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A method for the rapid determination of urea in minute amounts of blood.

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This method involves the digestion of urea by urease (Marshall-Van Slyke), the precipitation of the proteins (Folin-Wu), the direct Nesslerization of the filtrate (Myers) and determination of the color in the micro-colorimeter previously described.[1] For this color comparison a wedge, containing 1 per cent. potassium dichromate, mounted on a deep yellow ground-glass plate, is used.

The technique is as follows: A 0.2 c.c. pipette is rinsed with 20 per cent. potassium oxalate solution. The residual fluid is blown out well and 0.2 c.c. of blood is drawn up from the pricked finger or ear-lobe and discharged into a small test-tube. The pipette is then rinsed twice with exactly 0.2 c.c. of water and the washings are added to the blood. Three or four milligrams (knife-point) of powdered urease are now added to the blood and, after shaking, a stopper is inserted and the tube is kept at 50° for 10 minutes or at room temperature for 30 minutes or longer. Then 1.0 c.c. of water is added, followed by 0.2 c.c. of 10 per cent. sodium-tungstate solution and 0.2 c.c. of 2/3 N sulphuric acid.

[1] Kleiner, *Journal A. M. A.*, 1921, lxxvi, 172.

The mixture is immediately shaken and, after the precipitate has darkened, it is filtered into another small test-tube, using a 2.5–3 cm. funnel and small thin filter paper. With a dry 1 c.c. pipette, graduated in 1/100ths, a definite volume of the filtrate is discharged into one of the 5 c.c. graduated test-tubes with which the micro-colorimeter is provided. It is convenient to take 0.5 c.c. but one need not wait for this amount to filter through. Two volumes of water are added and one volume of Nessler's solution (Bock-Benedict formula diluted 1 : 5). After thorough mixing the tube is placed in the micro-colorimeter, and matched to the standard "nitrogen" wedge, described above. The reading is now made on the scale and the amount of urea nitrogen per 100 c.c. blood found directly by consulting Table I. If the color is too deep,

TABLE I.

DILUTION,—1 VOL. SOLUTION : 2 VOLS. WATER : 1 VOL. NESSLER'S SOLUTION.

Reading.	Mg. N per 100 c.c.	Reading.	Mg. N per 100 c.c.	Reading.	Mg. N per 100 c.c.	Reading.	Mg. N per 100 c.c.
10	10						
11	10	36	22	61	34	86	45
12	11	37	22	62	34	87	45
13	11	38	23	63	34	88	46
14	12	39	23	64	35	89	46
15	12	40	24	65	35	90	47
16	13	41	24	66	36	91	47
17	13	42	25	67	36	92	48
18	14	43	25	68	37	93	48
19	14	44	26	69	37	94	49
20	15	45	26	70	38	95	49
21	15	46	27	71	38		
22	15	47	27	72	39		
23	16	48	27	73	39		
24	16	49	28	74	40		
25	17	50	28	75	40		
26	17	51	29	76	40		
27	18	52	29	77	41		
28	18	53	30	78	41		
29	19	54	30	79	42		
30	19	55	31	80	42		
31	20	56	31	81	43		
32	20	57	32	82	43		
33	20	58	32	83	44		
34	21	59	33	84	44		
35	21	60	33	85	44		

five volumes of water are added and, after mixing, a comparison is again made, the result this time being found by consulting Table II. These tables cover a range from 10 to 100 mg. of urea N per 100 c.c. blood.

TABLE II.

DILUTION,—1 VOL. SOLUTION : 7 VOLS. WATER : 1 VOL. NESSLER'S SOLUTION.

Reading.	Mg. N per 100 c.c.	Reading.	Mg. N per 100 c.c.	Reading.	Mg. N per 100 c.c.
20	31				
21	32	46	62	71	92
22	33	47	64	72	94
23	34	48	65	73	95
24	36	49	66	74	96
25	37	50	67	75	97
26	38	51	68	76	98
27	39	52	70	77	99
28	41	53	71	78	100
29	42	54	72		
30	43	55	73		
31	44	56	74		
32	46	57	76		
33	47	58	77		
34	48	59	78		
35	49	60	79		
36	50	61	80		
37	51	62	82		
38	53	63	83		
39	54	64	84		
40	55	65	85		
41	56	66	86		
42	58	67	88		
43	59	68	89		
44	60	69	90		
45	61	70	91		

The tables represent the averages of many determinations made on sunny and on cloudy days. This minimizes the slight differences due to variations in intensity of light.

Results obtained with this method agree closely with analyses of the same specimens by the aeration method in which 2 c.c. of blood were used. The micro method may be performed easily in 20–30 minutes with sufficient accuracy for clinical purposes.