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[x] To whom inquiries should be directed.

Isolation and Identification of Morphine 3- and 6-Glucuronides, Morphine 3,6-Diglucuronide, Morphine 3-Ethereal Sulfate, Normorphine, and Normorphine 6-Glucuronide as Morphine Metabolites in Humans

S. Y. YEH [x], C. W. GORODETZKY, and H. A. KREBS

Abstract □ Morphine metabolites were isolated with column chromatography on a resin and neutral aluminum oxide and TLC from the urine of morphine-dependent subjects maintained on morphine sulfate at a dose of 240 mg/day. These metabolites were characterized as morphine 3-glucuronide, morphine 6-glucuronide, morphine 3,6-diglucuronide, morphine 3-ethereal sulfate, normorphine, normorphine 6-glucuronide, and, possibly, normorphine 3-glucuronide by free phenol and glucuronide tests, enzymatic hydrolysis, GLC, TLC, UV spectroscopy, and GLC–mass spectrometry.

Keyphrases □ Morphine metabolites, various—column chromatographic and TLC isolation from human urine □ Chromatography, column—isolation of various morphine metabolites from human urine □ TLC—isolation of various morphine metabolites from human urine □ Narcotic analgesics—various morphine metabolites, column chromatographic and TLC isolation from human urine

Studies with animals and humans have shown metabolism of morphine by the following pathways: (*a*) conjugation to give morphine 3-glucuronide, morphine 6-glucuronide, and morphine 3-ethereal sulfate (1–8); (*b*) *N*-demethylation to yield normorphine, which was then conjugated (9–15); (*c*) *O*-methylation to form codeine (16, 17); and (*d*) oxidation to form dihydromorphinone (18). Recently, a minor metabolite, tentatively identified as morphine 2,3-quinone, was reported in the urine of rats given morphine and after incubation of morphine with rat brain homogenates (19). Also, one study was unable to confirm the conversion of morphine to codeine (20).

Only morphine 3-glucuronide has been isolated as a morphine metabolite in humans (2), and a small amount of morphine 6-glucuronide was detected by TLC (5). The present paper reports the isolation and identification of morphine 3- and 6-glucuronides, morphine 3,6-diglucuronide, morphine 3-ethereal sulfate, normorphine, normorphine 6-glucuronide, and, possibly, normorphine 3-glucuronide from the urine of morphine-dependent human subjects.

EXPERIMENTAL

Materials and Subjects—The morphine sulfate USP used was a commercial product; normorphine hydrochloride was used as received[1]. Pseudomorphine and morphine *N*-oxide were synthesized according to the procedures of Fulton (21) and Freund and Speyer (22), respectively. Morphine 3-glucuronide, codeine 6-glucuronide, and morphine 3-ethereal sulfate were isolated from the urine of dogs and cats given morphine and codeine, respectively (3, 9, 23).

Four adult postaddict males[2] were judged to be in good health from recent history and physical and laboratory examinations, including tests of hematological, renal, hepatic, and cardiac functions. The subjects had an average age of 33 years (range of 27–41) and an average weight of 66 kg (range of 61–71). All subjects were given morphine sulfate injections in gradually increasing doses from 10 to 60 mg sc four times daily. Urine was collected from subjects during maintenance on morphine sulfate at 60 mg after several months and stored in a refrigerator.

Extraction of Morphine and Its Metabolites—Morphine, normorphine, and other possible metabolites were extracted from samples according to the procedure described previously (14). Samples were adjusted to about pH 10, buffered at pH 10.4 with 2 ml of 40% phosphate buffer (37% K_2HPO_4 and 3% K_3PO_4), salted, and extracted with 1,2-dichloroethane (glass distilled) containing 30% (v/v) 2-propanol.

The organic phase was shaken with 1 *N* HCl; then the acidic aqueous phase was separated and adjusted to pH 10.4, buffered, salted, and extracted again with the organic solvent. The residue obtained upon evaporation of the organic phase to dryness was used for TLC and GLC identification of morphine metabolites. For GLC identification, a derivative was prepared using 25% trimethylsilylimidazole in pyridine[3].

TLC—TLC was performed with either 250-μm silica gel plates with a preadsorbent area[4] or instant TLC sheets impregnated with silica gel[5].

[1] Courtesy of Dr. E. L. May, National Institutes of Health.
[2] Federal prisoner volunteers, incarcerated at the National Institute on Drug Abuse Research Center. Informed consent was obtained in writing in the presence of a witness.
[3] Pierce Chemical Co., Rockford, Ill.
[4] Quantum Industries, Fairfield, N.J.
[5] Gelman Instrument Co., Ann Arbor, Mich.

