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Note

Potential errors in benzoylecgonine and cocaine analysis

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Benzoylecgonine is considered to be the major metabolite of cocaine in man. Many methods have been reported for its detection and measurement in urine[1–13] and some workers have reported its detection in blood. Others, however, suggest that benzoylecgonine may not be a true metabolite of cocaine, in the sense that it is produced by non-enzymic hydrolysis[14–16]. These workers have shown that conversion of cocaine to benzoylecgonine occurs at a substantial rate in plasma and urine at alkaline pH.

Since most of the methods reported for benzoylecgonine analysis have involved an alkaline extraction stage, it may be that some proportion of the amount detected could be due to conversion of cocaine during such extractions. In order to check this possibility it was considered essential to determine the rate of conversion of cocaine to benzoylecgonine at different pH values.

EXPERIMENTAL

Solutions of cocaine hydrochloride were made in distilled water at a concentration of 50 μg/ml. These were adjusted to pH values ranging from 2.0 to 9.4 with 0.1 *M* HCl or 0.1 *M* NaOH. The solutions were analysed immediately, and after standing for various times at room temperature, by high-performance liquid chromatography (HPLC).

HPLC was carried out using a Waters Assoc. (Northwich, Great Britain) M6000A pump with a reversed-phase support, Hypersil-5-ODS (5 μm; Shandon Southern Products, Runcorn, Great Britain) column (10 cm $\times$ 4.6 mm I.D.) and an eluent composition of methanol–water (55:45) (adjusted to pH 3.8 with syrupy phosphoric acid). The eluate was monitored by determining UV absorbance at 232 nm with a CE212 UV monitor (Cecil Instruments, Cambridge, Great Birtain). Flow-rate was 2 ml/min. Under these conditions benzoylecgonine eluted at a retention time of 1.4 min and cocaine at 3.4 min. Conversion of cocaine to benzoylecgonine was calculated from the peak heigths for the two compounds.

The precision of the method was estimated by replicate (10) determinations of a solution containing 10 μg/ml cocaine and 10 μg/ml benzoylecgonine at pH 4.0. The means and standard deviations were, for cocaine, 9.7 $\pm$ 0.4; and for benzoylecgonine, 9.8 $\pm$ 0.5.

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RESULTS AND DISCUSSION

The degrees of conversion of cocaine to benzoylecgonine taking place at different pH values over the period of one hour are shown in Table I. The conversion taking place at pH 8 over a longer period is given in Table II.

TABLE I

THE EFFECT OF pH ON THE CONVERSION OF COCAINE TO BENZOYLECGONINE

pH of solution	*Conversion (%)**	
	Initial	*After 1 h*
2	0	0
4	0	0
6	0	0.6
7	0	1.4
8	1.7	16.7
9	3.2	27.5
9.4	3.9	40.7

* Mean of 3 determinations.

TABLE II

THE CONVERSION OF COCAINE TO BENZOYLECGONINE AT pH 8

Age of solution (h)	*Conversion (%)**
0	0.6
1.0	15.6
2.0	21.3
3.0	26.2
4.5	34.7
6.75	45.1

* Mean of 3 determinations.

These results clearly indicate that cocaine is susceptible to hydrolysis in slightly alkaline conditions. Since most published methods for cocaine and benzoylecgonine analysis involve an alkaline extraction step the results obtained with these methods must be reconsidered. It is likely that cocaine levels will have been underestimated and benzoylecgonine levels overestimated. Relevant details of the extraction procedures of most of the methods are given in Table III.

Significant cocaine hydrolysis at neutral and slightly alkaline pH has important implications in toxicological analysis since the delay in analysing samples after they have been taken can be considerable. Blood samples generally become alkaline (to pH 8–9) on standing and alkaline urine is often encountered. Unless such samples are stored frozen, or adjusted to acid pH upon receipt, any cocaine present may have disappeared by the time analysis is performed.

TABLE III

DETAILS OF ALKALINE CONDITIONS USED FOR THE EXTRACTION OF COCAINE OR BENZOYLECGONINE FROM BLOOD AND URINE BY VARIOUS WORKERS

Ref.	*pH of extraction stage*	*Contact time with alkaline phase (min)*
1	8.0	?
2	8.5	?
3	8.9	10
4	9.5	4
5	sat. Na_2CO_3	10
6	*ca.* 9.6	5
7	*ca.* 10.0	15
8	sat. $NaHCO_3$	20
9	9.5	?
10	sat. $NaHCO_3$	10
11	8.9	5
12	9.0	?
13	8.5	11

The evidence presented here does not exclude the possibility of enzymic hydrolysis of cocaine in the body, but it could explain the widely divergent estimates of how much cocaine is excreted unchanged.

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