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GAS CHROMATOGRAPHIC STUDY OF THE URINARY CODEINE-TO-MORPHINE RATIOS IN CONTROLLED CODEINE CONSUMPTION AND IN MASS SCREENING FOR OPIATE DRUGS

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SUMMARY

The urinary codeine-to-morphine ratios in fifteen volunteers administered codeine tablets at intervals were studied by gas chromatography (GC) and compared with one month's GC results for enzyme multiplied immunoassay technique (EMIT)-screened urine specimens in a mass-screening programme for abuse of opiate drugs, particularly heroin. It appears that when $M < 2$ and $C/M > 0$ or when $M > 2$ and $C/M > 0.5$, where C and M are codeine and morphine concentrations in μg per 10 ml of urine, codeine consumption has to be presumed.

INTRODUCTION

In large-scale screening programmes for abuse of opiate drugs, including heroin, detection of morphine in urine is evidence of their consumption[1]. Together with morphine, however, codeine, which is a natural ingredient of opium and impure heroin, is also excreted[2]. Even if pure heroin is consumed it has been shown to lead to the excretion of some codeine in addition to morphine[3]. The presence of codeine together with morphine in urine specimens would not have mattered in concluding that an opiate drug had been consumed if codeine was associated only with opiate drugs. Codeine, however, is also consumed either by itself in tablet form or in cough syrups and leads to the excretion of both codeine and morphine[2] and in some instances to morphine only[4], via the *o*-demethylation of codeine[5].

Studies on the excretory pattern of codeine and morphine following codeine consumption had sought to establish criteria for its exclusion when interpreting excretion results. In one study a codeine to morphine ratio of greater than 3:1 for urine was proposed[2] and in another a maximum ratio of 1.3:1 was established for plasma[6]. The method used in the former study was thin-layer chromatography (TLC) analysis and therefore lacked sufficient precision and sensitivity, and in the latter immunological methods were used, which lacked specificity. Gas chromatographic (GC) methods, on the other hand, are precise, sensitive and specific.

This present study was undertaken to determine accurately, by GC, the codeine

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to morphine ratios in urine specimens of volunteers administered codeine at intervals and to compare them with ratios from one month's GC results for enzyme multiplied immunoassay technique (EMIT)-screened urine specimens, verified not to belong to codeine consumers, in a mass-screening programme for abuse of opiate drugs. The object was to establish more accurate criteria to eliminate codeine consumption in mass-screening programmes for abuse of opiate drugs.

EXPERIMENTAL

Chemicals and reagents

Codeine phosphate, morphine hydrochloride, nalorphine hydrobromide and codeine phosphate tablets (containing an equivalent of 22.6 mg of codeine base), all of British Pharmacopoeia (1980) grade, were supplied by the Pharmaceutical Department, Singapore. Bis(trimethylsilyl)trifluoroacetamide (GC grade) was purchased from E. Merck (Darmstadt, G.F.R.). Ammonia solution, isopropanol, chloroform and methanol (all of analytical-reagent grade) and hydrochloric acid (of laboratory-reagent grade) were purchased from manufacturers.

A standard nalorphine solution was prepared, containing 1 mg/ml of equivalent base in water.

Chromatography

All GC separations were performed with a Perkin-Elmer 900 instrument equipped with a flame-ionization detector and an SP 4100 computing integrator.The stationary phase was 3% OV-1 on Chromosorb W HP (100–120 mesh) (Supelco, Bellefonte, PA, U.S.A.) in a glass column (1.83 m × 6.4 mm O.D.). The oven temperature was 240°C and the injector and detector temperatures 300°C. The carrier gas was nitrogen at a flow-rate of 40 ml/min. The attenuation was 8 and the chart speed 30 cm/h.

Collection of urine specimens

Fifteen male volunteers 19–37 years old were divided into three groups according to the number of codeine phosphate tablets (22.6 mg of free base per tablet) administered, as follows:

Group I: ten volunteers were each administered one codeine phosphate tablet.

Group II: two volunteers were each administered one codeine phosphate tablet followed 6 h later by another.

Group III: three volunteers were each administered one codeine phosphate tablet followed 6 h later by a second and a further 6 h later by a third.
continued until the urinalysis showed no codeine or morphine.

Urine samples were collected whenever the volunteers needed to void and the time and volume voided were recorded. For all except one, collection of samples continued until the urinalysis showed no codeine or morphine.