When silica gel plates with a preadsorbent area were used, samples were spotted on the preadsorbent area. The R_f value was calculated using the beginning of the silica gel section as the origin. Two solvent systems were used for development of the chromatograms: A, 1-butanol–acetic acid–water (35:3:10); and B, ethyl acetate–methanol–ammonium hydroxide (17:2:1). Chromatograms were visualized by spraying with iodoplatinate reagent.

GLC—A gas chromatograph[6] equipped with dual columns and dual flame-ionization detectors was used. A 0.9-m × 2-mm glass column was packed with 3% OV-17 coated on 60–80-mesh Gas Chrom Q. A 1.5-m × 2-mm stainless steel column was packed with 3% SE-30 coated on 100–120-mesh Varaport. The temperatures of the injector, column, and detector were 255, 220, and 295°, respectively; gas flow rates were 30 ml/min for nitrogen and hydrogen and 300–400 ml/min for dried compressed air.

GLC–Mass Spectrometry—Chemical-ionization mass spectral data were obtained on a gas chromatograph–mass spectrometer[7] equipped with an interactive data system[8] and a glass column (1.5 m × 2 mm) packed with 3% OV-17 coated on Gas Chrom Q. The gas chromatograph was coupled to the mass spectrometer by a 0.31-cm (0.125-in.) glass-lined stainless steel tube and a venting valve. The electron energy was 80 ev, and the ion repeller voltage was 3 v.

Methane with a flow rate giving an ion chamber pressure of about 1000 μm was used as the carrier gas as well as the chemical-ionization reagent gas in the ion source. The temperatures of the injector, column, and ion source were 230, 210, and 100°, respectively. Aliquots of 3–5 μl of the silylated sample were injected. After injection, the venting valve was opened for the first 30 sec, allowing excess derivatizing reagent and solvent to escape without entering the ionization chamber.

Hydrolysis of Conjugated Metabolites—Conjugated morphine metabolites were hydrolyzed to free base alkaloids by acid hydrolysis, incubation with β-glucuronidase (type II, bacterial powder)[9], or incubation with β-glucuronidase–sulfatase[10] according to a procedure described previously (14). After hydrolysis, the samples were adjusted to about pH 10, buffered, and extracted according to the described procedure.

Glucuronic Acid Determination—The content of glucuronic acid in the conjugated morphine metabolites was determined according to the procedure of Dische (24).

Free Phenol Test—Free phenol was determined by a modified procedure of Volterra (25) using the reagent of Folin and Ciocalteu (using one-tenth of the volume of samples and reagents stated).

Purification of Morphine Metabolites with Column Chromatography—Aliquots (200 ml) of urine from each subject were centrifuged to remove the insoluble material and passed at a flow rate of 2–4 ml/min through a resin[11] column (2.1 × 40 cm) previously washed with methanol and water. The column was washed with 150 ml of distilled water and eluted with 300 ml of methanol. The first 100 ml of washing was discarded. The remaining washing and methanol eluate were collected separately and concentrated separately to about 2 ml under reduced pressure in an evaporator[12] in a water bath at about 50°. (All evaporations in these studies were done in this manner.) A total of 6 liters of urine was thus chromatographed through the resin.

An aliquot of the methanol eluate was chromatographed on an instant TLC sheet, and the eluate was diluted with 15 ml of 5% NH_4OH. Each 5-ml aliquot of eluate was extracted three times with 15 ml of 1,2-dichloroethane containing 30% 2-propanol. The organic and aqueous phases were separately evaporated to dryness, and the residue of the organic phase was dissolved in methanol.

Organic Phase—An aliquot of the organic phase was chromatographed on an instant TLC sheet impregnated with silica gel, and the chromatogram was developed with System B. The region with R_f 0.4 (0.3–0.5), corresponding to morphine 3-ethereal sulfate and morphine *N*-oxide, was eluted with methanol and water (Fraction I). The region with R_f 0.85 (0.7–1.0), corresponding to morphine and normorphine, was eluted with methanol (Fraction II).

Another aliquot was concentrated, adsorbed on 10 g of neutral alumina, and added on a neutral alumina (50 g) column (2.1 × 150 cm). The column was sequentially eluted with 100 ml each of benzene, chloroform, acetone, acetone–methanol (50:50), and 500 ml of methanol, and the eluate was collected in 20-ml fractions. Morphine and its metabolites in each fraction were monitored by chromatographing an aliquot (100 μl) on TLC silica gel plates with System B. Spots with R_f 0.4, corresponding to morphine, were observed in fractions 21–35. In addition to morphine, spots with R_f ~0.2, corresponding to normorphine, were observed in fractions 26–30. Other spots whose R_f values did not correspond to any hypothesized morphine metabolites, probably due to nicotine and caffeine and their metabolites, were observed in fractions 15–19.

