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Studies in Non-Protein Nitrogen. I. A Convenient Method for Measuring Urea in Blood. (19073)

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The acid-heat hydrolysis method of measuring urea in biological fluids, initiated by Benedict and Gephart(1) and further developed by later workers(2-4), has recently been revised by Kibrick and Skupp(5). The procedure as applied by these authors to serum and plasma filtrates would appear to represent a highly convenient way of determining urea nitrogen. They did not extend the method to whole blood filtrates because of cloudiness which developed after addition of Nessler's solution, an observation known since the work of Folin and Denis(6). However, for reasons of convenience and ease of manipulation, the routine use of whole blood often is preferable in the clinical laboratory. Indeed, application of the method to whole blood was thought to be possible by employing the combined gluconate-persulfate nesslerizing reagent which was shown by Gentzkow(7) to prevent turbidity in filtrates from blood samples subjected to urease hydrolysis. This expectation was confirmed in our experiments.

Experimental. In order to test the accuracy of Kibrick and Skupp's method, it was applied at first to prepared urea nitrogen solutions. One ml of urea solution (or of distilled water for the blank) and 0.5 ml of 1 M phosphoric acid in a tin foil-covered test tube were subjected to either 15, 20 or 22 lb of pressure for

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2. Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, v38, 81.
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5. Kibrick, A. C., and Skupp, S., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 432.
6. Folin, O., and Denis, W., *J. Biol. Chem.*, 1916, v26, 473.
7. Gentzkow, C. J., *ibid.*, 1942, v143, 531.

TABLE I. Percent Recoveries of Urea Nitrogen from Prepared Urea Solutions.

Urea N solutions	Kibrick-Skupp method			New method			
	15	20	22	15	20	22	Lb pressure
	90	60	90	90	60	90	Min
mg/100 ml	% recovered						
11.66	94.1	97.4	102.2	90.6	89.5	100.3	
23.32	93.2	93.7	100.8	86.7	87.9	98.6	
46.64	93.3	93.5	97.7	85.8	88.5	99	
75	92	92.1	—	84.1	87.3	98.7	

Each % value represents average of triplicate analyses. The overall standard experimental error was computed as: Overall S. E. $= \sqrt{\frac{\Sigma\ \Sigma\ r\ n\ (x-m)^2}{r(n-1)}}$, where x represents the individual value; n, the number of replicates per analysis; m, the arithmetical mean of replicates, or $\frac{\Sigma x}{n}$; r, the number of sets of replicates. Overall S. E. for Table I was 1.12.

TABLE II. Percent Recoveries of Urea Added to Serum.

Urea N added to serum containing 11 mg/100 ml	Kibrick-Skupp method			New method			
	15	20	22	15	20	22	Lb pressure
	90	60	90	90	60	90	Min
mg/100 ml	% recovered						
30	91.6	93.3	98.9	91.6	99.7	101	
50	89.8	91.9	97.3	89.2	93.6	97	
100	87.7	92.6	97.8	83.1	95.6	97.8	

In setting up recovery experiments, urea standard solutions of appropriate concentrations were substituted for some of the distilled water used in making tungstic acid filtrates. Each figure expressing % recovery represents an average of the analyses of three filtrate aliquots. Overall S. E. = 1.19.

60 to 90 minutes (Table I) in a hospital autoclave.* After cooling, 1 ml of 1 N sodium hydroxide was added and thereafter distilled water to the 10 ml mark on the tube. This was followed by 1 ml of Nessler's reagent prepared according to Koch and McMeekin (8). After allowing the well-mixed solution to stand for 10 minutes, readings were taken within 10 minutes in a Beckman model DU spectrophotometer at a wave length of 500 millimicra and in a Klett-Summerson colorimeter with filter 54. (In view of the greater precision of the former instrument, only results obtained by it will be presented.) Standard solutions containing 2 and 5 mg of nitrogen, as ammonium sulfate, per 100 ml, treated in like manner but without the autoclaving step, served as the bases for photometric comparison and calculation of results.

