

derivatives. The stereoselective reduction of the carbonyl group adjacent to the benzene ring of bupropion is reported to favor the *threo* amino alcohol metabolite in humans (1, 2). We believe that bupropion use can cause false-positive results in an assay designed to detect amphetamine abuse, as evidenced by the following data.

A patient in no acute distress was admitted to this hospital's inpatient psychiatry unit for treatment of depression. The patient had a history of polysubstance abuse and had been prescribed bupropion (300 mg per day) three weeks before admission. On admission a drug screen (3) detected bupropion metabolites in the patient's blood. The patient's urine was positive for "amphetamines" in the Emit II monoclonal immunoassay (Syva Co., San Jose, CA) performed on a Hitachi 717 analyzer (Boehringer Mannheim, Indianapolis, IN), but was negative for methamphetamine and amphetamine by liquid chromatography (3). The patient denied use of drugs other than bupropion and ethanol within the past month. We then quantitatively analyzed the urine by liquid chromatography as stated (3), except that we used a 150 × 4.6 mm ABZ column (Supelco, Bellefonte, PA) and a mobile phase of 350 mL of acetonitrile mixed with 550 mL of a pH 6.4, 30 mmol/L phosphate buffer. Retention times for the analytes were: morpholinol metabolite, 2.7 min; *threo* amino metabolite, 3.0 min; *erythro* amino metabolite, 3.4 min; bupropion, 4.3 min; and the internal standard (proprietyline), 6.3 min. Urine concentrations of bupropion-related substances were: bupropion, 12 mg/L; *erythro* amino metabolite, 45 mg/L; *threo* amino metabolite, 335 mg/L; and morpholinol metabolite, 19 mg/L.

We further investigated the role of bupropion in the immunoassay by preparing saline solutions of bupropion and three of its major metabolites and analyzing these solutions with the monoclonal Emit II immunoassay. The results (Table 1) indicate that all four compounds cross-react to some extent. The *erythro* amino alcohol metabolite appeared to cross-react the most; the *threo* and morpholinol metabolites and bupropion itself were less reactive. On a molar basis, the assay is quite selective: even the *erythro* metabolite at 417 μmol/L gave only about the same response as the 6.7 μmol/L methamphetamine calibrator.

The patient's urine exhibited reac-

Table 1. Reactivities of bupropion and metabolites in Emit II assay of amphetamines.

Conc tested, mg/L	Reactivity, mA/min at 340 nm			
	Bupropion	Morpholinol metab.	Erythro metab.	Threo metab.
0	-1	2	-1	3
10	0	6	6	1
20	3	8	18	9
50	12	16	33	15
100	19	28	47	27
200			63	48
300	52	58		57
400			85	

The reactivity of the negative calibrator was set at 0 on the Hitachi 717. The calibrator containing 1 mg/L *d*-methamphetamine gave a reading of 48 mA/min; urine samples with values greater than that of this calibrator are considered to be "positive" according to the manufacturer.

tivities (1 unit of reactivity being defined as 1 mA/min at 340 nm) in the 60–65 range (see Table 1), significantly greater than the 48 mA/min for the 1 mg/L methamphetamine calibrator. The combination of the high concentration of the *threo* metabolite and its modest cross-reactivity (Table 1) explains a large portion of the assay reactivity observed in the patient's urine. Despite its much lower concentration, the *erythro* metabolite may also have been a significant contributor; the morpholinol metabolite and the parent drug probably were not.

This patient's bupropion dosage regimen was not high. Welch et al. (4) reported urinary bupropion metabolite concentrations after a single 200-mg oral dose given to seven healthy male volunteers; the concentrations of unconjugated urinary metabolites were lower than those reported here. It is possible, however, that at steady-state the urinary bupropion metabolite concentrations will be higher than Welch et al. reported (4). We recently encountered a random urine sample from a patient receiving 450 mg per day bupropion and found: *erythro* metabolite, 20 mg/L; *threo* metabolite, 90 mg/L; morpholinol metabolite, 29 mg/L; and bupropion, 7 mg/L. This patient's urine was also positive for amphetamines by the Emit II assay, suggesting that typical doses may generate sufficient metabolites to cause "false positives."

From these findings, we believe bupropion should be added to the list of psychotropic drugs/metabolites that may cross-react in certain amphetamine drug-of-abuse immunoassays (5, 6).

References

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Demonstration of Macroamylasemia by Polyethylene Glycol (PEG) Precipitation Requires Correct PEG Concentration

To the Editor:

We recently encountered an anomaly while following a method published in this journal for confirming macroamylasemia by polyethylene glycol (PEG) precipitation (1).

A 43-year-old man (patient A) complaining of abdominal pain was found to have persistently increased amylase activity. The surgeon could find no cause for this and suspected macroamylasemia. Analysis of a serum and a urine sample gave: serum amylase 388 IU/L (reference range 10–90), serum creatinine 110 μmol/L (50–120), urine amylase 156 U/L (25–550), and urine creatinine 12 780 μmol/L (9000–12 000). The fractional excretion of amylase (amylase clearance/creatinine clearance) was calculated as 0.3% (reference range 2.3–6.3%), which was regarded as consistent with reduced renal clear-

ance of amylase due to macroamylasemia.

As a confirmatory measure, we carried out PEG precipitation as described by Isham et al. (1), based on the method of Levitt and Ellis (2). Isham et al. added 0.2 mL of a 120 g/L solution of PEG 6000 to one 0.2-mL aliquot of serum and 0.2 mL of water to another (1). Both aliquots were incubated at 37 °C for 10 min, then centrifuged (5000g, 10 min), and the supernates were analyzed for amylase activity. If 73% or more of the amylase activity was precipitated by PEG as compared with water, macroamylase was considered to be present; PEG precipitation of <52% of amylase activity was expected with amylase of normal molecular mass (1-3).

We were surprised to find that only 7% of the amylase activity was precipitated by the 120 g/L PEG (Table 1), which strongly suggested amylase of normal molecular mass and was at variance with the fractional excretion result. In view of the apparent anomalous result, we investigated serum samples sent for amylase measurement in eight patients with pancreatitis, all of whom showed a similar precipitable amylase (6-8%).

The PEG precipitation method initially described by Levitt and Ellis (2) and subsequently by van Deun et al.

Table 1. Percentage of amylase activity precipitated from patients' serum by PEG solutions of different concentrations.

	PEG added, g/L	
	120	240
Patient A	7	80
Patient B	21	93
Pancreatitis patients	6-8 ^a	15-23 ^a

^a Range for eight patients.

(3) used a PEG solution of 240 g/L (with a final concentration of 120 g/L) to precipitate any macroamylase. When we used this higher concentration of PEG (i.e., 240 g/L instead of the 120 g/L described by Isham et al.), 80% of the amylase in the serum of patient A was precipitated, indicating the presence of macroamylasemia and consistent with the results of the fractional amylase excretion.

In a second patient (B) with a consistently increased serum amylase and a fractional amylase excretion (1.2%) suggestive of macroamylasemia, significant precipitation of the serum amylase activity was achieved with 240 g/L PEG but not with 120 g/L PEG (Table 1). Although the proportion of amylase in patients with pancreatitis was a little higher with 240 g/L PEG than with 120 g/L PEG,

the results were all well below the maximum 52% precipitation expected for amylase of normal structure (Table 1).

We conclude that care needs to be taken to ensure that the correct PEG concentration is used to demonstrate absence or presence of macroamylasemia.

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