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Cocaine and Benzoylecgonine Excretion in Humans

Cocaine is rapidly and extensively metabolized. Studies with ^{3}H-labeled cocaine have demonstrated that the drug is biotransformed to at least ten metabolites in the rat, with the concentration of unchanged cocaine in the urine being less than 10% that of the metabolite benzoylecgonine [*1*]. Information on the metabolism and excretion of cocaine in man is limited. Montesinos [*2*] has reviewed investigations performed in the 1950s and 1960s on chewers of coca leaf. However, these studies not only used analytical methods less specific and sensitive than those available today, but also employed routes of administration different from those currently used for therapy [*3*] or abuse. Although several recent investigations have demonstrated that cocaine is extensively metabolized to benzoylecgonine in man [*4,5*] and that plasma cocaine levels diminish rapidly [*6*], knowledge concerning the extent and rate of metabolism and excretion of parent drug and metabolite is almost nonexistent. Wallace et al [*7*] measured the urinary excretion of cocaine and benzoylecgonine in ten patients who had been administered cocaine hydrochloride prior to rhinoplastic surgery. These studies were limited to the initial 24 h after drug administration and consisted of three consecutive 8-h collective specimens per patient. It was observed that the excretion of cocaine and benzoylecgonine diminishes rapidly, that benzoylecgonine concentrations in urine consistently exceeded the corresponding cocaine concentrations by a significant amount, and that the benzoylecgonine/cocaine ratios of urine concentrations varied significantly, demonstrating the impracticability of attempting to predict cocaine concentrations from benzoylecgonine data, or conversely.

The difficulties of analyzing benzoylecgonine are well documented. This polar, highly hydrophilic compound resists efficient extraction by simple organic solvents, ion-exchange resins, or styrene-divinylbenzene copolymers [*8,9*]. Separation by gas chromatography requires derivatization of the molecule [*7*], and single detection reagents commonly employed in thin-layer chromatography often do not provide sufficient sensitivity [*10*]. In recent years, several methods have been developed which provide for the specific and sensitive determination of benzoylecgonine, including thin-layer chromatographic (TLC) [*10*], gas-liquid chromatographic (GLC) [*7*], enzyme-immunologic (EMIT) [*11*], radioimmunoassay (RIA) [*12*][4] and hemagglutination inhibition techniques.[5] Wallace et al [*13*] recently conducted a detailed

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[4]J. G. Christenson, E. Squires, R. Cleeland, and E. Grunberg, unpublished data.

[5]Toxi-Pak® Immune HI® Cocaine, J. T. Baker Chemical Co.

evaluation of four of the more sensitive and specific analytical methods, assaying human urine spiked with varying concentrations of cocaine or benzoylecgonine. It was observed that each of the four methods examined had unique advantages and limitations, indicating that each method was particularly applicable to specific analytical requirements. For example, gas chromatography was the most precise method, but it was also the most time-consuming; radioimmunoassay was the most sensitive method, but its inability to differentiate between parent drug and metabolite limited its use in pharmacokinetic studies.

This report presents urinary cocaine and benzoylecgonine concentration data for six individuals who received acute administrations of 1.5 mg cocaine hydrochloride per kilogram body weight via nasal inhalation. The dosage is representative of therapeutic use, and the route of administration is one that is used frequently by cocaine abusers. The study provides a more discrete (six urine collections within the initial 24 h) and a more extensive profile, with collections continuing for seven days following administration, than that of any previously compiled on human subjects. The principal means of analysis was the gas chromatographic procedure of Wallace et al [*7*], previously shown to provide a superior precision [*13*]. In addition to the GLC analyses, the specimens were also analyzed by TLC [*10*], EMIT,[6] and RIA to provide an evaluation of these analytical techniques. Particular emphasis was placed on determining the duration with which cocaine or its metabolites may be detected with confidence; therefore, this was a study with major forensic implications.

Materials and Methods

Subjects, Dosage, and Sampling

The subjects were six healthy adults, three males and three females, ranging in age from 23 to 34 years (Table 1). Three of the subjects received no medication during the study or in the week immediately prior; two subjects (A and D) on occasion ingested small amounts of Tylenol® or alcoholic beverages, and one subject (E), experiencing a mild cold, received aspirin, Robitussin®, and Sudafed® in addition to moderate amounts of various alcoholic beverages during the study.