Results. It is apparent from Table I that under the conditions of pressure and time recommended by Kibrick and Skupp(5), namely 15 lb and 90 minutes or 20 lb and 60 minutes, recoveries of urea nitrogen were quite deficient. However, they were very satisfactory with the use of 22 lb for 90 minutes. This was true with and without the modification using the gluconate-persulfate reagent described below. Data on the recov-

* A horizontal, direct steam-heated type of 14000 cu in. capacity made by the American Sterilizer Co., Erie, Pa. (Company names are used as a means of identifying the products under discussion, and do not represent an endorsement of the products by the Public Health Service.)

8. Hawk, P. B., Oser, B. L., and Summerson, W. H., Practical Physiological Chemistry, 12th Edition, The Blakiston Co., Philadelphia and Toronto, 1942, p. 1230.

TABLE III. Percent Recoveries of Urea Added to Whole Oxalated Blood.

Urea N added to blood, mg/100 ml	Urea N found, mg/100 ml	% urea N recovered	Hydrolyzing conditions
0	7.5	—	15 lb
27	31.1	87.4	90 min
40.5	37.6	74.4	
81.1	51.3	54	
0	15.6	—	20 lb
19.9	35.8	101.5	90 min
39.9	54.9	98.7	
59.8	75.9	100.7	
99.7	114.9	99.6	
0	12.7	—	22 lb
30	43.8	103.5	90 min
50	62.5	99.6	
100	111.8	99.1	
150	167	102.8	

See footnote of Table II. Overall S. E. = .95.

ery of urea added to serum or blood as listed in Tables II and III, respectively, warrant the same conclusions. At 20 lb or more applied for 90 minutes, recoveries were practically complete.

These results appeared to indicate that hydrolysis of urea may not be complete at the pressure values originally suggested(5); hence they were increased in the procedure as finally adopted.

New method. One ml of tungstic acid filtrate from either whole blood, plasma or serum and 0.5 ml of 1 M phosphoric acid in a tube covered with tin foil are placed in an autoclave or pressure cooker and exposed to a minimum of 20 lb of pressure for 90 minutes. Into the cooled tube, 1 ml of 0.9 N sodium hydroxide is pipetted,† followed by distilled water to the 10 ml mark. After mixing, 2 ml of Gentzkow's nesslerizing reagent(7) are added (2 parts of Folin Nessler's solution(2), 1 part of 2.5% aqueous nitrogen-free potassium persulfate and 1 part of 1% aqueous potassium gluconate. The mixture must be used within 15 minutes. Both the persulfate and gluconate solutions must be made up fresh weekly and kept under refrigeration). The sample is shaken, allowed to stand for 15 minutes and read within the next 90 minutes in a spectrophotometer or photoelectric colorimeter at a wave length of between 490 and 510 millimicra. A distilled water blank and an ammonium sulfate standard (containing 2 mg of nitrogen per 100 ml) are treated identically except that the standard is not autoclaved. For convenient computation of results, a calibration curve may be prepared by means of a series of ammonium sulfate solutions ranging in concentration between 1.5 and 8 mg of nitrogen per 100 ml. Within this range there exists a straight-line relationship between concentration and optical density. Filtrates of blood samples containing more than 80 mg of urea nitrogen per 100 ml must be diluted with distilled water and the results multiplied by the dilution factor. In order to test for completeness of hydrolysis, particularly when the pressure gauge is not reliable, it is desirable to include a urea solution of

† This results in a pH of about 7.0 while the use of 1 N sodium hydroxide, as previously recommended by Kibrick and Skupp(5), produces an unduly high pH which may enhance turbidity after nesslerization.

TABLE IV. Comparison of Blood Urea Nitrogen Values as Determined by the New and by the Urease Methods.