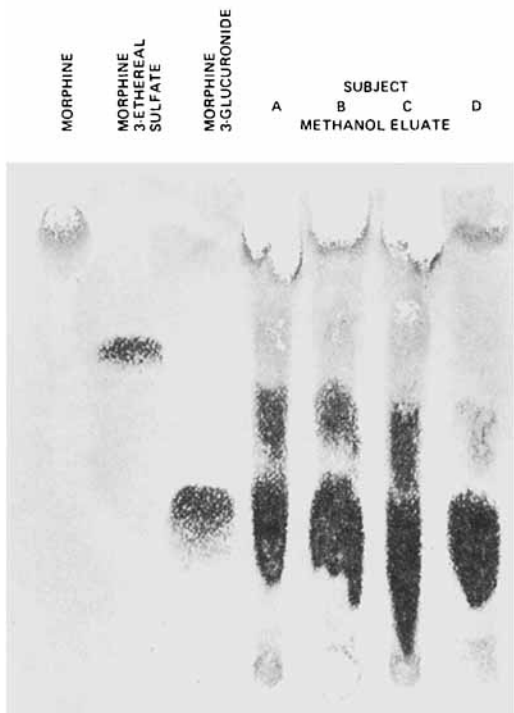

Figure 1—*Chromatogram of the methanol eluate obtained from a resin column on an instant TLC sheet impregnated with silica gel and developed with 1-butanol–acetic acid–water (35:3:10).*

The eluates collected in fractions 21–25, 26–30, and 31–35 were streaked on TLC silica gel plates, and the chromatograms were developed with System B. The regions with R_f 0.2 and 0.4 were separately eluted with methanol to yield Fractions III and IV, respectively.

Aqueous Phase—The residue of the aqueous phase was triturated with methanol to yield methanol-soluble and methanol-insoluble portions. The methanol-insoluble material was recrystallized in aqueous methanol to yield Fraction V and a mother liquid, which was combined with the methanol-soluble portion. This portion was again evaporated to a syrupy residue and triturated with methanol to yield new methanol-soluble and methanol-insoluble portions. These processes were repeated several times to separate the maximum of the methanol-insoluble material from the methanol-soluble portion.

The methanol-soluble portion was adsorbed on neutral alumina and placed on a 50-g neutral alumina column (2.1 × 150 cm). The column was sequentially eluted with 100 ml each of methanol and methanol–water (in proportions of 75:25, 50:50, and 25:75) and, finally, with 500 ml of water. The eluate was collected in 20-ml fractions, and morphine and its metabolites were monitored by chromatographing 100-μl aliquots of each fraction on instant TLC sheets impregnated with silica gel. The chromatograms developed with System A showed that fractions 5–9 contained one spot with R_f 0.85, corresponding to morphine; fractions 11–14 showed two spots with R_f 0.4 and 0.65; and fractions 17–35 showed four spots with R_f 0.1, 0.3, 0.5, and 0.6.

The eluates collected in fractions 17–35 were evaporated to syrupy residues and triturated with methanol to yield methanol-soluble and methanol-insoluble fractions. The methanol-insoluble fractions were combined with Fraction V. The methanol-soluble fractions were chromatographed on instant TLC sheets impregnated with silica gel (20 × 20 cm), and the chromatogram was developed with System A. The zones corresponding to the spots with R_f 0.1, 0.3, 0.5, and 0.6 were horizontally cut off and eluted with aqueous methanol. The eluates were evaporated to dryness and crystallized from aqueous methanol to yield Fractions VI,

[6] Varian model 2700.
[7] Finnigan model 3300.
[8] Finnigan model 6000.
[9] Sigma Chemical Co., St. Louis, Mo.
[10] Glusulase, Endo Laboratories, Garden City, N.Y.
[11] Amberlite XAD-2, Rohm & Haas Chemical Co., Philadelphia, Pa.
[12] Buchler Instruments, Fort Lee, N.J.

Table I—TLC, GLC, and Chemical-Ionization GLC–Mass Spectrometric Characteristics of Morphine Metabolites Isolated from Human Urine

