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S. C. Harris,[1] *M.D.; H. E. Hamilton,*[2] *M.S.; and*
J. E. Wallace,[3] *Ph.D.*

Incidence of Cocaine Metabolites in Urine Specimens from Medical Examiners' Cases

In their recent report on the toxicology and epidemiology of cocaine, Finkle and McCloskey summarized data from 27 medical examiners' offices [*1*]. Methods used by the contributing laboratories varied widely in sensitivity and specificity and included thin-layer chromatography, gas-liquid chromatography, mass spectrometry, spectrophotometry, radioimmunoassay, and enzyme-linked immunoassay. The sensitivity range generally reported by participating laboratories was 1 to 2 μg/ml cocaine. Sixteen laboratories relied on detecting only parent cocaine, while eleven laboratories also analyzed for benzoylecgonine, the principal cocaine metabolite.

This report describes the use of a radioimmunoassay procedure to detect cocaine metabolites in urine specimens obtained from medical examiners' laboratories. Prior evaluation in our laboratory had shown that this technique reliably detects urinary cocaine metabolite concentrations of 0.025 μg/ml [*2,3*]. Concentrations of this magnitude had been found in the urines of human volunteers as long as three to six days after nasal inhalation of a single dose (1.5 mg/kg body weight) of cocaine hydrochloride [*2*].

Materials and Methods

Analyses

Radioimmunoassay was performed according to the procedure of Cleeland et al [*4*]. This assay determines both cocaine and benzoylecgonine with an approximately equivalent sensitivity, but it detects neither ecgonine nor structurally related compounds. Therefore, results from this assay, although reported herein as μg/ml of benzoylecgonine, are actually μg/ml of benzoylecgonine equivalents, representing the combined concentrations of cocaine and benzoylecgonine.

Antibody and ^{125}I-antigen were obtained from Hoffman-La Roche Inc., Nutley, N. J. The procedure involves sequential addition of urine specimen, labeled antigen, and antibody, followed by a brief incubation at ambient temperature. The bound label is then precipitated with ammonium sulfate and the supernatant is removed for counting of the unbound label. One-minute counts were obtained on a Packard AutoGamma scintilla-

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[1]Chief, Special Chemistry, Department of Pathology and Medical Research Service, Audie L. Murphy Veterans Administration Medical Center, San Antonio, Tex., and assistant professor, Department of Pathology, The University of Texas Health Science Center at San Antonio.

[2]Assistant instructor, Department of Pathology, The University of Texas Health Science Center at San Antonio; currently, supervisor, Toxicology Laboratory, Sealy Hospital, The University of Texas Medical Branch, Galveston.

[3]Associate professor, Department of Pathology, The University of Texas Health Science Center at San Antonio.

tion spectrometer, Model 5320. Benzoylecgonine standards were prepared with cocaine-negative pooled human urine. All assays were performed in duplicate. Prior studies in this laboratory indicated that 0.005 μg/ml of cocaine or benzoylecgonine in urine may be detected by this radioimmunoassay technique [3]. However, to avoid reporting "false positives," a 0.025 μg/ml threshold level was used for evaluating clinical specimens [2]. All specimens determined to have an apparent benzoylecgonine concentration of 0.005 μg/ml or greater were subjected to confirmatory radioimmunoassay at a later date. All specimens determined to have an apparent benzoylecgonine concentration of greater than 0.1 μg/ml were also analyzed in duplicate by EMIT® (Enzyme-Multiplied Immunoassay Technique, Syva Corp., Palo Alto, Calif.), an immunologic assay which shares a number of similarities with radioimmunoassay (RIA) in principle and technique and which has an advertised detection limit for benzoylecgonine of 1.6 μg/ml for qualitative work.

Specimens

Autopsy urine specimens were obtained from the medical examiners' laboratories of three major Texas metropolitan areas, Fort Worth, San Antonio, and Houston. The samples were randomly selected from specimens that had been stored at −20°C, a temperature at which the formation of ecgonine from benzoylecgonine is minimal. Specimens were not submitted on the basis of suspected drug use. To preserve the privacy of the subjects, all specimens were submitted identified only by code numbers. Most of the specimens were from cases originating during 1974 through 1977, but some dated back to 1972. Case reports were examined by personnel of the respective submitting laboratories for all specimens having a benzoylecgonine concentration of 0.025 μg/ml or greater to determine if any pattern such as mode of death or usage of other drugs was evident.

Results and Discussion

The geographic and chronologic distribution of all specimens is presented in Table 1. Of 585 autopsy urines analyzed, 15 contained more than 0.025 μg/ml benzoylecgonine (2.6%). None of these 15 specimens had been previously reported as containing cocaine metabolites. This high percentage of positive specimens would seem to justify making cocaine metabolite analysis a part of the routine forensic toxicologic examination.

The distribution of urinary benzoylecgonine concentrations found in the 15 positive specimens is presented in Table 2. All specimens shown by RIA to have greater than 0.4 μg/ml benzoylecgonine were also positive by EMIT. This confirmed our previous studies, using control urine samples with added known amounts of benzoylecgonine, that

TABLE 1—*Geographic and chronologic distribution of specimens.*[a]

Year	San Antonio	Fort Worth	Houston	Total
1972	5/70	...	...	5/70
1973	1/16	...	...	1/16
1974	1/60	1/53	...	2/113
1975	1/67	0/6	...	1/73
1976	1/79	0/36	0/9	1/124
1977	...	4/143	1/46	5/189
Total	9/292 (3.1%)	5/238 (2.1%)	1/55 (1.8%)	15/585 (2.6%)

[a]Presented as a ratio in which the numerator is the number of specimens having at least 0.025 μg/ml benzoylecgonine equivalents and the denominator is the total number of specimens assayed.