Extraction and analytical procedures

A 10-ml volume of urine together with 2 ml of 11.5 *M* hydrochloric acid was autoclaved at 120°C for 15 min to convert glucuronides of codeine and morphine into

the free bases[7]. The hydrolysed solution was cooled, made alkaline with 3 ml of 17 *M* ammonia solution (pH 9.3) and extracted with 15 ml of chloroform–isopropanol (9:1)[8]. The aqueous phase was discarded and the organic phase extracted with 4 ml of 0.5 *M* hydrochloric acid. The organic phase was discarded and the aqueous phase made alkaline with 5 ml of borate buffer (pH 9.3) and extracted with 15 ml of chloroform–isopropanol (9:1). The organic phase was evaporated in an evaporating dish by heating on a water bath.

The residue was quantitatively transferred with 4 ml of methanol into a 15-ml tapered test-tube and 20 μl of standard nalorphine solution (internal standard) were added. The solution was evaporated to dryness at 90°C in the test-tube in an electric heating block. To the residue was then added 0.2 ml of bis(trimethylsilyl)trifluoroacetamide and heating was continued at 90°C for another 2 h, when silylation of morphine, codeine and nalorphine was complete. A 2-μl volume of the resulting solution was then injected into the gas chromatograph.

Recovery studies were conducted by adding known amounts of morphine and codeine, separately, to 10-ml blank (drug-free) urine samples, hydrolysing and carrying out the extraction procedure. A calibration graph was prepared prior to each analysis by silylating a mixture of known amounts of morphine and codeine and a fixed volume of nalorphine standard solution (20 μl).

RESULTS

The retention times of silylated codeine, morphine and nalorphine were 3.45, 4.02 and 5.41 min, respectively. The peak-height ratios of both silylated codeine and morphine to silylated nalorphine were linear over the concentration range 0–60 μg and sensitivity was 0.2 μg per 10 ml for each of the drugs. Urine samples whose codeine and morphine content fell outside the range 0.2–60 μg per 10 ml were rejected and re-analysed. The standard deviations for codeine and morphine at 3 and 6 μg (as the base) were found to be 1.9 for codeine ($n = 5$) and 1.2 for morphine ($n = 5$).

The extraction recoveries of codeine and morphine from blank urine, spiked with 3 and 6 μg per 10 ml of each, were 51.7–54.2% for codeine and 60.1–67.4% for morphine.

Fig. 1 shows typical chromatograms of extracted and silylated blank urine, standard silylated codeine, morphine and nalorphine (internal standard) and a volunteer's urine specimen that had been extracted and silylated. The last chromatogram shows that in spite of a low morphine content of 0.6 μg per 10 ml of urine and an almost 6-fold higher codeine content of 3.4 μg per 10 ml of urine, the peaks are well resolved.

A specimen of urine with a morphine content as low as 0.3 μg per 10 ml was also analysed by combined GC–mass spectrometry and the presence of silylated morphine confirmed by its characteristic spectra.

Table I summarizes the results for all the volunteers. The total amount of codeine excreted (after correcting for extraction recovery by a factor of 1.71) is between 21 and 76% of the amount administered and is considerably higher than the 7–30% range reported previously[2]. Between 0.5 and 6.6% of the codeine administered is excreted as morphine (corrected for extraction recovery by a factor of 1.93). The excretion figures for both codeine and morphine are comparable to those reported by