Sample	Instant TLC, R_f Solvent A	Instant TLC, R_f Solvent B	TLC, Solvent B, R_f	Phenol Test	Glucuronide Test	As Trimethylsilyl Derivative: GLC R_t, min	Chemical-Ionization GLC–Mass Spectrometry, m/e: M − 15	M + 1	M + 29
Authentic:									
Morphine	0.85	0.9	0.4	+	−	6.0	414	430	458
Normorphine	0.85	0.85	0.2	+	−	7.2	400	416	444
Morphine 3-glucuronide	0.3	0	0	−	+				
Morphine 3-ethereal sulfate	0.6	0.4	0	−	−	6.0	414	430	458
Codeine 6-glucuronide	0.5	0	0	−	+				
Isolated:									
Fraction I	0.6	0.4	0	−	−	6.0	414	430	458
Hydrolyzed I	0.85	0.9	0.4	+	−	6.0	414	430	458
Fraction II	0.85	0.9	0.2[a]	+	−				
			0.4[b]	+	−	6.0	414	430	458
Fraction III	0.85	0.8	0.2	+	−	7.2	400	416	444
Fraction IV	0.85	0.9	0.4	+	−	6.0	414	430	458
Fraction V	0.3	0	0	−	+				
Hydrolyzed V	0.85	0.9	0.2[a]	+	−	7.2	400	416	444
			0.4[b]	+	−	6.0	414	430	458
Fraction VI	0.1	0	0	−	+				
Hydrolyzed VI	0.85	0.9	0.4	+	−	6.0	414	430	458
Fraction VII	0.3	0	0	−	+				
Hydrolyzed VII	0.85	0.9	0.2[a]	+	−	7.2	400	416	444
			0.4[b]	+	−	6.0	414	430	458
Fraction VIII	0.5	0	0	+	+				
Hydrolyzed VIII	0.85	0.8	0.4	+	−	6.0[b]	414	430	458
						7.2[a]	400	416	444
Fraction IX	0.6	0	0	+	+				
Hydrolyzed IX	0.85	0.85	0.2	+	−	6.0[a]	414	430	458
						7.2[b]	400	416	444
Fraction X	0.4			−	−				
Fraction XI	0.6	0.4	0	−	−	6.0	414	430	458
Hydrolyzed XI	0.85	0.9	0.4	+	−	6.0	414	430	458

[a] Minor. [b] Major.

VII, VIII, and IX, respectively. The metabolite with R_f 0.1, Fraction VI, could not be crystallized from methanol. After evaporation to dryness, this residue was triturated with ether and dried under vacuum to yield about 10 mg of hygroscopic material.

The eluates collected in fractions 11–14 were concentrated and streaked on instant TLC sheets impregnated with silica gel and the chromatogram was developed with System A. The zones corresponding to R_f 0.4 and 0.65 were horizontally cut off and eluted with aqueous methanol. The eluates were separately evaporated to dryness, and the residues were dissolved in water to yield Fractions X and XI, respectively.

RESULTS

The chromatogram of the methanol eluate from a resin column developed with System A showed five iodoplatinate positive spots with R_f values equal to 0.1 (unknown), 0.3 (corresponds to morphine 3-glucuronide), 0.5 (unknown), 0.6 (corresponds to morphine 3-ethereal sulfate), and 0.85 (corresponds to morphine) (Fig. 1). Results of TLC, GLC, GLC–mass spectrometry, and phenolic and glucuronide tests for all fractions are shown in Table I.

Identification of Morphine 3-Glucuronide—A major morphine metabolite, located in Fractions V and VII, was isolated in crystalline form in a yield of 600 mg. The metabolite on TLC had the same R_f values as authentic morphine 3-glucuronide and was negative in the free phenol test. Its UV spectrum in an alkaline medium did not show a bathochromic shift. This metabolite was positive in the glucuronide test and hydrolyzed with β-glucuronidase to yield morphine, identified by TLC, GLC, and GLC–mass spectrometry. The ratio of glucuronic acid to morphine was about 1:1. Based on these data, the identity of this metabolite was established as morphine 3-glucuronide.

Identification of Morphine 6-Glucuronide—The metabolite located in Fraction VIII was isolated in crystalline form in a yield of 40 mg. This metabolite was positive in both the free phenol and glucuronide tests. Its UV spectrum in an alkaline medium showed a bathochromic shift (Fig. 2). After hydrolysis with acid or β-glucuronidase, it yielded morphine and a small amount of normorphine (3–5%), identified by TLC, GLC, and GLC–mass spectrometry (Fig. 3). The ratio of morphine to glucuronic acid was about 1:1. The identity of this metabolite was thus established as morphine 6-glucuronide.

Identification of Morphine 3,6-Diglucuronide—The metabolite located in Fraction VI was positive in the glucuronide test and negative in the free phenol test. After hydrolysis with acid or β-glucuronidase, it yielded morphine, identified by GLC and GLC–mass spectrometry (Fig. 4). The ratio of morphine to glucuronic acid was about 1:2. The metabolite was thus identified as morphine 3,6-diglucuronide.

