to have concentrations for all the above that were within the normal range. FPG, TG, TC, and UA were measured with the SMA-12 Technicon multi-channel analyzer; HDL-C was measured by the method outlined in the Lipid Research Clinics Manual of Laboratory Operations (6). The intercorrelations are shown in Table 1.

The fact that significant correlation coefficients exist between the above metabolic variables does not prove that they are causally related. However, the measurement of any one of them may be a useful indicator of the presence of another "latent" abnormality. For example, of 18 individuals with a HDL-C of <0.4 g/L, 12 (66%) had a FPG >1 g/L. Only four of 33 subjects with a HDL-C of >0.6 g/L had a FPG of >1 g/L. Conversely, 36 of 45 men with a FPG of >1 g/L had an HDL-C of <0.5 g/L, and 37 of 48 men with a FPG of <1.0 g/L had an HDL-C of >0.5 g/L.

Dr. S. Reddy (Metropolitan Laboratories, Vancouver, B.C.) assisted in providing these data.

References

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<table>
<thead>
<tr>
<th>Table 1. Intercorrelations Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analyte</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>FPG</td>
</tr>
<tr>
<td>TG</td>
</tr>
<tr>
<td>TC</td>
</tr>
<tr>
<td>HDL-C</td>
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</table>

Significant at * p < 0.001; * p < 0.01; * p < 0.05.

Elimination of Interference by Acetobolol and Its Metabolites in Determinations of Urinary Metanephrines

To the Editor:

The method of Pisano (1) is extensively used to determine urinary metanephrines. Unfortunately, it is unreliable for use with patients who are taking β-blocking agents such as propanolol, whose urines show higher blank absorbances before periodization than after because of the simultaneous excretion of an oxidized metabolite (4-hydroxypropanol) (2). We have observed a similar phenomenon in patients who are taking acetobolol (Spectral, Laboratoire Specia, Paris, France), a β-blocking drug widely used as an antihypertensive agent. Such patients may be screened for phaeochromocytomas.

Thus, we examined the effects of acetobolol and its two main metabolites (acetamide and aniline derivatives) (3, 4) on the method and found we could eliminate the drug-related interference by simple extraction with dichloromethane. Our results suggest that the values obtained are on the same order of magnitude as those obtained with the unmodified method.

We measured metanephrines according to the procedure provided with 3.5 X 1 cm ion-exchange columns from Bio-Rad Labs., Richmond, CA 94804 (kit no. 166205). After filtration and acid hydrolysis at pH 0.5 to 1.0, pass 5 mL of urine (adjusted to pH 6.5) through the resin column and wash with boric acid (40 g/L), then with ammonium hydroxide (4 mol/L). To a 3.5-mL aliquot of the ammonium hydroxide eluate, add 100 μL of sodium metabsulfit (100 g/L) to stop the reaction and decrease the absorbance of the metabolite. To prepare the blank, first add 100 μL of the sodium metabsulfit to a second 3.5-mL aliquot of the ammonium hydroxide eluate, followed by 100 μL of the sodium metaperiodate 2 min later. Measure the absorbance of blank and sample at 350 nm and compare the results with values from a nor-metanephrine standard curve.

To study the effects of acetobolol and its metabolites, we added increasing amounts of the drugs to aliquots of urine, to obtain urinary concentrations corresponding to those observed in patients undergoing acetobolol therapy at 200 to 400 mg/day.

We calculated these concentrations from the excretion curves reported by Munn et al. (5), assuming that the compounds are eliminated in the same proportions as those reported by Andreason and Davis (3), i.e., acetobolol 40%, acetamide 50%, and aniline 10%.

We also recorded the absorbance spectra of acetobolol and its metabolites at pH 12 to 13, with a scanning spectrophotometer.

To eliminate the drug interference in urines of 15 patients who were taking acetobolol, we used a simple extraction procedure with dichloromethane (6). Dichloromethane (3.7 mL) was added to both the sample and the reaction blank just before the absorbance measurement. We made two successive extractions; the organic phase was discarded each time. The absorbance of the aqueous phase was measured at 350 nm.

Acetobolol - HCl, its acetamide metabolite [36903 R.P. (Rhone Poulenc Chemicals reference no.)], and its aniline metabolite (29589 R.P.) were obtained through the courtesy of Laboratoire Specia.

When acetobolol and its metabolites were boiled for 20 min at pH 0.5 to 1.0 and subsequently adjusted to pH 12 to 13, we observed (Figure 1) a significant absorbance between 280 and 380 nm (maximum, 325 nm). If the acid hydrolysis is not performed, the absorbance peak is shifted to a slightly lower wavelength (315 nm) and appears sharper, showing a much lower absorbance between 350 and 360 nm (not shown).

