by analysis of variance (repeated-measures design) and Student's t-test was used for paired and grouped observations. The level of significance was α=0.05.

The mean maximum heart rate (± SD) was 190.9 ± 7.1 beats/min, which was 95–102% of the maximum rate predicted for the volunteers. Baseline and peak systolic blood pressure were 121.0 ± 13.4 and 176.0 ± 13.8 mmHg, respectively. Corresponding baseline and diastolic blood pressure were 80.3 ± 6.0 and 73.3 ± 8.8 mmHg, respectively. VO₂ max was 53.5 ± 7.7 mL·kg⁻¹·min⁻¹. Peak V̇E was 143.5 ± 29.2 L/min. The respiratory exchange ratio at the time of maximum exertion was 1.24 ± 0.07. Serum lactic acid concentrations immediately after exercise ranged from 15.8 to 18.6 mmol/L (17.0 ± 0.85 mmol/L).

Plasma chromogranin A concentration rose from a baseline value of 40.94 ± 10.23 μg/L to a peak of 55.98 ± 3.54 μg/L (P <0.005) at 15 min postexercise (Figure 1). Baseline plasma chromogranin A concentration was similar to that reported in normal healthy subjects (45.0 ± 3.0 μg/L) (5). A peak chromogranin A concentration at 2 min after high-intensity exercise was reported by Takiyuddin et al. (15), indicating that peak chromogranin A concentrations may occur earlier than 15 min postexercise. The increase in chromogranin A concentrations after exercise in our study was modest, reaching values less than those previously reported in patients within essential hypertension (5) and considerably less than the plasma concentrations seen in patients with proven pheochromocytomas who have plasma concentrations generally >100 μg/L and often >1000 μg/L (6). Because strenuous exercise did not increase plasma chromogranin A concentrations into the range seen in patients with pheochromocytomas, restrictions on physical activity before measuring chromogranin A in hypertensive patients with suspected pheochromocytoma may be unnecessary, and physical activity before such measurements should not obscure the diagnostic utility of plasma chromogranin A measurement in the screening process.

We thank J. Boysian, M. Haruno, and C. Healy for their expert assistance in conducting the treadmill studies.

Fig. 1. Plasma concentrations of chromogranin A after acute exercise in eight physically active men
Mean ± SD; *P<0.005
-properties of an acidic protein from chromaffin granules of bovine adrenal medulla.

References
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REMEDY Drug Profiling System
Readily Distinguishable between Cyclobenzaprine and Amitriptyline
In Emergency Toxicology Urine Specimens

To the Editor:
Cyclobenzaprine (Flexeril) is a skeletal muscle relaxant indicated for relief of spasms associated with acute or chronic skeletal muscle pain. Structurally, cyclobenzaprine differs from amitriptyline only by a double bond in the central cyclohexyl ring; therefore, the drug is often difficult to distinguish from amitriptyline by methods used in emergency toxicology testing. Cyclobenzaprine cross-reacts in the EMIT and Abbott ADx tricyclic antidepressant immunoassays (1, 2). It has retention times similar to amitriptyline in popular gas-chromatographic and high-pressure liquid-chromatographic (HPLC) systems and is difficult to resolve from amitriptyline in classical thin-layer chromatographic systems (3). Cyclobenzaprine has staining characteristics in the TOXI-LAB commercial thin-layer chromatography system different from those of amitriptyline. In stage III, TOXI-LAB A, amitriptyline fluoresces pink, whereas cyclobenzaprine gives an orange color. However, the intensity of the fluorescence is concentration-dependent and identification may be questionable.

The extra double bond in cyclobenzaprine produces an ultraviolet spectrum readily distinguishable from amitriptyline or other common tricyclic antidepressants. Cyclobenzaprine has a maximum absorbance at 290 nm in aqueous acid with no alkaline shift. Amitriptyline has a maximum absorbance at 239 nm in aqueous acid. HPLC with photodiode array detection readily distinguishes between cy-
clobenzaprine and other tricyclic amines (3). With variable-wavelength detectors, the analyst can monitor the HPLC effluent at two wavelengths and then calculate the ratio of the absorbance of one wavelength to the other (3, 4). For example, the ratios of absorbances of clobenzaprine and amitriptyline at 250 and 260 nm are 2.06 and 2.60, respectively (3). Similarly, questionable spots may be eluted from thin-layer plates and analyzed in a spectrophotometer scanning from 320 to 220 nm. However, this time-consuming, labor-intensive procedure is seldom applied in laboratories performing emergency toxicology testing.

