with the structurally similar 4-biphenylyl *N*-methylcarbamate (1, 2) indicated that k_{OH^-} in water was six times that of the value in 50% ethanol. Thus, it may reasonably be predicted from the data in Table I that considerable hydrolysis of I would occur over the pH range encountered in the small intestine and that absorption of the intact drug could be variable from dose to dose. In addition, nonenzymatic hydrolysis at the pH of the blood, following absorption, would be rapid. The half-life of I at pH 7.3, 37°, calculated from the data in Table I is 73 min. Comparison of these results with those obtained earlier with 4-biphenylyl *N*-methylcarbamate (1) indicates that substitution of the 4-benzoyl group for the 4-phenyl group increases the hydrolysis rate nearly 200-fold.

Following oral administration of I to the dog, traces of intact I were detected in the urine but were below the level of reasonable quantification (<0.1% of the dose). The only drug-related materials detected in the urine were the hydrolysis product, II, and glucuronide and/or sulfate conjugates of II (Table II). Overall excretion ranged from 8.7 to 17.3% of the dose in the two experiments. These results are consistent with erratic absorption and the predicted extensive hydrolysis of I based on the *in vitro* kinetic studies.

Urinary excretion of 47–92% of orally administered II as a glucuronide conjugate in the rabbit was reported by Robinson (5). The present results for II (produced from I) in the dog are qualitatively similar.

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Mass Fragmentographic Detection of Normorphine in Urine of Man after Codeine Intake

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Abstract □ The biological disposition of codeine in urine was studied after intake of a single therapeutic dose of 20 mg. of codeine phosphate. Human urine after ingestion of codeine was previously reported to contain codeine as well as two of its metabolites, morphine and norcodeine. The presence of an additional metabolite, normorphine, was detectable after acid hydrolysis of an aliquot of human urine collected for 10 hr. after intake. Analysis was performed using a combined GC–mass spectrometer, with an additional accelerating voltage alternator unit as a specific detector. Presence of normorphine was indicated by mass fragmentography. A partial mass spectrum for identification was obtained by repeated scanning across the normorphine peak area.

Keyphrases □ Normorphine—mass spectroscopic detection as a metabolite of codeine in human urine □ Codeine—mass spectroscopic detection of normorphine as a metabolite, human urine □ Mass spectroscopy—detection of normorphine as a metabolite of codeine in human urine

Researchers have investigated the biological disposition of codeine in man, monkey, dog, rat, mouse, rabbit, and guinea pig (1, 2). The metabolic pathway of codeine in different species includes conjugation, *O*- and *N*-dealkylation to morphine and norcodeine, and conjugation of these metabolites.

In 1962, Way and Adler (3) reported: "Thus far there has been no direct evidence to indicate that the same codeine molecule is demethylated at both the *O*- and the *N*-positions to yield normorphine." However, during the same year, Kuhn and Friebel (4) identified traces of normorphine in urine of rats after treatment with codeine. In 1970, Yeh and Woods (5) reported the detection of normorphine by paper and thin-layer chromatography in urine and bile of rats after subcutaneous injection of large amounts of codeine phosphate. In the present investigation, traces of normorphine were detected, by means of mass fragmentography, in addition to the already known metabolites in urine of man after intake of a single therapeutic dose of either 10 or 20 mg. of codeine phosphate.

MASS FRAGMENTOGRAPHY

In 1968, mass fragmentography was introduced and its application has been demonstrated for the identification of chlorpromazine and its metabolites in human blood (6). With mass fragmentography, it is possible to take advantage of certain physicochemical properties of compounds or a group of compounds with similar chemical structures. The mass spectrometer is used as a GC detector. Compounds are detected by the presence of characteristic mass numbers (their molecular ion or fragments).

The accelerating voltage alternator[1] allows the detection and simultaneous recording of up to three mass numbers of a compound by keeping the magnetic field constant while switching the accelerating voltage. With this technique, amounts as small as 5 pg. can be detected. Another advantage is the high selectivity of mass fragmentography. There is very little chance that two different compounds have the same retention time and correspond at the same time to preselected fragments with identical peak ratios. The information for identification is, however, less than that obtained when a complete mass spectrum is recorded.

[1] LKB Produkter AB, Bromma, Sweden.