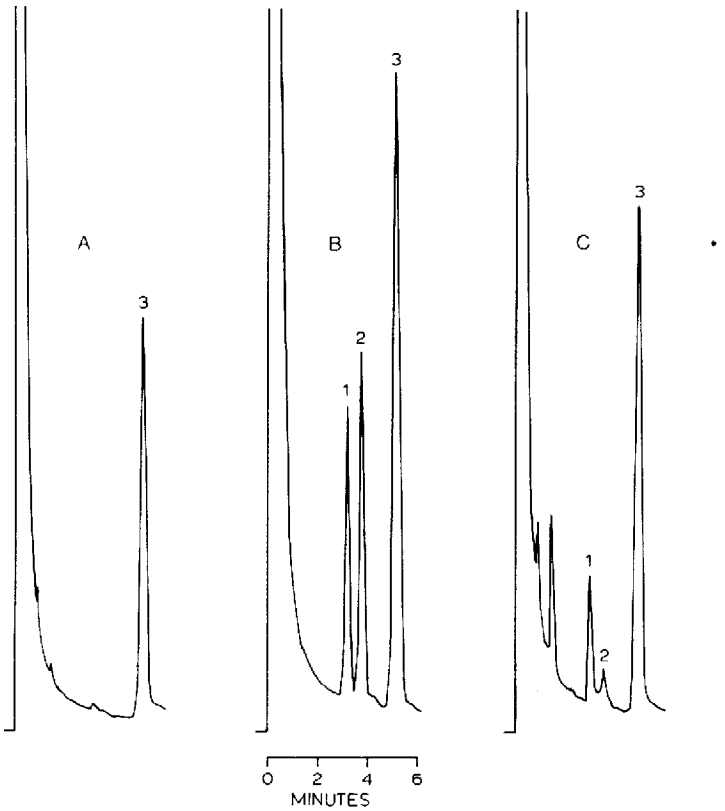

Fig. 1. Gas chromatograms of (A) silylated drug-free urine containing 20 μg of nalorphine (internal standard); (B) 6 μg each of silylated codeine and morphine and 20 μg of nalorphine; (C) 3.4 μg of codeine and 0.6 μg of morphine in the urine of a volunteer administered 1 tablet followed 6 h later by another, and urine collected 36 h later. Peaks: 1 = Codeine (retention time 3.45 min); 2 = morphine (4.02 min); 3 = nalorphine (5.41 min).

Adler *et al.*[9] of 31–63% unchanged and bound codeine and 5–17% morphine in a 24 h urine sample.

Both codeine and morphine could be detected in urine at a level of 0.2 μg per 10 ml up to 72 h after cosumption of the first codeine tablet. Similar results have been reported by other workers[4].

DISCUSSION

The results of the controlled codeine consumption study show that although the codeine to morphine ratios are initially high, they decrease with time until, for eleven of the volunteers, they went below the 1:1 mark (*i.e.*, the morphine concentration was higher than that of codeine) and for four of them there were instances when only morphine was detected, as observed by Solomon[4]. The criterion that a codeine to morphine ratio of greater than 3:1 is evidence of codeine consumption, as proposed by Lim and Ng[2], in fact held true for only six of the volunteers and even then

TABLE I

URINARY EXCRETION DATA OF CODEINE AND MORPHINE FROM VOLUNTEERS ADMINISTERED CODEINE PHOSPHATE TABLETS

Each tablet contained the equivalent of 22.6 mg of codeine base.

Dose	*Volunteer No.*	*Concentration range of drugs excreted (μg per 10 ml of urine)*		*Range of codeine-to-morphine ratios*	*Drugs last detected (h)*		*Drugs recovered (%)**	
		Codeine	*Morphine*		*Codeine*	*Morphine*	*Codeine*	*Morphine***
1 tablet	1	0.3–95	0.3–5.1	0–30.5	50	54	50.6	3.0
	2	0.2–164	0.2–9.2	0.9–42.3	50.5	50.5	62.8	4.3
	3	0.3–58	0.4–3.5	0.7–19.7	44.2	44.2	37.7	4.0
	4	0.4–134	0.4–14.3	0–12.4	42.0	50.0	38.8	6.6
	5	0.7–94	0.2–3.1	3.1–31.2	72.2	54.0	52.7	2.4
	6	0.2–222	0.2–3.5	0.8–20.9	65.0	65.0	46.6	1.6
	7	0.2–320	0.8–6.3	0.2–51.7	66.1	66.1	49.1	1.6
	8	0.2–195	0.2–12.5	0.7–20.5	60.0	60.0	21.4	2.0
	9	0.2–197	0.4–3.6	0.5–54.3	66.5	66.5	76.2	4.2
	10	0.2–373	0.3–6.9	0–55.7	58.3	58.3	45.6	2.3
1 tablet, 6 h later 2nd tablet	11	0.2–168	0.4–16.2	0.4–11.1	68.0	68.0	26.9	3.8
	12	0.3–116	0.2–2.7	2.0–16.5	52.5	52.5	36.3	1.6
1 tablet, 6 h later 2nd tablet, further 6 h later 3rd tablet	13	0.4–170	0.3–11.7	1.1–35.3	52.7	52.7	25.2	2.0
	14	0.3–305	0.3–9.7	0–45.5	62.7	70.5	48.2	2.0
	15	1.8–182	0.4–3.0	17.5–81.9	33.9	31.1	30.5	0.5

* Corrected for extraction recovery by a factor of 1.71 for codeine and 1.93 for morphine.
** Calculated as percentage of codeine dose administered.

only when the morphine concentration was above 1 μg per 10 ml, the sensitivity at which Lim and Ng based their observations. For concentrations below this level only for two volunteers did the ratio of 3:1 hold for all their urine specimens. Therefore, at least when GC is used, Lim and Ng's criterion is not universally applicable.