Identification of Morphine 3-Ethereal Sulfate—The metabolite located in Fraction XI on TLC had the same R_f value as authentic morphine 3-ethereal sulfate, was negative in the free phenol and glucuronide tests, and was not hydrolyzed with β-glucuronidase, but it was hydrolyzed with arylsulfatase to yield morphine, identified by TLC. The metabolite prior to or after hydrolysis and derivatization with 25% trimethylsilylimidazole in pyridine showed a peak having the same retention time and

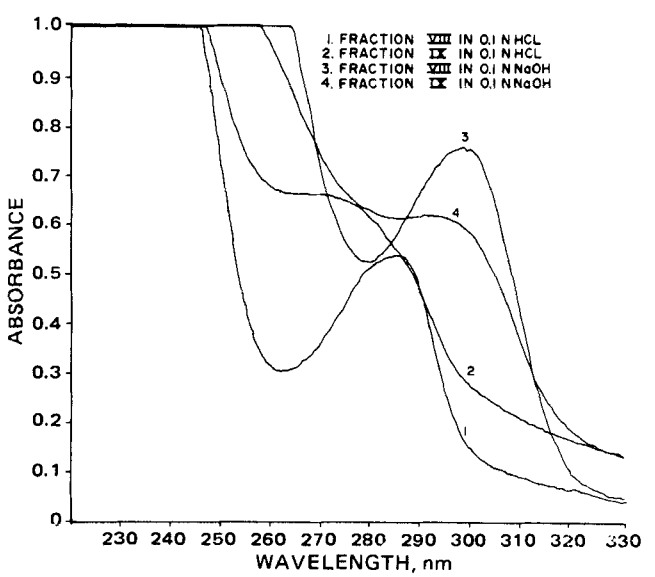

Figure 2—*UV spectrum of Fractions VIII and IX in 0.1 N HCl and 0.1 N NaOH.*

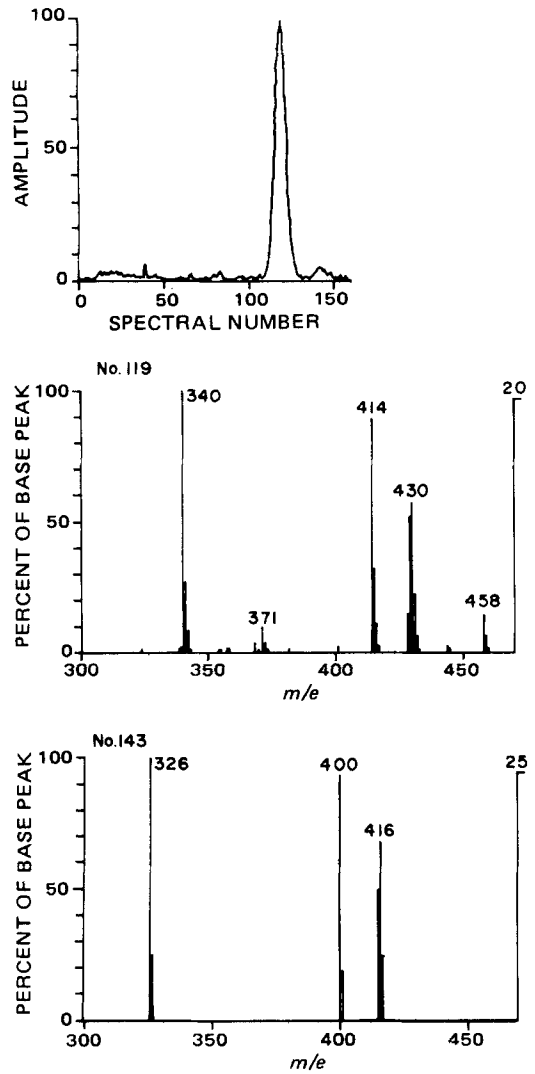

Figure 3—*Integrated total ion current chromatogram (top) of an extract of hydrolyzed Fraction VIII as the trimethylsilyl derivative and mass spectra No. 119, identified as morphine, and No. 143, identified as normorphine.*

mass fragmentation pattern as morphine. Authentic morphine 3-ethereal sulfate, derivatized with 25% trimethylsilylimidazole in pyridine, also showed a peak with the same retention time as morphine. The result indicated that morphine 3-ethereal sulfate was probably hydrolyzed to morphine during the derivatization.

The metabolite located in Fraction I on TLC had the same R_f value as morphine 3-ethereal sulfate as well as morphine *N*-oxide, was not reduced with sodium bisulfite, was negative in the free phenol and glucuronide tests, and was tentatively identified as morphine 3-ethereal sulfate.

Identification of Normorphine—The metabolite located in Fraction III on TLC had the same R_f value as authentic normorphine (Fig. 5). After derivatization with 25% trimethylsilylimidazole in pyridine or acetic anhydride, it showed a peak on GLC with the retention time and mass fragmentation pattern of normorphine (Fig. 6, lower panel).