Table I—The 10 Strongest Peaks above *m/e* 200 of the Mass Spectra of Morphine, Codeine, Normorphine, and Norcodeine as Trifluoroacetyl Derivatives

Trifluoroacetyl-Morphine, mol. wt. 477			Trifluoroacetyl-Codeine, mol. wt. 395			Trifluoroacetyl-Normorphine, mol. wt. 559			Trifluoroacetyl-Norcodeine, mol. wt. 577		
	Relative Intensity, %			Relative Intensity, %			Relative Intensity, %			Relative Intensity, %	
m/e	a[a]	b[b]	*m/e*	a	b	*m/e*	a	b	*m/e*	a	b
364	100(28)[c]	100	395	100(20)	100	305	100(11)	100	477	100(15)	100
477	57(16)	55	282	94(19)	98	559	47(5)	45[d]	305	48(7)	52
365	22(6)	33	281	25(5)	26	306	44(5)	50	223	38(6)	40
478	12	13	396	23	26	319	30	32	478	26	25
311	10	9	283	21	23	332	27	28[d]	351	21	22
380	8	9	225	10	19	317	22	22	237	19	23
363	6	7	229	10	16	433	18	17[d]	224	18	25
307	6	6	266	10	10	333	14	13[d]	337	15	16
476	5	5	223	8	13	560	13	13	235	11	16
362	3	2	280	8	9	235	13	—	306	10	15

[a] Reference compounds. [b] Extract of urine. [c] Number in parentheses is relative total ionization. [d] Recorded but not shown in Fig. 2.

EXPERIMENTAL

Twenty milligrams of codeine phosphate (14.1 mg. base) was given to a healthy male of 83 kg. body weight. The subject did not take any drugs previous to these studies. A total of 400 ml. of urine was collected during the 10 hr. after intake. Urine used in all experiments as a blank control was collected before intake of the codeine phosphate. Since most of the administered codeine and its main metabolite, morphine, are excreted in urine as glucuronides, all samples were hydrolyzed before analysis. One milliliter of urine was transferred to a 10-ml. culture test tube, 1 ml. of 6 *N* HCl was added, and the sample was hydrolyzed in a paraffin bath for 30 min. at 100–110°. After hydrolysis, 1 ml. of borax–hydrochloric acid buffer (0.04 *M*, pH 8.85) was added and the mixture was titrated by careful addition of 8 *N* NaOH to pH 8.85. It was then extracted for 20 min. with 4 ml. of a 3:1 mixture of chloroform–isoamyl alcohol, using a mechanical shaker. After centrifugation at 3000 r.p.m. for 5 min., 3 ml. of the organic layer was transferred to a glass-stoppered 3-ml. centrifuge tube and evaporated to dryness in a paraffin bath at 80–90° under a stream of nitrogen. The dry residue was reacted with approximately 100 μl. of trifluoroacetic acid anhydride for 30 min. After evaporating to dryness, the dried residue was taken up in 100 μl. of chloroform and samples of this solution were injected into the GC column.

GC–mass spectroscopy analysis was performed with a gas chromatograph–mass spectrometer[2] with an accelerating voltage alternator. GC was done on a silanized 1.8-m. (6-ft.) × 1.5-mm. i.d. glass column, packed with 3% SE-30 on 100–120-mesh Gas Chrom Q. Temperatures were: column, 210°; flash heater, 250°; separator, 255°; and ion source, 290°. The carrier gas was helium, with a 20-ml./min. flow rate. All mass spectra and accelerating voltage alternator recordings were registered at an ionizing potential of 70 ev. and a basic accelerating voltage of 3.5 kv.

Table I represents, in a tabulated form, the relative abundance of the 10 strongest peaks above *m/e* 200 in the mass spectra of the trifluoroacetyl derivatives from: (*a*) the reference compounds and (*b*) hydrolyzed urine after codeine intake. The peaks are tabulated in order of decreasing abundance.

Figure 1 displays the recordings of the trifluoroacetyl derivatives. The accelerating voltage alternator recording was performed at 70 ev. The analyzer/collector slit setting was 0.1/0.3. The multiplier gain setting was at position 8 (range 1–11 = 1.2–3.5 kv.).

The switching frequency of the accelerating voltage alternator unit was 2 Hz. The range of the accelerating voltage alternator unit is relatively small. By adjusting the magnetic field to *m/e* 282 (at a given basic accelerating voltage of 3.5 kv.), the accelerating voltage alternator unit allows a maximum simultaneous recording of *m/e* 310 (282 + 10%). Since we had to have a registering range from *m/e* 282 to *m/e* 560, we had to readjust the magnetic field (and, subsequently, the accelerating voltage) to obtain at least three or four characteristic mass fragments of all four derivatives.