Addition of acetobolol to urines at concentrations corresponding to those observed in patients undergoing therapy at a daily dose of 200 to 400 mg showed little effect on the results for metanephrines (ΔA between samples and blanks was 0.010 to 0.015). For all the concentrations tested we observed a slight increase in the blank absorbance (increasing from 0.035 to 0.081 when acetobolol was increased from 0 to 75 mg/L), but we never obtained any negative difference between sample and blank absorbances. Addition of the acetylated metabolite showed no effect on metanephrine values.

Addition of the aniline metabolite led to a negative difference between blank and sample absorbance: ΔA = -0.003 at 5 mg/L and -0.020 at 25 mg/L. When a mixture of the three compounds was added, we obtained approximately the same results as with aniline metabolite alone. If the acidic hydrolysis step is not performed, however, the three compounds have no effect on the metanephrine values, ΔA values between samples and blanks being 0.008 for
acebutolol (15 mg/L), 0.015 for acetamide (20 mg/L), 0.014 for aniline (5 mg/L), and 0.023 for a mixture of the three. Extraction with dichloromethane had no effect on the values obtained during the preparation of the normetanephrine standard curve.

Metanephrine determinations on urines of patients given acebutolol therapy showed either a negative or a negligibly small difference between blank and sample absorbances. After extraction with dichloromethane, in all the cases the blank absorbance decreased (Table 1); this decrease occurred in both the blank and the sample, but was much more pronounced in the blank. The metanephrine concentrations measured after extraction were in the same range as in patients not taking acebutolol (i.e., 300 to 1500 μg/24 h).

Our results demonstrate that acebutolol and its two main urinary metabolites show a significant absorbance at pH 12 to 13 in the 350–360 nm region. Furthermore, hot acidic hydrolysis leads to an increase in absorbance, confirming that acebutolol is unstable in these conditions (3); therefore, the acidic step of the Pisano method might be an important factor in the development of drug-related interference, by releasing compounds that absorb at 350 and 360 nm.

That the absorbance of the mixture of the three compounds is lower than the absorbance of the acetyl metanephrine alone might be attributed to the butyramide moiety of acebutolol.

When this procedure is applied to urine of patients taking acebutolol, the drug and its metabolites do not interfere and the absorbance differences observed correspond only to urinary metanephrine content in the physiological range. We conclude that dichloromethane extraction can be used routinely for determinations of urinary metanephrines in patients who are undergoing therapy with acebutolol.

Table 1. Absorbance Readings for Urines of 15 Patients Receiving Acebutolol, with and without Dichloromethane Extraction

<table>
<thead>
<tr>
<th>ΔA (sample - blank)</th>
<th>Metanephrine, μg/24 h</th>
<th>Extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>No extraction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.005</td>
<td>220</td>
<td>0.015</td>
</tr>
<tr>
<td>0.005</td>
<td>140</td>
<td>0.011</td>
</tr>
<tr>
<td>0</td>
<td>490</td>
<td>0.026</td>
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<tr>
<td>0.005</td>
<td>1300</td>
<td>0.082</td>
</tr>
<tr>
<td>0.053</td>
<td>750</td>
<td>0.023</td>
</tr>
<tr>
<td>0.005</td>
<td>350</td>
<td>0.015</td>
</tr>
<tr>
<td>0.002</td>
<td>150</td>
<td>0.011</td>
</tr>
<tr>
<td>0.004</td>
<td>530</td>
<td>0.038</td>
</tr>
<tr>
<td>0.021</td>
<td>210</td>
<td>0.013</td>
</tr>
<tr>
<td>0.018</td>
<td>290</td>
<td>0.017</td>
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<tr>
<td>0</td>
<td>150</td>
<td>0.009</td>
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</table>

References


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Factors Affecting Ultrafiltration-Assessed Values for Drug Binding

To the Editor:

Several techniques have been reported for assessing drug binding to plasma proteins, the two most popular being equilibrium dialysis and ultrafiltration. Both of these procedures rely on a membrane that is permeable to small (drug) molecules but impermeable to macromolecules; however, they differ considerably with regard to their underlying assumptions, method of separation, and speed of assay. The literature on the binding of the anti-asthmatic drug, theophylline, in human serum or plasma as assessed by equilibrium dialysis (1, 2) and ultrafiltration (3-6) reveals large intra- and inter-technique variability, with binding values ranging from 28 to 80%. Several investigators have alluded to the possibility that nonspecific drug binding may distort the values obtained in both equilibrium dialysis (7-10) and ultrafiltration (3, 11, 12). However, these methodological considerations are almost always disregarded when such data are used to explain clinical observations. We therefore compared the two techniques with respect to the binding of four drugs in human serum and plasma.

The materials (and their sources) were as follows: theophylline and acetaminophen (Sigma Chemical Co., St. Louis, MO 63178); warfarin (Frosst, Pointe, Claire, P.Q. H9R 4P8, Canada); mannitol (J. T. Baker Chemical Co., Phillipsburg, NJ 08865); [14C]mannitol (New England Nuclear, Lachine, P.Q. H8T 3C9); HPLC-grade solvents (Cal-