Identification of Normorphine 6-Glucuronide—The metabolite located in Fraction IX was positive in the free phenol and glucuronide tests. Its UV spectrum in an alkaline medium showed a bathochromic shift (Fig. 2). After hydrolysis with acid or β-glucuronidase, it yielded mainly normorphine and a small amount of morphine (5%), identified by GLC and GLC–mass spectrometry (Fig. 7). The ratio of normorphine to glucuronic acid was about 1:1. The R_f value of the metabolite (0.6) on instant TLC sheets, impregnated with silica gel and developed with System A, was close to that of morphine 3-ethereal sulfate (0.65). However, when these two metabolites were cochromatographed, the chromatogram showed two definite spots. The metabolite was identified as normorphine 6-glucuronide.

Identification of Normorphine 3-Glucuronide—Fractions V and VII were hydrolyzed and extracted. The extract was chromatographed on TLC and developed with System B. The chromatogram showed a major spot of morphine and a minor spot of normorphine (Fig. 5). The normorphine conjugate, crystallized together with morphine 3-glucuronide, had a different R_f value than that of normorphine 6-glucuronide and was enzymatically hydrolyzed with β-glucuronidase. It was tentatively identified as normorphine 3-glucuronide.

Analysis of Fraction X did not reveal any hypothesized morphine metabolites.

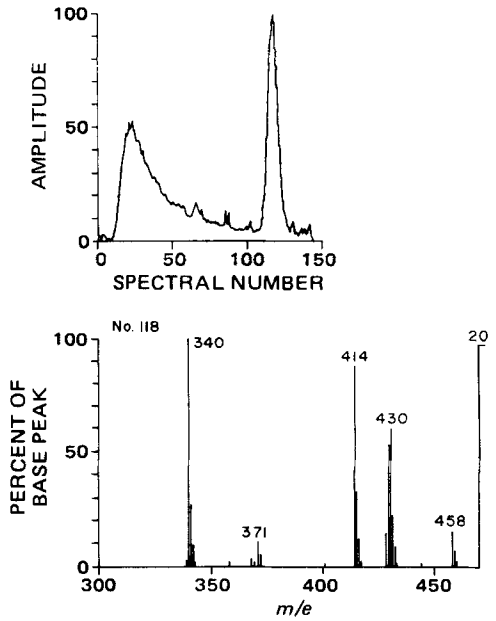

Figure 4—*Integrated total ion current chromatogram (top) of an extract of hydrolyzed Fraction VI as the trimethylsilyl derivative and mass spectrum No. 118, identified as morphine.*

DISCUSSION

The finding of morphine 3- and 6-glucuronides in the urine of morphine-dependent subjects confirmed previous observations (2, 5). In addition to morphine 3- and 6-glucuronides, morphine 3,6-diglucuronide was isolated. In an earlier study on the metabolism of morphine in the dog (1), one morphine conjugate was crystallized from aqueous methanol and was identified as morphine 3-glucuronide dihydrate, but another conjugate was more polar and could not be obtained in crystalline form. It was postulated to be morphine 3-ethereal sulfate 6-glucuronide. The amount of morphine conjugates was reported to account for 55–64% of the administered dose (14).

Morphine 6-glucuronide could result from enzymatic conjugation *in vivo* or hydrolysis of morphine 3,6-diglucuronide either *in vivo* or during analysis, since the glucuronide conjugate at the 3-position hydrolyzes easier than that at the 6-position (26). Although the R_f value of isolated morphine 6-glucuronide on instant TLC sheets, impregnated with silica gel and developed with System A, was close to that of codeine 6-glucuronide, after hydrolysis of the isolated material followed by extraction and derivatization, analysis by GLC and GLC–mass spectrometry indicated only morphine and a small amount of normorphine but no codeine.

A previous study demonstrated that the administered morphine sulfate sample contained about 0.04% codeine (20), which could be biotransformed to morphine and norcodeine, which could then be conjugated (27). However, no codeine or norcodeine was found in the present study. The amount of these conjugates was probably too small to be detectable. The amount of morphine 6-glucuronide and morphine 3,6-diglucuronide was estimated to be about 1% that of morphine 3-glucuronide.

