Adaptation of the Berthelot Color Reaction for the Determination of Urea Nitrogen in Serum and Urine to an Ultramicro System

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Existing methods for the estimation of urea and ammonia nitrogen in biologic fluids by means of the Berthelot color reaction have been adapted to an ultramicro system for routine clinical analysis. The resulting procedure is convenient and accurate, and can be carried out using 5 μl. of sample or less.

THE PHENATE-HYPOCHLORITE procedure for the determination of urea nitrogen in serum has been demonstrated to have distinct advantages over existing, more commonly employed, procedures (*1–4*). The following modification of this procedure, devised in our laboratories for use with the Beckman-Spinco ultramicro system,* has been in routine use for several months with very satisfactory results.

Experimental

Reagents and Equipment

The concentrated reagents of Chaney and Marbach (*3*) and Searcy *et al.* (*4*) were used. All solutions and aliquots were measured and dispensed, using polyethylene micropipets. The pipets, centrifuge tubes, microspectrophotometer, microcentrifuge, and related supporting equipment were used without modification.

Procedure

Place 150 μl. of buffered urease and 5 μl. of serum or plasma in a microfuge tube. Mix, centrifuge for 15 sec., and incubate for 5 min. at

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50–55°. Cool and place 10 μl. of incubated solution into a second microfuge tube. Add 30 μl. of phenol color reagent, shake to the bottom of the tube, and then add 30 μl. of alkali-hypochlorite. Mix immediately, centrifuge for 15 sec., and incubate for 5 min. at 50–55°. Remove from the waterbath and add 250 μl. of water, mix well, and read in the microspectrophotometer at 640 mμ against a reagent blank adjusted to read 100% T. Reagent blanks and standards are prepared by processing 5 μl. aliquots of water (or buffer) and working standard (20 mg. N/100 ml.) in the same manner as given for serum.

To measure urinary ammonia nitrogen, mix 5 μl. of urine with 150 μl. of water in a microfuge tube. Place 10 μl. of this urine dilution in a second microfuge tube and develop color directly, exactly as in the analysis of serum. A color development carried out on 10 μl. of water serves as a reagent blank. Measurement of urinary urea and ammonia nitrogen is made by treating 5 μl. of the urine dilution prepared above exactly as in the analysis of serum. Reagent blanks and standards are identical to those used for the analysis of serum.

Results and Discussion

The validity of the use of the phenate-hypochlorite reaction for the measurement of urea and ammonia nitrogen has been amply demonstrated by previous investigators (*1–4*). Our primary concern was to demonstrate that no unsuspected source of error was introduced in adapting the reaction to the ultramicro system. Three commercial control serums from two different suppliers were analyzed in replicate. The values obtained of 11, 50.5, and 52 mg./100 ml. are in reasonable agreement with the respective stated analyses of 11.5, 51, and 53 mg./100 ml. Recoveries of ammonia nitrogen added at various levels to serum and urine ranged from 99–104%. Table 1 presents a series

Table 1. Reproducibility of Analytical Results

Specimen	*No. of replicates*	*Absorbance ± S.D.*	*mg./100 ml. ± S.D.*	*Coeff. of variation*
Serum pool No. 18	8	—	23.1 ± 0.3	1.3
Serum pool No. 18	8	—	22.6 ± 0.46	2.1
Serum pool No. 18	35*	—	22.4 ± 0.6	2.7
Serum pool No. 19	9*	—	22.8 ± 1.2	5.3
20 mg./100 ml. Std.	8	0.152 ± 0.004	—	2.6
20 mg./100 ml. Std.	25*	0.149 ± 0.008	—	5.4

*Average results from daily use in the routine laboratory by varied personnel. (All others obtained by same operator.)

of replicate analyses on two control pooled serums and on the nitrogen standard. The routine daily results are of particular interest. At least 8 technicians participated in obtaining these results. The somewhat higher deviation for the standard is, at least in part, self-compensating, since it corrects for minor variations in technic from one operator to the next.

Figure 1 demonstrates the linear nature of the microspectrophotometer response to increasing amounts of ammonia nitrogen. When values in excess of 80 mg./100 ml. are obtained, dilution of the final color development solution can be employed, and is satisfactory up to at least 160 mg./100 ml. However, a relatively large volume of urease-incubated serum dilution is available that can be employed directly in the color-development step, using 5-μl., in place of 10-μl., aliquots. This means that little time is lost in checking and/or rerunning occasional very high levels. Table 2 demonstrates the close agreement between the analytical results when serums are analyzed by the original and the modified procedures.

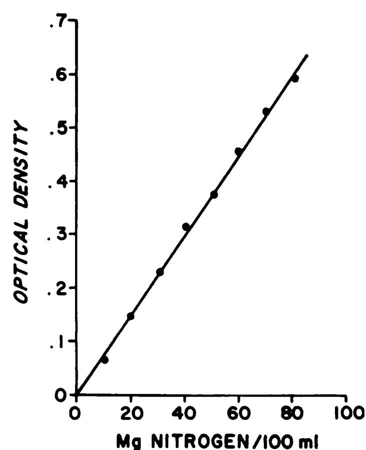

Fig. 1. Microspectrophotometer response to increasing concentrations of ammonia nitrogen.

Table 2. COMPARATIVE SERUM ANALYSES USING THE METHOD OF SEARCY ET AL. AND THE DESCRIBED MODIFICATION

Specimen No.	*Searcy* et al.	*Ultramicro system*	*Specimen No.*	*Searcy* et al.	*Ultramicro system*
1	23	22	6	44	41
2	40	41	7	20	20
3	6	6	8	10	10
4	58	60	9	80	78
5	21	22	10	22	25
			11	15	16

The dilutions employed in the measurement of urinary ammonia and urea nitrogen have the convenience of being identical with those used in the serum procedure. Separate pipets were employed for the urine ammonia step to avoid urease contamination.

The sensitivity of the over-all procedure is best demonstrated by the fact that the final color development for urea is carried out using $^{1}/_{3}$ and $^{1}/_{93}$ of a μl. of serum and urine, respectively. The 5 μl. specimen volume employed in the initial step of the procedure was based on commercial availability of this type of polyethylene transfer pipet. Volumes of 1 μl. or less could be used, if desired.

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