In order to establish a credible criterion we recognized the need for equivalent parallel studies on controlled consumption of opiate drugs. However, as such studies are difficult to undertake, we decided to study the results of mass screening of suspected opiate consumers and of those who had undergone rehabilitation and came under an after-care programme that required them to undergo a urine test varying from once in 2 days to once in 2 weeks.

All urine samples received by the Department from all the enforcement agencies in Singapore for the month of December 1982 were used for the study. The total of 22,100 samples were first screened by the EMIT–morphine system (Syva Corp., U.S.A.). All those that recorded as "positive" against a 400 ng/ml morphine calibrator were then extracted and silylated as described earlier and subjected to GC analysis.

Fig. 2 shows the distribution of the codeine to morphine ratios against morphine concentration for all the results in the codeine consumption study (the reason for combining all the data from all the volunteers, irrespective of the number of

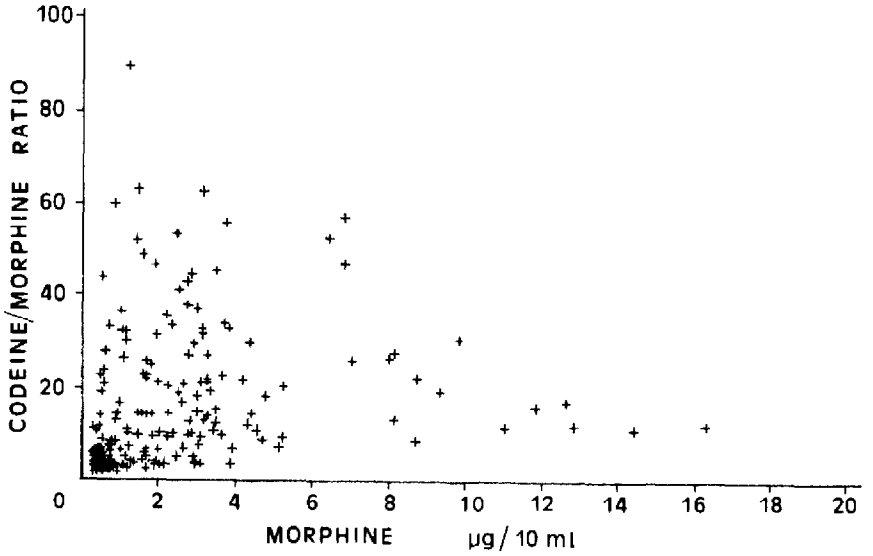

Fig. 2. Combined distribution of urinary codeine to morphine ratios against morphine concentration of 15 volunteers administered either one tablet of codeine phosphate (22.4 mg of base), one tablet followed 6 h later by another, or by one tablet followed 6 h later by a second and a further 6 h later by a third.

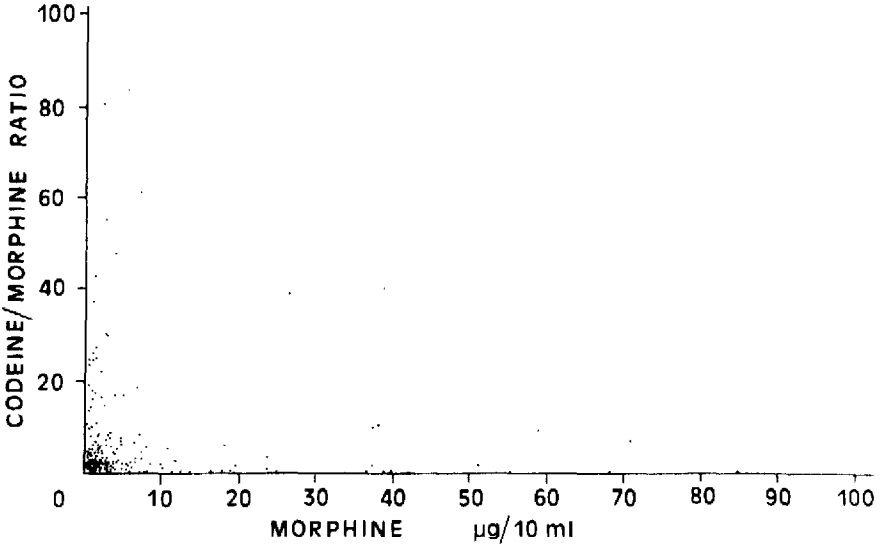

Fig. 3. Distribution of urinary codeine to morphine ratios against morphine concentration of 830 EMIT–morphine-positive and GC-positive specimens in a mass-screening programme.