Blood sample No.	mg of urea N per 100 ml — Urease methods — Gentzkow's(7)	Karr's(9)	New method
1	5	3	6.5
2	9.3	8	10.3
3	9.9	7	9.7
4	10.7		10.9
5	13.4		13.8
6	14.8	13	17.3
7	15.5	12	14.5
8	16.2	18	20.8
9	17.4	13	15.9
10	22.8	20	22.8
11	37.3	33	32.5
12	38.8		39
13	40		39.7
14	53.3		55.5
15	53.5		54.5
16	59	59	59.8
17	61.8		62
18	75	70	77.6
19	75		73.4
20	99.6	110	104

Values given for Gentzkow's and for the new method are averages of duplicate analyses determined by means of the Coleman Jr. Spectrophotometer. Overall S. E. for the former method was estimated to be .22, for the latter, .16. Values for Karr's method represent single analyses performed in the course of routine hospital laboratory work with the use of a Klett-Summerson colorimeter.

9. *Ibid.*, p. 505.

known concentration in the series of unknowns analyzed.

Application of the method to a series of blood samples and comparison with Gentzkow's(7) and Karr's(9) methods using urease hydrolysis (Table IV) resulted in satisfactory agreement of values.

Summary. The acid hydrolysis method of measuring urea has been revised so that it will furnish accurate and reliable results when applied to either whole or cell-free blood.

The computation of standard errors was carried out by Mr. Myron J. Willis of the Statistics Section, Epidemiologic Services, Communicable Disease Center.

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Abrupt Improvement of Serum Electrophoretic Pattern in Nephrosis After ACTH-Induced Diuresis. (19074)

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This paper deals with the partial correction of the abnormal electrophoretic pattern, which is characteristic of the serum in nephrosis(1-4), in patients who responded to ACTH with intense diuresis. In the nephrotic syndrome there occurs a progressive accumulation of fluid in the interstitial spaces and the serous cavities, the edema increasing until, quite unpredictably, it is relieved by massive diuresis. Until recently the onset of diuresis has been fortuitous, and it has therefore been difficult to follow the chemical and physical changes which occur in blood just before and after diuresis. It is now possible to produce diuresis in many nephrotic patients with a variety of drugs such as ACTH, cortisone, urea, cation exchange resins, and occasionally by nitrogen mustard. The effect of the diuresis thus produced upon some chemical constituents of the blood has been described(5-7).

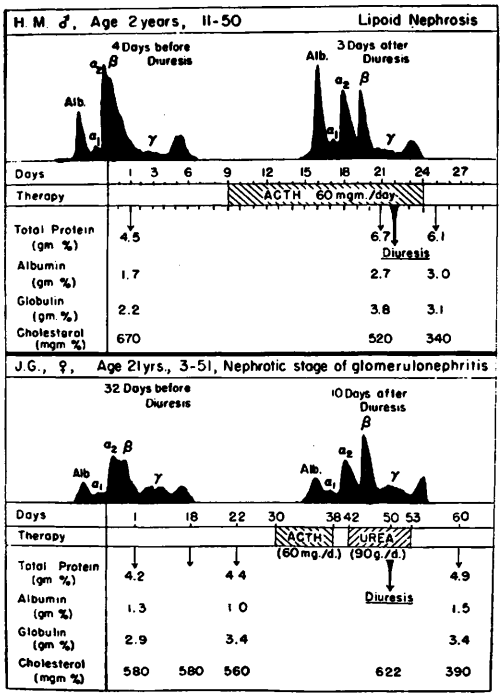

FIG. 1. Total protein, albumin, globulin results listed were determined by chemical analyses.

This paper describes the marked changes observed in the electrophoretic pattern of the serum proteins.

Methods and materials. Observations are presented from 4 cases, 3 infants suffering from lipoid nephrosis and one adult female, 21 years of age, suffering from chronic glomerulonephritis. Her nephrotic syndrome

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