[2] LKB 9000.

The accelerating voltage alternator recordings in Fig. 1B indicate, by retention time and peak ratio, the presence of the trifluoroacetyl derivatives of morphine, codeine, normorphine, and norcodeine.

For further identification, the complete mass spectra of codeine, morphine, and norcodeine as their trifluoroacetyl derivatives could be recorded. Since the amount of normorphine present was very small, a 30-ml. sample of urine was prepurified by filtering off the

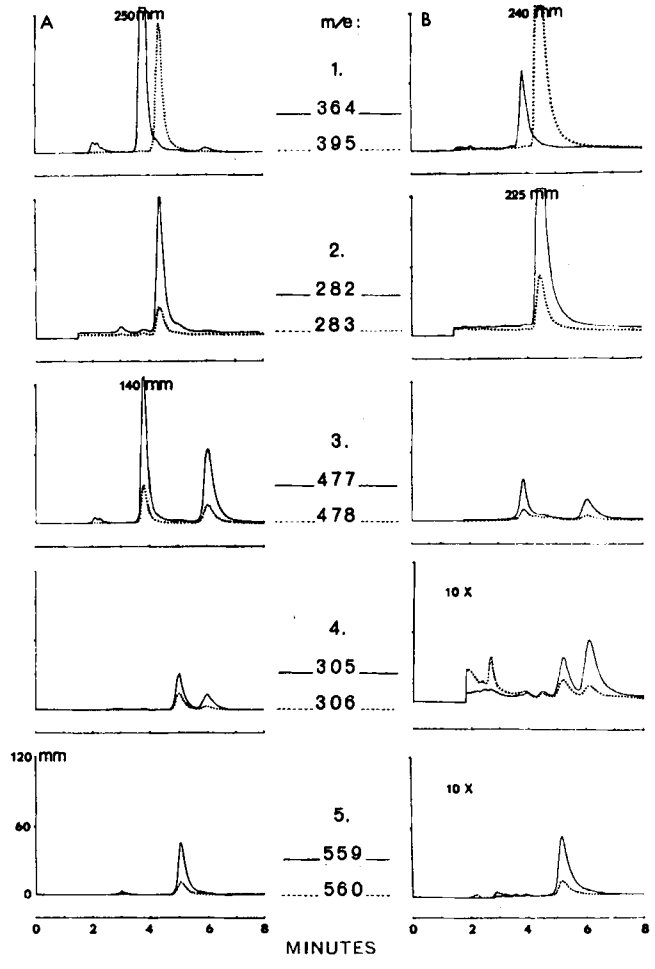

Figure 1—*Accelerating voltage alternator recording of indicated mass units (trifluoroacetyl derivatives, 200 ng. each) of: A1, morphine (364) and codeine (395); A2, codeine (282/283); A3, morphine and norcodeine (477/478); A4, normorphine and norcodeine (305/306); A5, normorphine (559/560); and B1–5, corresponding compounds after urine extraction.*

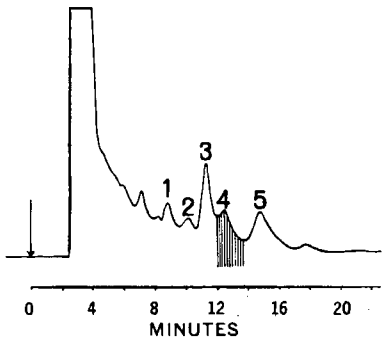

Figure 2—*Gas chromatogram of urine extract after codeine intake. For GC details, see text. Key: trifluoroacetyl derivatives of 1, morphine; 2, codeine; 3, unknown impurity; 4, normorphine; and 5, norcodeine. Strokes under 4 indicate the area where the repetitive scanning was performed.*

sediments and extracting it three times with an equal volume of ethyl ether. No normorphine was detected in the ether extract. The aqueous layer was then extracted twice with 25 ml. of the chloroform-isoamyl alcohol (3:1) mixture. The residue, after evaporation of the solvent and derivative formation, was taken up in 50 μl. of chloroform.

The column temperature was decreased to 188° and 5 μl. of the solution was injected into the GC column. The retention times of the trifluoroacetyl derivatives of morphine, codeine, normorphine, and norcodeine were found to be 8.8, 10.1, 12.4, and 14.7 min., respectively (Fig. 2, peaks 1, 2, 4, and 5, respectively). Small amounts of morphine and codeine were left in this prepurified mixture. By focusing the accelerating voltage alternator on mass units 559 and 560, the M^+ and $M^+ + 1$ ions of the trifluoroacetyl derivative of normorphine, the compound appeared as a shoulder on the tailing side of a somewhat larger peak due to impurities (Fig. 2, peaks 2 and 4).