Cocaine hydrochloride, 1.5 mg/kg body weight, was applied to the nasal mucosa via inhalation through a short straw; U.S. Pharmacopeia grade crystalline cocaine hydrochloride was previously pulverized into a fine powder. The subjects remained under the observation of a physician from 30 min before to 2 h after drug administration; blood pressure and respiration were monitored regularly during this time. The subjects were free to pursue normal activities throughout the remainder of the study.

Urine specimens were collected in polyethylene bottles containing 100 to 200 mg sodium fluoride. A specimen was collected prior to cocaine administration and inclusive pools were obtained 1, 2, 4, 8, 12, 24, 48, 72, 120, 144, and 168 h subsequent to medication. After the specimens were received in the laboratory, urine volume and pH were recorded and the specimens were aliquoted into 20-ml vials and stored at −20°C to await analysis.

To ascertain the extent to which cocaine remained unabsorbed on the nasal mucosa, nasal swabs were obtained at 4 and 8 h. These samplings consisted of cotton-tip applicators (Johnson & Johnson cotton buds) wetted in sterile water being rubbed against the nasal mucosa by the subject. The cotton-tip ends were placed in glass vials and stored at −20°C to await gas chromatographic analysis.

Analysis

Five-millilitre aliquots of urine were analyzed for cocaine and benzoylecgonine by the GLC [*7*] and TLC [*10*] procedures of Wallace et al. Benzoylecgonine was also determined

[6] Syva Corp., Palo Alto, Calif.

TABLE 1—*Clinical data summation.*

Patient	Sex	Age	Weight, kg	Cocaine Dose, mg	Cocaine Absorbed, mg[a]	Initial 24 h		168 h
						Cocaine Excreted, mg	Benzoylecgonine[b] Excreted, mg	Cocaine + Benzoylecgonine[b] Excreted, mg
A	f	29	50.0	66.96	65.78	1.21	10.50	14.16
B	f	23	43.2	57.86	55.86	0.10	13.52	13.62
C	m	28	68.2	91.34	90.24	0.86	22.10	26.65
D	f	34	72.7	97.40	95.91	4.28	18.63	24.33
E	m	34	79.9	107.14	104.29	1.84	39.11	44.07
F	m	28	81.8	109.73	109.60	0.87	38.14	41.87
Mean,%	...	...	...	...	98.2 ± 1.2	1.7 ± 1.5	26.1 ± 8.5	29.4 ± 7.0
Range,%	...	...	...	...	96.5–99.9	0.2–4.5	16.0–37.5	21.5–38.2

[a] Based on analysis of nasal swabs.
[b] Adjusted for molecular weight.

by the EMIT system. Cocaine and benzoylecgonine combined were determined by a radioimmunoassay recently developed by Hoffman-La Roche Inc. Determinations by each method were performed in duplicate.

Results and Discussion

The administration of cocaine produced no significant changes in the status of any of the subjects; respiration, blood pressure, and pupil size remained normal. None of the subjects expressed feelings of euphoria. One subject (D) briefly exhibited minimal symptoms of anxiety and hostility.

The absorption of cocaine by the nasal mucosa was nearly complete, as evidenced by analyses performed on the nasal swabs (Table 1). Quantities of cocaine detected on the 4 and 8-h swabs represented 1.6 ± 0.4% and 0.2 ± 0.2% of the administered doses, respectively (detection of any cocaine in the latter swab attests to incomplete sampling in the earlier swab). Van Dyke et al [*6*] stated that cocaine was detected on nasal swabs 6 h after the administration of 1.5 mg/kg body weight of cocaine hydrochloride to the nasal mucosa of patients; however, quantitative information was not presented.

The baseline specimens before drug administration for all six subjects were negative for cocaine, benzoylecgonine, or both by the chromatographic and radioimmunoassay techniques. One subject (E) was determined by EMIT to have a baseline concentration of 2.1 μg benzoylecgonine equivalent per millilitre urine. This was the patient who ingested quantities of aspirin, Robitussin, Sudafed, and various alcoholic beverages during the study. Studies in our laboratory with urine specimens spiked with the above indicated they did not cause EMIT "positives"; the effect of their metabolites was not examined, however.