TABLE 2—*Distribution of urinary benzoylecgonine concentrations.*

Source and Date of Specimen	Radioimmunoassay[a,b]	EMIT[b]
San Antonio, 1976	0.03	NA[c]
San Antonio, 1973	0.04	NA
San Antonio, 1972	0.06	NA
San Antonio, 1972	0.07	NA
Fort Worth, 1977	0.08	NA
San Antonio, 1972	0.14	NDA[d]
San Antonio, 1972	0.20	NDA
Fort Worth, 1977	0.22	NDA
San Antonio, 1974	0.23	NDA
San Antonio, 1972	0.28	NDA
Fort Worth, 1974	0.47	0.6
Fort Worth, 1977	0.70	0.9
San Antonio, 1975	>1.00	1.7
Houston, 1977	>1.00	1.7
Fort Worth, 1977	>1.00	4.2

[a]Each value given here is the mean of the initial duplicate determinations and of all subsequent confirmatory assays.
[b]Reported as μg/ml benzoylecgonine equivalents.
[c]Not analyzed.
[d]No detectable amount.

had suggested that benzoylecgonine concentrations as low as 0.4 to 0.5 μg/ml could be detected by EMIT [*3*].

It should be noted that only 5 of the 15 cocaine-positive urines identified in this study had benzoylecgonine concentrations exceeding 0.4 μg/ml (Table 2). Thus as many as two thirds of the positive specimens from this study would have been missed by EMIT or by routine thin-layer or gas-liquid chromatographic procedures, all of which have detection limits of 0.4 to 1.6 μg/ml [*3,5,6*]. Nonradioisotopic procedures applicable to measuring concentrations of benzoylecgonine below 0.5 μg/ml are generally confined to gas-liquid chromatographic methods that use electron-capture, nitrogen-specific, or mass-spectrometric detection.

Examination of the case information for the 15 subjects whose urinary benzoylecgonine concentrations exceeded 0.025 μg/ml revealed an association, in all but possibly one case, with other drugs of abuse, violence, or both (Table 3). Furthermore, it was noted that heroin/morphine was the other drug most frequently found in these subjects. Morphine had not been tested for in 4 of the 15 cases, but significant amounts of morphine had been detected in the bile or urine in 8 of the 11 other cases. The four specimens not initially examined for morphine were therefore assayed in duplicate by EMIT; two of these specimens were positive. Thus 10 of the 15 cocaine-positive specimens also contained morphine. This result confirms the previously reported high incidence of opiate use among cocaine users [*1*]. It is thus apparent that if opiate metabolites are not looked for in routine specimen evaluation, then a history of cocaine use in a case or the detection of cocaine metabolites in a sample should automatically result in examination for opiate metabolites to provide a more accurate and comprehensive toxicologic evaluation.

To examine this association of cocaine and opiate abuse from another perspective, 1447 urine specimens from a Fort Worth, Tex. methadone maintenance program were screened for benzoylecgonine by RIA. Ninety-four of the 1447 urine specimens were positive. Thus a significantly higher incidence of cocaine use was found in the opiate user population (6.5%) than in the population reflected in the medical examiners' case load (2.6%), $P < 0.001$ by Fisher's exact test. Once again, the need for sensitive detec-

TABLE 3—*Case information for cocaine-positive specimens.*

Source and Date of Specimen	Benzoylecgonine Concentration, μg/ml	Morphine Concentration, μg/ml[a]	Comments from Medical Examiners' Records
San Antonio, 1976	0.03	NA[b] (−)	"natural death"; quinine detected
San Antonio, 1973	0.04	67, 87[c]	heroin
San Antonio, 1972	0.06	27[c]	heroin
San Antonio, 1972	0.07	57	heroin
Fort Worth, 1977	0.08	NA (+)	gunshot
San Antonio, 1972	0.14	304[c]	heroin
San Antonio, 1972	0.20	120[c]	heroin
Fort Worth, 1977	0.22	NDA[d]	accidental carbon monoxide poisoning; marijuana present[e]
San Antonio, 1974	0.23	NA (−)	gunshot
San Antonio, 1972	0.28	108[c]	heroin
Fort Worth, 1974	0.47	NDA	dextroamphetamine detected
Fort Worth, 1977	0.70	NDA	accidental carbon monoxide poisoning; marijuana present[e]
San Antonio, 1975	>1.00	131[c]	heroin overdose
Houston, 1977	>1.00	45	heroin, gunshot
Fort Worth, 1977	>1.00	NA (+)	gunshot

[a]Morphine concentration in urine, unless otherwise noted.
[b]Not initially analyzed for morphine; sign in parentheses indicates EMIT results from subsequent analysis.
[c]Morphine concentration in bile.
[d]No detectable amount.
[e]These two individuals were found together.

tion methods was seen; 75 of the 94 urines contained benzoylecgonine concentrations of less than 1.0 μg/ml.

Summary

A radioimmunoassay procedure was used to determine the incidence of cocaine metabolites in postmortem urine specimens obtained from three medical examiners' laboratories in Texas. Approximately 2.6% of the 585 specimens contained cocaine or benzoylecgonine. The need to assay medicolegal specimens for cocaine and its metabolites with sensitive methods was demonstrated; none of the positives detected had been reported as containing cocaine or its metabolites. Morphine was detected in 67% of the cocaine-positive samples, suggesting that a thorough toxicologic examination should include an assay for opiate if cocaine use is suspected or found.

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Address requests for reprints or additional information to
Steven C. Harris, M.D.
Department of Pathology
Audie L. Murphy Veterans Administration Medical Center
7400 Merton Minter Blvd.
San Antonio, Tex. 78284