When confirming results by gas chromatography/mass spectrometry (GC/MS), both clobenzaprine and amitriptyline produce a base peak at m/e 58; however, when scanning from 60–500 mass units, clobenzaprine gives a base peak at m/e 215, whereas amitriptyline has a base peak at m/e 202 (5). Both m/e 215 and 202 ions are present in the spectrum of each drug. Therefore, if ion scanning is performed below m/e 60, the abundance of the m/e 58 ion common to both drugs may be so great that the differences in the relative abundance at 215 and 202 m/e are not immediately obvious. Amitriptyline also produces mass ions at 214 and 217 m/e, but this cluster around the 217 ion may not always be evident with clobenzaprine. However, this type of sophisticated full-scan GC/MS drug screening is beyond the capabilities of the vast majority of clinical laboratories.

We have observed that the automated HPLC REMEDI Drug Profiling System from Bio-Rad Labs (Hercules, CA), which uses a fast-scan ultraviolet detector, will consistently and correctly identify clobenzaprine or amitriptyline. In the REMEDI system the relative retention times (RRTs) of clobenzaprine and amitriptyline are the same: 3.018 and 3.023, respectively. However, the absorbance spectra are markedly different (Figure 1) and the identification algorithm of the system correctly chooses which of the drugs is present. We have also observed, in a case of clobenzaprine overdose, two peaks with RRT values in the REMEDI system similar to those of nortriptyline and 10-hydroxyamitriptyline but having absorbance spectra indistinguishable from that of clobenzaprine. Pure reference material was not available to enable us to verify these peaks with RRTs of 2.268 and 0.521. Nevertheless, we suspect they are the major urinary metabolites of clobenzaprine, nor-clobenzaprineline and 10-hydroxyclobenzaprineline. In cases of clobenzaprine overdose, analysts who use the REMEDI system should look not only for the parent drug but also for the additional metabolite peaks at RRT values given above and compare their absorbance spectra with that for clobenzaprineline. Should a sample contain both clobenzaprineline and amitriptyline at relatively equal concentrations, correct identification of either drug may be impossible by REMEDI or other methods routinely available in clinical toxicology laboratories.

References

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No Time-Dependent Change in Cytochrome P-450 2D6 Phenotyping Results during HPLC Analysis

To the Editor:
O-Demethylation of the cough suppressant dextromethorphan (DM) to form its main metabolite, dextrorphan (DX), is mediated by the polymorphic cytochrome P-450 2D6. After a single dose of DM, urinary molar concentrations of DM and DX can be determined by HPLC, and the urine molar ratio can be used to define the extensive metabolizer (EM) and poor metabolizer (PM) phenotypes for P-450 2D6 polymorphism (1, 2). Because our HPLC assay takes 20 min per sample, we were concerned that, during batch analysis of a large number of urine samples, drug degradation could occur while the samples loaded onto the autosampler were awaiting injection. Such degradation could potentially affect measured concentrations of DM and DX and therefore the oxidation phenotyping of subjects. The objective of this study was to investigate the potential for sample degradation during automated HPLC analysis for DM and DX and the influence of batch analysis on the phenotyping results.

Four-hour urine specimens were collected from 32 subjects after an oral 30-mg dose of DM. Each urine sample was divided into portions and frozen at −20 °C until HPLC analysis for DM and DX. On the day before analysis, a portion was thawed and 1 mL was incubated overnight with β-glucuronidase at 37 °C for 18 h to ensure complete hydrolysis of the conjugates. The samples were then extracted with a mixture of hexane and butanol (96:5, by vol). The organic layer was evaporated at 37 °C under a stream of nitrogen. The resulting residue was reconstituted with mobile phase and separated into two equal portions.

To evaluate whether sample degradation might occur during the HPLC analysis, we placed the two portions of the reconstructed extract in the autosampler at positions that gave an interval of 8 h between the injection of each portion (to simulate analysis time on a normal work day). From each portion, 40 μL was injected into the column and analyzed by fluorescence detection. The results for the sample portions were labeled HPLC analysis 1 and 2, respectively. Standard curves for DM and DX were plotted as peak areas vs concentration. Peak areas of the subjects’ samples and the regression equation of each standard curve were used to calculate the DM and DX concentrations, which were then converted to molar concentrations. The molar ratios of DM to DX from each pair of split urine sample extracts were then compared by a Student’s t-test (two-tailed). To establish the reproducibility of the results.

Fig. 1. REMEDI comparison of the ultraviolet spectra of clobenzaprineline (dotted line) and amitriptyline (solid line) in plasma or urine.

![Graph](image-url)