The excretion of unchanged cocaine peaked early and diminished rapidly. Three of the subjects exhibited maximum concentrations in the 0 to 1-h and the other three subjects showed maximum concentrations in the 1 to 2-h urine specimens (Table 2). Of twelve

TABLE 2—*Cocaine excretion, μg/ml.*

Specimen	Patient						Mean ± SD
	A	B	C	D	E	F	
0-1 h	1.4	0.3	4.8	7.9	24.1	1.5	6.7 ± 9.0
1-2 h	0.7	0.7	5.9	3.5	1.4	4.3	2.8 ± 2.2
2-4 h	0.4	0	0.4	2.0	0.5	1.6	0.8 ± 0.8
4-8 h	0.2	0.3	0.5	1.8	0.2	0.4	0.6 ± 0.6
8-12 h	0	0.3	0.3	1.2	0.2	0.2	0.4 ± 0.4
12-24 h	0	0	0	0.1	0	0	0
24-48 h	0	0	0	0	0	0	0

specimens collected after drug administration for each of the six subjects, only one specimen had a cocaine concentration ≥8 μg/ml (E, 0 to 1 h, 24.1 μg/ml), and no specimens exceeded 2 μg/ml after the second hour. Adjusted for urine volumes, peak excretion of unchanged cocaine occurred in the 0 to 1-h specimen for three subjects, in the 1 to 2-h specimen for two subjects, and in the 2 to 4-h specimen for one subject (Table 3).

Benzoylecgonine excretion, expressed both as concentration (Table 4) and as amount excreted per unit time (Table 5), was maximal at 1 to 12 h following cocaine administration, with subjects exhibiting peak excretions randomly within this period. Mean peak excretion occurred during the 4 to 8-h interval, with several specimens exceeding 70 μg/ml.

The mean excretions of unchanged cocaine and benzoylecgonine are depicted in Figs. 1 and 2, respectively. For both parent drug and metabolite, the intersubject variation was

TABLE 3—*Cocaine excretion, μg/h.*

Specimen	Patient A	B	C	D	E	F	Mean ± SD
0-1 h	573	12	269	1469	1542	124	665 ± 678
1-2 h	307	25	471	1232	84	164	380 ± 447
2-4 h	122	1	16	355	159	212	144 ± 132
4-8 h	20	6	8	160	10	27	38.5 ± 60.0
8-12 h	0	8	13	38	9	12	13.6 ± 13.0
12-24 h	0	0	0	6	2	0	1.4 ± 2.5
24-48 h	0	1	0	0	3	0	0.7 ± 1.4
48-72 h	0	0	0	0	0	0	0

TABLE 4—*Benzoylecgonine excretion, μg/ml.*

Specimen	Patient A	B	C	D	E	F	Mean ± SD
0-1 h	0	4.2	12.2	1.2	37.4	25.5	13.4 ± 15.1
1-2 h	0	29.4	10.9	2.2	66.6	49.9	26.5 ± 27.2
2-4 h	0.8	75.3	13.6	4.7	3.9	27.7	21.0 ± 28.3
4-8 h	8.1	44.3	70.8	15.9	55.4	17.3	52.0 ± 39.1
8-12 h	5.0	32.3	42.2	21.1	41.4	35.9	29.6 ± 14.3
12-24 h	5.6	4.1	16.7	7.2	17.5	17.6	11.4 ± 6.5
24-48 h	1.2	0	3.4	0.6	1.8	2.9	1.6 ± 1.3
48-72 h	0.3	0	0.7	0.2	0.3	0.6	0.4 ± 0.3
72-96 h	0	0	0	0	0.2	0	0
96-120 h	0	0	0.4	0	0	0.2	0.1 ± 0.2
120-144 h	0	0	0.2	0	0	0.1	0
144-168 h	0	0	0.2	0	0	0	0

TABLE 5—*Benzoylecgonine excretion, μg/h.*

Specimens	Patient A	B	C	D	E	F	Mean ± SD
0-1 h	0	161	734	220	1943	2091	858 ± 932
1-2 h	0	1118	869	756	3932	1897	1429 ± 1370
2-4 h	232	1468	483	838	1277	3769	1344 ± 1275
4-8 h	788	941	1274	1413	2979	1627	1504 ± 785
8-12 h	480	889	1647	690	1937	2469	1352 ± 786
12-24 h	373	113	569	559	767	706	514 ± 239
24-48 h	98	0	120	56	124	100	83 ± 47
48-72 h	28	0	17	13	10	14	12 ± 11
72-96 h	3	0	0	0	6	0	2 ± 2
96-120 h	0	0	17	0	0	5	4 ± 7
120-144 h	0	0	5	0	0	2	1 ± 2
144-168 h	0	0	5	0	0	0	1 ± 2