In subsequent studies, repetitive scans were taken at intervals of 10 sec. during a retention time period from 12 to 13.67 min. The mass range encompassed in one scan was *m/e* 250–600, and a fast scan speed was chosen. Figure 3 shows the relative abundance of the most important and characteristic fragments of the trifluoroacetyl derivative of normorphine plotted *versus* elution time. It shows, furthermore, that *m/e* 277 and 386, which decrease in relative abundance with time, originate from the concurrently eluting unknown impurity peak, tailing all over the trifluoroacetyl derivative of normorphine peak area (Fig. 3, peak 3). The recording of *m/e* 355, derived from the SE-30 column coating, keeps essentially constant over the whole area. It is evident that the peak ratios of the different mass units are nearly constant during the whole elution from the GC column.

RESULTS AND DISCUSSION

The results demonstrate the detection and identification of normorphine in human urine after intake of codeine phosphate. The detection of codeine and morphine has been reported in the literature. The presence of normorphine as a metabolite of codeine has been confirmed by analyzing the urine of five individuals after intake of 20 or 10 mg. of codeine phosphate.

A corresponding sample of urine, to which 20 mg. of codeine phosphate was added, was allowed to stand for 12 hr. at room temperature and then subjected to the above-mentioned hydrolysis, extraction, and analysis procedures. Except for codeine, none of the identified metabolites was detected.

To estimate the quantities of excreted metabolites, the peak heights of identified compounds in urine (Fig. 1B) were compared with those of the known standards (200 ng. each) in Fig. 1A. An excretion of approximately 70% total codeine, approximately 9% total norcodeine, and approximately 10% total morphine was thus calculated in a sample of urine (400 ml. collected for 10 hr. after intake of 20 mg. codeine phosphate). Although we are presently unable to do exact quantitative determinations, these findings are very close to those reported by Adler *et al.* (7). The excretion of normorphine was calculated to be less than 4% of the administered dose of codeine.

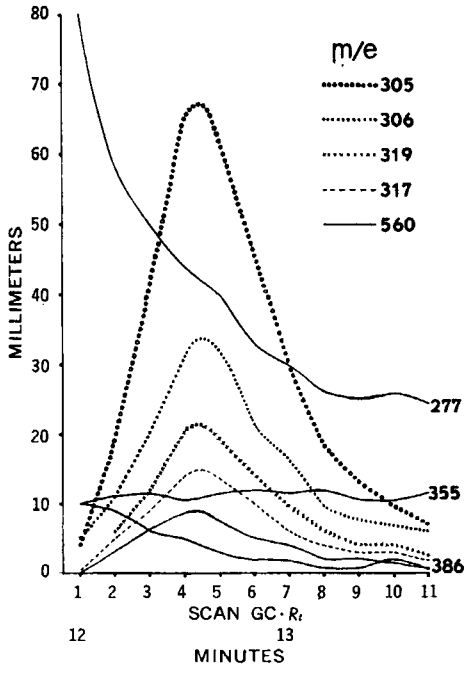

Figure 3—*Response of peaks* m/e: *305, 306, 317, 319, and 560 of the trifluoroacetyl derivative of normorphine plotted* versus *elution time from GC column;* m/e *277 and 386 indicate tailing of previous eluting impurity peak (see Fig. 2, peak 3). The SE-30 column background is shown at* m/e *355. For more details, see text.*

A more quantitative investigation of the excretion of these metabolites with these techniques remains to be done. As can be seen from Fig. 1A, the response of the base peaks of each compound (morphine, codeine, normorphine, and norcodeine as trifluoroacetyl derivatives) to the accelerating voltage alternator recorder is very different since the contribution of the base peaks to the total ion current differs for each compound. A slight drifting of the focusing and variations in the valve opening between the separator and the ion source may adversely affect quantitative determinations. It would, therefore, be preferable to use an internal standard in the test mixture. The ideal internal standard would be an isotopically labeled compound with the same chemical structure (8). For mass fragmentography, no chemical differences are necessary and the mass difference alone is sufficient to discriminate between the substance under investigation and the internal standard. No variations concerning extraction, derivative formation, column adsorption, *etc.*, will be obtained.

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