Morphine 3-ethereal sulfate was isolated from the urine of humans and located in Fractions I and XI. The R_f value of the spot of Fraction I corresponded to the values of both morphine *N*-oxide and morphine 3-ethereal sulfate. However, it was not reduced with sodium bisulfite, ruling out morphine *N*-oxide. Use of an authentic standard confirmed that morphine 3-ethereal sulfate could be extracted into the organic phase under the experimental conditions. The ethereal sulfate group of morphine 3-ethereal sulfate, like dopamine 3- and 4-ethereal sulfates (28),

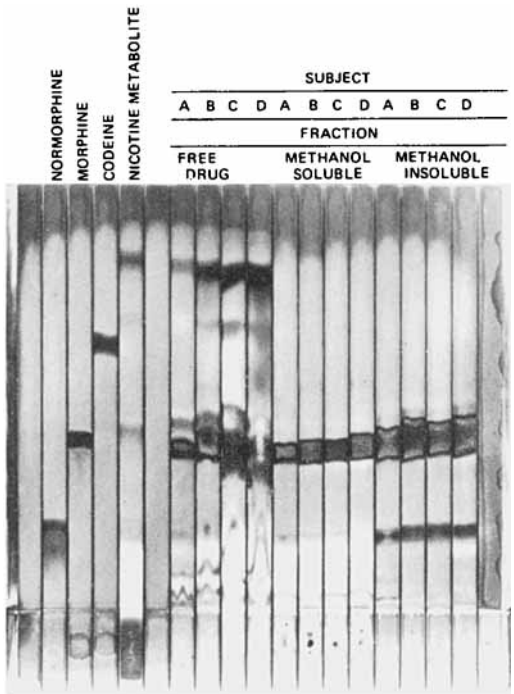

Figure 5—*Thin-layer chromatogram of the organic phase, the methanol-soluble and the acid-hydrolyzed methanol-insoluble fractions on TLC silica gel plates developed with System B. The methanol eluate of nicotine metabolites was obtained by chromatographing 200 ml of drug-free cigarette smoker's urine through a resin column and treating it as for the isolation of morphine metabolites.*

can be hydrolyzed during derivatization with 25% trimethylsilylimidazole in pyridine. The amount of morphine 3-ethereal sulfate was estimated to be about 1% that of morphine 3-glucuronide.

Although *N*-demethylation of morphine in humans was reported using radioisotopes (9, 15) and GLC (14), no report on the isolation of free and conjugated normorphine appears in the literature. The amount of free and conjugated normorphine was reported to account for about 1 and 4%, respectively, of the administered dose (14). Normorphine conjugates, like morphine glucuronides, could exist in two forms: one conjugated as glucuronide at the 3-position and the other conjugated at the 6-position.

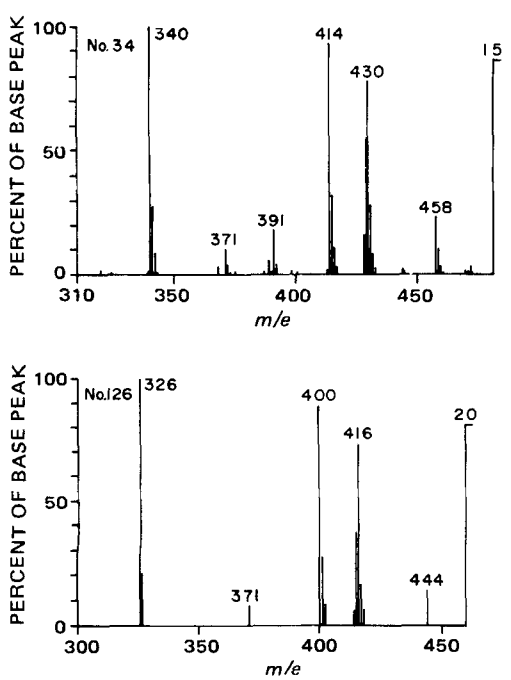

Figure 6—*Upper panel: mass spectrum (No. 34) of GLC peak for Fraction IV, identified as morphine. Lower panel: mass spectrum (No. 126) of GLC peak for Fraction III, identified as normorphine.*