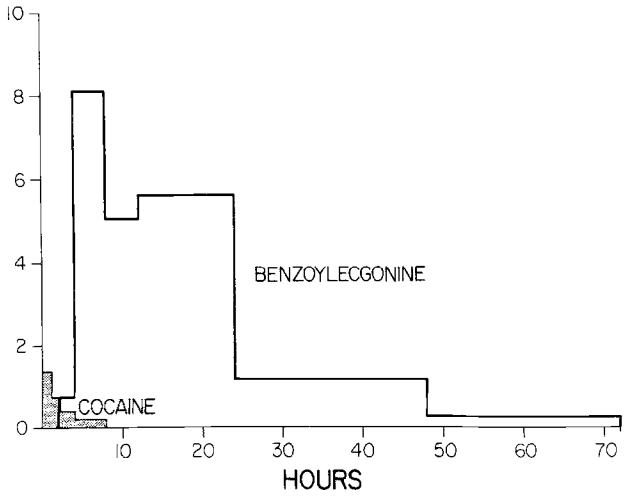

FIG. 1—*Mean excretion in μg/ml of cocaine and benzoylecgonine.*

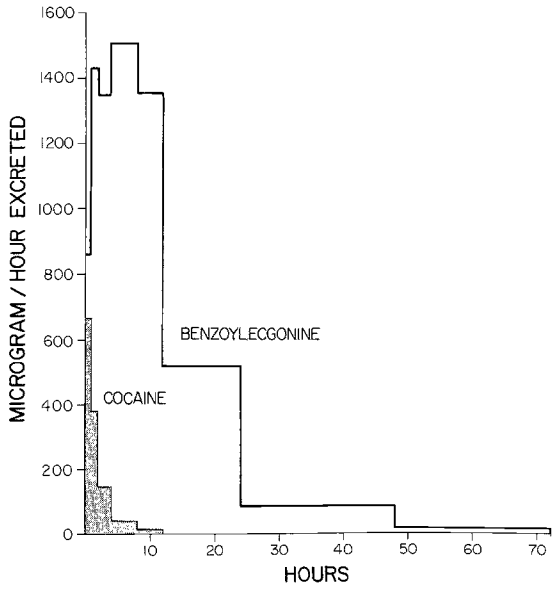

FIG. 2—*Mean excretion in μg/h of cocaine and benzoylecgonine.*

large (Tables 2–5). This is in good agreement with earlier studies by Wallace et al [7], in which 8-h collective urine specimens were analyzed for cocaine and benzoylecgonine during the initial 24 h following intranasal absorption of cocaine (Table 6). Intersubject variation during the initial 4 h was comparable for cocaine and benzoylecgonine; after 4 h intersubject variation for benzoylecgonine was significantly less than that noted for cocaine.

The benzoylecgonine/cocaine ratios, which generally greatly exceeded one, varied significantly both between subjects and between various specimens for given subjects (Table 7). It would be unrewarding to attempt to estimate cocaine concentrations from benzoylecgonine concentration data, or vice versa. For one individual (A), no benzoylecgonine was detected in the 0 to 1-h and the 1 to 2-h specimens, although cocaine excretion was com-

TABLE 6—*Benzoylecgonine/cocaine ratios.*

Specimen	Patient						Mean ± SD[a]
	A	B	C	D	E	F	
0-1 h	0	14.0	2.5	0.2	1.6	17.0	5.9 ± 7.6
1-2 h	0	42.0	1.8	0.6	47.6	11.6	17.3 ± 21.8
2-4 h	2.0	...	34.0	2.4	7.8	17.3	12.7 ± 13.4
4-8 h	40.5	147.7	141.6	8.8	277.0	43.2	109.8 ± 99.8
8-12 h	...	107.7	140.7	17.6	207.0	179.5	130.5 ± 73.5
12-24 h	...	...	...	72.0	...	...	...

[a] For specimens for which cocaine was detected.

parable to that of the other subjects during this interval (Fig. 3). It is believed that this is the first time cocaine excretion in the absence of benzoylecgonine has been reported (when analytical techniques specific for benzoylecgonine were used). With an experimental population of only six subjects, the question is raised whether this individual is indeed unique or if excretion of cocaine alone might be exhibited by a significant number of users of cocaine provided adequate sampling techniques are employed. The potential for false negatives when assaying specimens by techniques sensitive only to the metabolite is also demonstrated.

