Chemical Assay of p-Aminohippuric Acid Simplified by Use of Dimethylaminocinnamaldehyde in Ethanol

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We have developed a new and simple method of p-aminohippuric acid determination by use of dimethylaminocinnamaldehyde (DACA). It differs from previous methods using DACA in that the reaction is carried out in ethanol rather than in dilute acid. This results in deeper and more stable color development. We have used this method successfully to determine effective renal plasma flow in a clinical study of this variable in pregnant women.

The clearance of p-aminohippuric acid (PAH) continues to be used to measure "effective" renal plasma flow in research settings. Current methodology for this analyte involves reacting the specimen with a chromogenic aldehyde, dimethylaminocinnamaldehyde (DACA), in dilute acid (1). We have found that by using DACA in ethanol instead, the color is formed more directly, and is deeper and more stable.

Materials and Methods

Centrifuge, for 4 min at 100 × g, PAH standard solutions and samples of plasma or urine with equal volumes (0.25 mL is sufficient) of a 150 g/L trichloracetic acid solution in water. Combine 0.25 mL of the clear supernate with 1.75 mL of DACA (Aldrich Chemical Co., Milwaukee, WI; cat. no. D14040-6) solution, 10 g/L, in 95% ethanol, and shake vigorously for 15 s. Measure the absorbance at 550 nm after 10 min, using a 10 mm (pathlength) glass cuvette. We used a Model 24 spectrophotometer (Beckman Instruments, Brea, CA) and assayed all samples in duplicate.

To test the reliability of measuring PAH in biological fluids, we added 0.2 mL of a 1.0 g/L stock solution of aqueous PAH to 9.80 mL of plasma obtained from heparinized whole blood (25 USP units of heparin per milliliter of blood); we also added 4.0 mL of the same stock solution to 100 mL of 20-fold diluted urine. We tested for possible interference from p-amino compounds, including sulfanilamide, procainamide, and p-aminobenzoic acid at approximate therapeutic concentrations.

In collaboration with the Departments of Pathology and Obstetrics, we used this method of PAH determination to evaluate effective renal plasma flow in pregnant women at 28–34 weeks of gestation. We used the clearance tests described by Smith (2), with the following modifications: (a) Urine was collected by spontaneous voiding. (b) Urine flow was maintained within the range of 10 to 20 mL/min by water loading before and during the procedure. (c) Final clearance values were not adjusted for surface area, given the difficulty of accurate body surface area approximation for this patient population. (d) Three 30-min periods of timed urine collection were used; however, for four patients, urines from only two periods were actually analyzed, because of obvious errors in urine volumes resulting from spontaneous voiding. These tests were performed as part of an experimental protocol investigating alterations in renal function after dietary manipulation (2). The results presented are those obtained during "control" periods for subjects with normal renal function.

Results and Discussion

At PAH concentrations in the range of 3 to 70 mg/L, the maximum red color is formed after 10 min and is stable to within 0.2% of the maximum value for 1 h. The standard curve yields a slope of 26.74 absorbance units (A) per gram per liter PAH concentration, with a y-intercept of −0.001 A. Standard curves were fit by using Deming's method of linear regression and yielded \( S_{\text{xy}} = 0.010 \) A and 0.2 mg/L, with a resulting Pearson product-moment correlation coefficient >0.9999. Using solutions of known PAH concentration, we could account for 100.1% of the PAH in plasma \( (n = 12, \text{CV} = 0.9\% , \text{range} = 19.8–20.3 \text{mg/L}) \) and 100.4% in dilute urine \( (n = 12, \text{CV} = 0.7\% , \text{range} = 39.9–40.5 \text{mg/L}) \). At concentrations of 20, 100, and 50 mg/L, respectively, the three candidate interferent compounds yielded color formation that was roughly equivalent to that formed by 200, 200, and 50 mg of PAH per liter. Therefore, the concomitant use of other p-amino compounds precludes the performance of accurate PAH clearance tests.

In the renal-clearance studies, samples collected from 14 patients in 15 trials during six months yielded PAH clearances of 829 ± 47.4 mL effective renal plasma flow per minute (mean ± SEM, range 512–1145), which is close to published values (4). The CV for individual patients was 7.07 ± 1.31% (range 1.40–10.9%), which is similar to the previously reported value of 6.3% for normal, nonpregnant volunteers (5).

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References


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