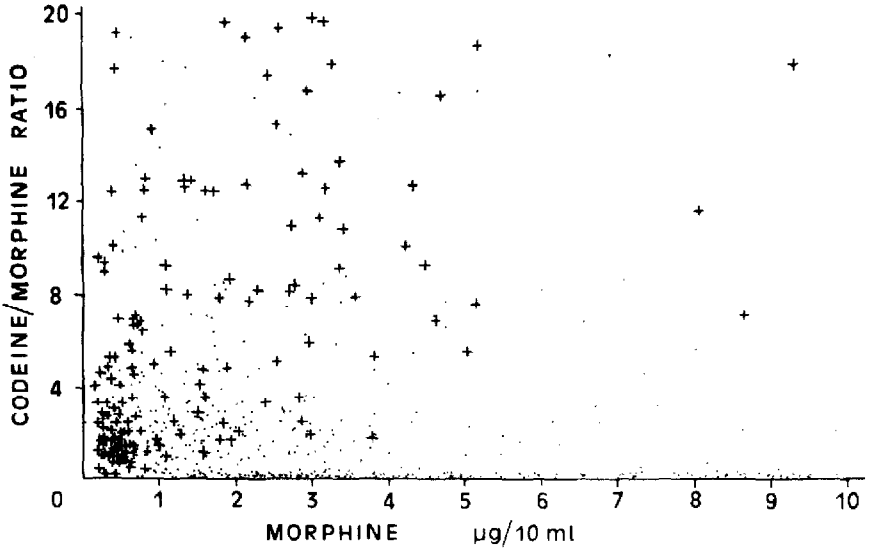

Fig. 4. Combined data of Figs. 2 and 3 over a selected range: + + represents codeine consumption results; dots represents mass-screening results.

codeine tablets consumed, is that no significant features, other than additional concentration peaks, were observed). Fig. 3 shows a similar distribution for all the positive GC results (830) of the mass-screening programme as described above. Fig. 4 shows the combined distribution for a selected range of ratios (0–20) and morphine concentration (0–10 μg).

An examination of these figures shows that for the mass-screening tests, most of the points lie below a codeine to morphine ratio of 0.5, irrespective of the morphine concentration (Fig. 4) and that at low morphine concentrations (below 2 μg per 10 ml) there is considerable overlap of points between those of controlled codeine consumption and those of mass-screening results (Fig. 4). The figures also show that there is no overlap of such points for morphine concentrations greater than 2 μg per 10 ml and codeine to morphine ratios less than 0.5 as well as morphine concentrations greater than 20 μg per 10 ml and codeine to morphine ratios of any value.

An obvious deduction from these observations is that morphine consumption is indicated at least for points lying within the area bounded by $M > 2$ and $C/M < 0.5$, where C and M are codeine and morphine concentrations in μg per 10 ml of urine. The validity of this deduction was verified when the Central Narcotics Bureau confirmed that none of the suspects in these cases claimed that they had consumed codeine. With this confirmation, a criterion for codeine consumption now presents itself: codeine consumption is presumed either when M is between 0 and 2 and the corresponding C/M ratio has any value or when M is greater than 2 and the corresponding C/M ratio is greater than 0.5.

It must be stressed that the proposed criteria are only guidelines and allowances must be made for unusual cases.

With a morphine cut-off at 2 μg per 10 ml of urine, as proposed above, the need for GC analysis, with its higher sensitivity, may appear to be superfluous. It has to be stressed that whilst TLC techniques may suffice for cases where the codeine concen-

tration is considerably higher than the morphine concentration and the latter is clearly greater than 2 μg per 10 ml, for cases where their concentrations produce results close to the boundaries of the proposed criteria, or when background interference on the TLC plate makes the determination of strengths difficult, GC techniques are imperative.

CONCLUSION

It has been shown through controlled studies on fifteen volunteers that consumption of codeine tablets leads to the urinary excretion of both codeine and morphine and, in some instances, to morphine only. This complicates the interpretation of urinary morphine or morphine and codeine results in mass screening for abuse of opiate drugs, including heroin. In such a programme, a 1-month's study of 830 EMIT–morphine-positive and GC positive specimens showed that when the codeine-to-morphine ratios were plotted against morphine concentration and compared with those of the controlled codeine consumption study there were zones where there was no overlap of points. After verifying that these zones were not from codeine consumers, a criterion has been proposed for codeine consumption: when $M < 2$ and $C/M > 0$ or when $M > 2$ and $C/M > 0.5$, codeine consumption has to be presumed.

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