The duration during which cocaine, benzoylecgonine, or both were detected in this study is depicted in Fig. 4. The criteria for this data were the "cut-off" concentrations previously determined based on assays of spiked urine specimens obtained from a number of subjects: 0.5 μg/ml for TLC [*10*], 0.4 μg/ml for GLC [*7*], 1.0 μg/ml for EMIT, and 25 ng/ml for RIA [*13*]. Cocaine was generally detected for 2 to 4 h by TLC and for 4 to 8 h by GLC; the maximum duration unchanged cocaine was detected was for 12 h, and for that long in one subject only. By contrast, benzoylecgonine was generally detected by the chromatographic techniques and by EMIT for 48 to 72 h, and cocaine/benzoylecgonine was generally detected by the radioimmunoassay for 120 h. When the criterion for a "positive" assay was the corresponding baseline value rather than the predetermined 25 ng/ml, all six subjects were still positive by RIA for cocaine/benzoylecgonine in the 144 to 168-h specimen.

A linear regression analysis was performed on the urine concentrations obtained for both cocaine and benzoylecgonine as determined by each of the four analytical techniques (Table 8). For unchanged cocaine, a comparison could be made only for the chromatographic techniques since EMIT is insensitive to cocaine and the RIA procedure used also detected benzoylecgonine. For comparison of benzoylecgonine concentrations determined by RIA with values derived by the chromatographic and enzyme immunologic assays, only specimens after the initial 12 h were considered to avoid difficulties of interpretation caused by the detection of unchanged cocaine. Correlation between the chromatographic and enzyme immunologic assays was excellent, ranging from 0.88 to 0.97. Correlation between the radioimmunoassay and the other analytical techniques was less, ranging from 0.72 to 0.79. This was not surprising, since previous studies [*13*] had demonstrated that although the precision and accuracy of the RIA technique were comparable to those achieved by the chromatographic techniques at very low concentrations, they were reduced at higher concentrations because of the narrow range of the RIA method and the resultant need for multiple dilutions.

Summary

Maximal urinary excretion of unchanged cocaine occurred within 2 h of the intranasal absorption of 1.5 mg/kg body weight of cocaine hydrochloride, and diminished rapidly

TABLE 7—*Results of an earlier investigation [7] of cocaine and benzoylecgonine excretion.*

Patient	Cocaine, μg/ml			Benzoylecgonine, μg/ml		
	0-8 h	8-16 h	16-24 h	0-8 h	8-16 h	16-24 h
A	0	0	0	34.5	22.9	8.6
B	1.4	0	0	123.1	11.3	1.0
C	0.7	0	0	18.0	20.4	5.7
D	0.2	0	0	10.3	16.8	6.8
E	0.3	0	0	34.0	5.2	0.4
F	0.5	0	0	15.0	6.1	1.2
G	2.6	0.8	0	17.3	46.9	14.7
H	1.4	0.3	0.1	85.6	75.7	43.5
I	2.8	0.4	0	82.4	49.8	11.6
J	0	0	0	30.0	17.0	7.2
Mean	1.0 ± 1.0	0.2 ± 0.3	0	45.0 ± 38.3	27.2 ± 22.9	10.1 ± 12.6
Range	0-2.8	0-0.8	0-0.1	10.3-123.1	5.2-75.7	0.4-43.5

