CHROM. 5928

AN IMPROVED TECHNIQUE FOR THE ANALYSIS OF AMINO ACIDS AND RELATED COMPOUNDS ON THIN LAYERS OF CELLULOSE

VII. A SIMPLE TECHNIQUE FOR THE ESTIMATION OF BLOOD UREA AND OF ITS CLEARANCE FROM PLASMA

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(Received January 24th, 1972)

SUMMARY

A simple method is described for the rapid determination of blood urea which can be applied to the estimation of renal function. When combined with the concurrent determination of urea in urine, accurate urea clearance values are obtained.

INTRODUCTION

The measurement of glomerular filtration rate is fundamental in the examination of renal function and is most accurately determined by the measurement of inulin clearance. However, because this procedure is difficult and time-consuming, either blood urea values or urea clearance values are often used as an estimate of this function[1]. At present, urea is usually determined by colorimetric analysis of the samples of plasma, or of the blood, using an autoanalyser[2,3].

We recently described a simple, one-dimensional chromatographic procedure for the determination of urea in urine[4] which depended on the production of a stable and reproducible colour with Ehrlich reagent[5].

The method has now been applied to plasma urea and the values are considered either directly as an indication of renal function or used with urinary urea values to determine the clearance. The results agree well with those obtained by an automatic procedure. The technique may be simplified further by the application of the visual comparison method of estimation. In this way effective analysis is achieved without the aid of complex instrumentation.

EXPERIMENTAL

Apparatus

The thin-layer chromatographic (TLC) equipment used was supplied by

Shandon*. The automatic recording and integrating double beam reflectance densitometer 'Chromoscan' with thin-layer attachment was used for the quantitative evaluation of the developed chromatograms**. The results obtained by densitometry following chromatography were compared with those obtained by an automatic procedure using the Technicon Instrument***.

Materials and methods

Clearance tests. These were carried out on ten normal adults after a period of overnight fasting. The subjects were each given 1 l of water to drink and then allowed to rest for 1 h. After this time the bladder was emptied, the specimen being discarded. The bladder was emptied after 30 min and 60 min, each sample being saved and the void volume noted. The urine samples were pooled for analysis. A sample of oxalated venous blood was drawn after 45 min. The blood cells were centrifuged off and the plasma was retained.

Preparation of thin layers of cellulose. Glass plates (20 × 20 cm) were coated with a slurry of cellulose powder MN 300 (without binder)§ at a thickness of 400 μ and the procedure described previously[4,6]. Pre-prepared§§ plates coated with Cellulose F were also used.

Solvent for chromatography. Chromatography was carried out using a freshly prepared solvent[6] of 2-propanol–butanone–1 *N* hydrochloric acid (60:15:25). All solvents were of AnalaR or M.F.C. grade§§§.

Chromatography. The plasma sample (2 μl) and the corresponding pooled urine sample (1 μl) were placed side by side along a line 1.5 cm up from the lower edge of the layer as described previously[4]. In this way clearance values and blood urea may be determined for up to five patients on one thin-layer plate. When the solvent front had travelled 13 cm from the origin (approximately 2.5 h for the manually prepared plates) the plate was removed from the tank. Hydrogen chloride was removed in a stream of warm air (15 min) and organic solvent by heating at 60° for 15 min in a convection oven.

Detection and determination of urea. When cool, the plate was sprayed with Ehrlich reagent[5] applied by means of a Shandon atomiser until the layer just appeared translucent. After heating at 60° for 45 min in the convection oven, the spots were scanned by densitometry at right angles to the direction of chromatography[4]. Because the spots were stained yellow by the Ehrlich reagent, the filter used in the densitometer (complementary colour) was 405 nm in wavelength.

The area under the densitometric curve was measured using the relationship: Area = peak height × width at half height and the value obtained was then related to the amount of urea present by reading from a prepared standard graph[4].

Automatic analysis of urea. The automatic analysis of urea was carried out according to the manufacturers manual using diacetylmonoxime as the chromogenic reagent[3].

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* Shandon Scientific Co. Ltd., 65 Pound Lane, London NW-10.
** Joyce Loebl and Co. Ltd., Gateshead-on-Tyne, Durham.
*** Technicon Instruments Co. Ltd., Hanworth Lane, Chertsey, Surrey.
§ Macherey Nagel and Co. Ltd., Agents, Camlab (glass) Ltd., Cambridge.
§§ E. Merck and Co. Ltd., Agents. Anderman and Co. Ltd. London.
§§§ Hopkin and Williams Ltd., Chadwell Heath, Essex.

TABLE I

DETERMINATION OF UREA CLEARANCE BY TLC (COMPARISON WITH AUTOMATIC ANALYSER (AA))

Patient No.	*Urinary output (ml/min)*	*Urea concentration (mg/100 ml)*				*(ml/min)*	
		Plasma		*Urine*			
		TLC	*AA*	*TLC*	*AA*	*TLC*	*AA*
1	2.45	28.6	29.0	736	795	65.4	67.2
2	1.82[a]	24.5	24.4	1291	1267	71.1	70.1
3	4.72	34.1	34.8	547	535	75.6	72.5
4	3.10	29.1	30.2	655	645	69.7	66.2
5	2.51	28.4	27.6	759	750	67.1	68.2
6	2.23	24.6	25.1	801	830	72.6	73.8
7	3.27	35.9	36.2	827	848	75.3	76.6
8	4.10	36.8	37.4	741	749	82.5	82.1
9	3.71	31.9	31.2	698	729	81.2	86.6
10	1.90[a]	26.0	26.7	1394	1418	73.9	73.2

[a] Urea clearances were calculated using the square root of the urinary output when it was below 2.0 ml/min[7].

RESULTS AND DISCUSSION

The blood urea, urinary urea and urea clearance of ten different adults have been obtained both by an automatic procedure and by a TLC method. The results, which are compared in Table I, show very close agreement between the two procedures. For fifty subjects, the coefficient of variation was 2.87%. Furthermore, adding known amounts of urea to both plasma and urine samples gave recoveries which averaged 99.7% and 100.3% respectively by the TLC procedure (Table II), while the variation in repeat analysis of the same sample was less than 3% in each case.

The method is therefore suggested as an alternative means of determining blood urea or urea clearance. Because of its speed and accuracy the new technique should prove to be useful when automatic procedures are not available or as an alternative when analysis is required on only a few samples.

TABLE II

PERCENTAGE RECOVERY FROM PLASMA AND URINE OF ADDED UREA USING TLC

Urea in sample (mg/ml)	*Urea added (mg)*	*Total urea expected (mg/ml)*	*Total urea found by TLC (mg/ml)*	*Percentage recovery (%)*
Plasma				
0.21	0.1	0.31	0.30	96.7
0.15	0.2	0.35	0.36	102.8
0.31	0.1	0.41	0.42	102.3
0.29	0.2	0.49	0.49	100.0
0.25	0.1	0.35	0.34	97.1
Urine				
7.41	1.0	8.41	8.50	101.0
14.62	1.0	15.62	15.43	98.7
11.93	1.0	12.93	12.77	98.8
8.41	1.5	9.91	10.10	102.0
6.21	2.0	8.21	8.32	101.3

If the chromatography is carried out on commercially available thin layers of cellulose and the coloured complexes which are obtained by the use of Ehrlich reagent are estimated by visual comparison[8,9], the procedure may be simplified even further. In this way the method may be applied, *e.g.*, by the general practitioner, and approximate, but reliable, estimates of blood urea and of urea clearance may be calculated by means of the commercial nomogram[10].

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