Fig. 1) showing the relation between quantity of urea and change in conductivity was prepared. Table II gives the readings obtained.

This graph could be used for obtaining urea values of different physiological fluids such as blood, urine and milk.

Applications. The method has been employed for the estimation of urea in blood, urine and milk. The soya enzyme used in these experiments was prepared by Van Slyke and Cullen's acetone precipitation method [1914]. To test the applicability of the method, two series of experiments were carried out: (1) known quantities of urea were added to a definite volume of blood, milk or urine and the quantity of urea thus added estimated by the conductometric method, and (2) the values obtained by the conductometric method were compared with those obtained by the well-known aeration-titration procedure of Van Slyke & Cullen [1916]. The results obtained with additions of known quantities of urea are tabulated in Table III.

Table III. *Estimation of added urea.*

Quantity of urea added mg.	mg. urea estimated after addition to		
	Blood (sheep) 0.20 ml.	Urine (human) 0.01 ml.	Milk (cow) 0.50 ml.
0.102	0.100	0.100	0.099
0.204	0.205	0.203	0.200
0.408	0.399	0.391	0.401

The difference in conductivities before and after addition of urea is a measure of the change due to the added urea. There is close agreement between the added and estimated values.

The results obtained by the conductometric and titrimetric methods are compared in Table IV. The quantities of blood and urine employed for the aeration-titration method are respectively 5 and 1 ml. The corresponding quantities for the conductometric method are 0.2 and 0.01 ml.

Table IV. *Comparison of aeration-titration and conductometric methods.*

Method	mg. of urea in			
	Blood (sheep) 5.0 ml.	Urine (human) 1.0 ml.		
		Sample 1	Sample 2	Sample 3
Aeration-titration	2.6	6.8	10.1	7.3
Conductometric	2.5	6.7	10.0	7.6

The close agreement between the two sets of values establishes the applicability of the conductometric method to the estimation of urea in physiological fluids.

The urea contents of some normal and pathological specimens of urine and

blood (human), as well as those of milks, determined by the conductometric method are tabulated below.

Table V.

Material	Urea* mg. in 10 ml.
Human urine (normal)	75
„ (albuminous)	178
„ (jaundice)	82
„ (albuminous)	113
Human blood (normal)	3.1
„ (acute nephritis)	8.5
„ (mild nephritis)	5.8
Milk (cow)	2.2
„ (ass)	4.9
„ (ass, ultrafiltrate)	4.9

* The values for blood and urine approximate to those furnished by the Bowring Hospital, Bangalore, from whom the samples were obtained.

DISCUSSION.

The results given above show that conductometry furnishes an elegant means for the accurate estimation of urea in physiological fluids. In Table VI, an attempt has been made to compare the method with others in vogue with respect to minimum quantities of experimental material, rapidity and accuracy of estimation.

Table VI. *Estimation of urea in physiological fluids. A comparative study of methods.*

	1	2	3	4	5	6
	Aeration- titration	Mano- metric	Colorimetric (ammonia distillation or Permutit absorption)	Hypo- bromite	Xanthidrol (Beattie, colori- metric)	Conducto- metric
Quantity of blood required (ml.)	3	0.2	0.5	0.5	<0.5	0.1
Accuracy (%)	1	1	2-3	Positive error >4	2-3	1
Time taken (min.)	Long time: involves isolation of ammonia	10	Long time: involves isolation of ammonia	3	Long time: involves filtration of ureide	5-10

It will be seen that the method, while it claims equality with the manometric procedure with respect to accuracy, possesses the merit of requiring smaller quantities of material. By choosing appropriate cells it should be possible to work with even smaller quantities. The molar concentration of ammonia in the cell, in our experiments, was as low as 1/1700; the cell contained 6 ml. of liquid and the distance between the electrodes was 1 cm.; by merely decreasing the distance between the electrodes, it will be possible to employ smaller quantities of fluid without greatly affecting the accuracy. The procedure is simple, does not require the use of standard solutions or many pieces of apparatus. There is hardly any need for determining the preformed ammonia by a duplicate experiment. The use of strong alkali carbonates for displacing the ammonia formed as a result of the hydrolysis of urea in physiological fluids involves the risk of the decomposition of associated amide-containing proteins with the liberation of ammonia therefrom. By reason of its simplicity

and reliability, the conductometric method can be adopted by the trained medical practitioner for the routine examination of bloods and urines.

Conductometry can be employed not only for the estimation of urea but also for the determination of urease activity and in a similar manner for the estimation of arginine and arginase activity. For the study of the kinetics of urease and arginase, particularly when they are available only in micro-quantities, the method offers exceptional advantages: thus it permits of a number of readings in the initial stages of the action, not possible by chemical methods. The possibility of adopting this procedure for the determination of urea and arginine or their respective enzymes in tissues is now being examined.

SUMMARY.

A micro-method for the estimation of urea based on the measurement of the change of conductivity resulting from the hydrolysis of urea by urease has been described. The method gives reliable values for quantities as low as 0.5×10^{-5} g. of urea.

The method has been applied to the determination of urea in various physiological fluids and the values obtained compared with those obtained by macro-methods.

Our thanks are due to Mr M. Sreenivasaya for many helpful suggestions, and to the authorities of the Bowring Hospital, Bangalore, for supplying the pathological specimens of urine and blood. Our thanks are also due to Dr V. Subrahmanyam for his interest in the work.

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