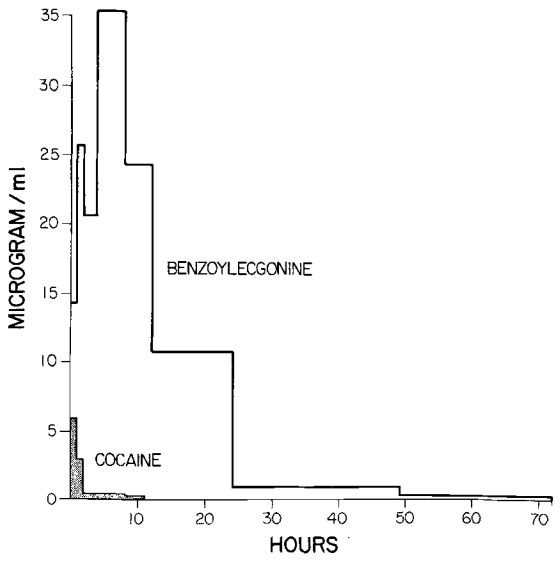

FIG. 3—*Excretion of cocaine and benzoylecgonine in Subject A.*

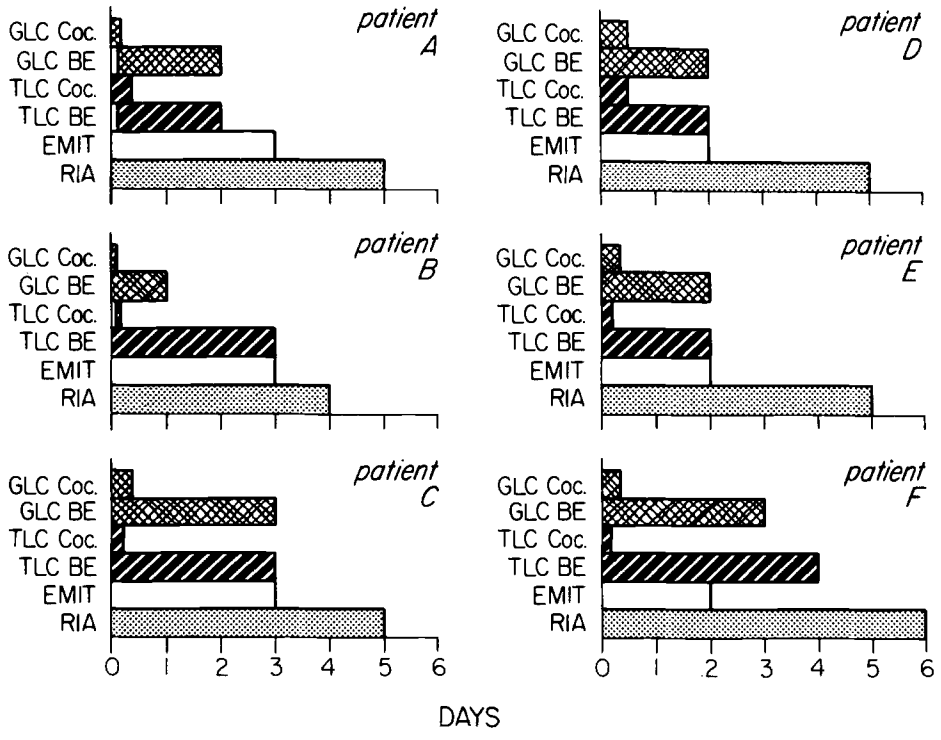

FIG. 4—*Duration of detection of cocaine, benzoylecgonine, or both by each of the four analytical techniques evaluated.*

TABLE 8—*Correlation of methods of analysis.*

Analysis	Slope	x-Intercept	Correlation Coefficient
Cocaine[a]			
GLC versus TLC	0.96	0	0.95
Benzoylecgonine[b]			
GLC versus TLC[b]	1.02	3.6	0.88
GLC versus EMIT[b]	0.94	1.3	0.97
TLC versus EMIT[b]	0.76	0.5	0.92
RIA versus GLC[c]	0.81	0.2	0.72
RIA versus TLC[c]	1.32	0.2	0.79
RIA versus EMIT[c]	1.04	0.4	0.75

[a] For specimens within 0 to 12 h of cocaine administration (n = 30).
[b] For specimens within 0 to 144 h of cocaine administration (n = 66).
[c] For specimens in the interval 12 to 144 h of cocaine administration (n = 36).

thereafter. Excretion of benzoylecgonine was maximal 4 to 8 h following administration of the drug and diminished slowly over an interval of several days. Peak cocaine and benzoylecgonine concentrations observed were 24 and 75 μg/ml, respectively. Benzoylecgonine/cocaine ratios were too varied to allow estimation of cocaine concentrations from benzoylecgonine concentration data or vice versa. Benzoylecgonine concentrations generally exceeded the corresponding cocaine values by a wide margin, but excretion of free cocaine in the absence of benzoylecgonine was observed in one subject. Cocaine was generally detected for only approximately 8 h, and for a maximum of 12 h, whereas benzoylecgonine was generally detected by chromatographic or enzyme immunologic assays for 48 to 72 h. Benzoylecgonine was positively identified in urine by radioimmunoassay for 96 to 144 h after dosing.

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