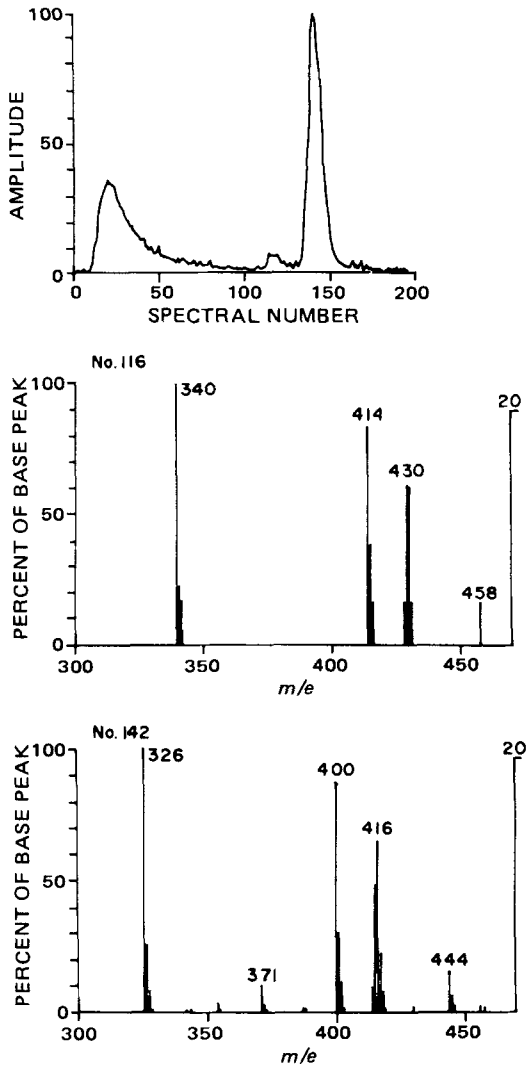

Figure 7—*Integrated total ion current chromatogram (top) of an extract of hydrolyzed Fraction IX as the trimethylsilyl derivative and mass spectra No. 116, identified as morphine, and No. 142, identified as normorphine.*

Normorphine 3-glucuronide was probably less soluble in methanol and crystallized together with morphine 3-glucuronide in aqueous methanol; normorphine 6-glucuronide, like morphine 6-glucuronide, was more soluble in methanol and was isolated from the methanol-soluble fraction. The present study was performed in morphine-dependent subjects. The extent of *N*-demethylation of morphine after acute administration in nondependent humans needs further study.

Although dihydromorphinone was detected in the urine of various animal species given morphine (18, 29), dihydromorphinone was not detected in the urine of morphine-dependent subjects in the present study.

The existence of morphine 2,3-quinone or 2-hydroxymorphine in the urine of humans is inconclusive. Samples were derivatized with 25% trimethylsilylimidazole in pyridine and scanned for morphine 2,3-quinone or 2-hydroxymorphine by GLC–mass spectrometry, and no evidence of their presence was found. In the organic phase, an iodoplatinate positive spot with R_f 0.1 was observed on instant TLC sheets impregnated with silica gel and developed with both Systems A and B. This spot was difficult to elute with methanol or water but could be eluted with 2.5% acetic acid in methanol. After derivatization with trifluoroacetic anhydride or 25% trimethylsilylimidazole in pyridine, it did not show any peak on GLC at a high column temperature (275° for 30 min). No peak was observed with the solid probe of the mass spectrometer at 300°. The chromatographic characteristics of this spot were similar to those of an authentic morphine 2,3-quinone sample[13] (19).

[13] Obtained from Dr. Misra, New York State Office of Drug Abuse Services Testing and Research Laboratory, Brooklyn, N.Y.

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[x] To whom inquiries should be directed.

Polymer Sorption of Nitroglycerin and Stability of Molded Nitroglycerin Tablets in Unit-Dose Packaging

M. J. PIKAL [x], D. A. BIBLER, and B. RUTHERFORD

Abstract □ The sorption of nitroglycerin by thermoplastic polymers and the stability of molded nitroglycerin tablets in strip packaging were studied. The polymers investigated varied greatly in their affinity for nitroglycerin, the order of decreasing affinity being: vinyls ≫ low density polyethylene > ionomers > high density polyethylene. With the proper choice of packaging, molded nitroglycerin tablets stabilized with povidone maintained acceptable potency for up to 2 years at 26° when strip packaged in unit doses. Chemical decomposition (hydrolysis) of nitroglycerin also was investigated. Povidone accelerated the decomposition of nitroglycerin; at high temperature, decomposition was a significant factor in tablet stability for tablets containing povidone.

Keyphrases □ Nitroglycerin—sorption by thermoplastic polymers and stability of molded tablets in various strip packages, effect of povidone □ Sorption—nitroglycerin by thermoplastic polymers in various packaging materials □ Stability—molded nitroglycerin tablets in various strip packages, effect of povidone □ Packaging materials, various—sorption by thermoplastic polymers and stability of molded nitroglycerin tablets, effect of povidone □ Povidone—effect on stability of molded nitroglycerin tablets in various strip packages □ Tablets, molded—nitroglycerin, sorption by thermoplastic polymers and stability in various strip packages □ Dosage forms—molded nitroglycerin tablets, sorption by thermoplastic polymers and stability in various strip packages □ Vasodilators, coronary—nitroglycerin, sorption by thermoplastic polymers and stability of molded tablets in various strip packages, effect of povidone

Recently, the stability of nitroglycerin tablets has been studied extensively (1–4). Nitroglycerin tablets potentially can lose potency in four ways: chemical decomposition, loss to the atmosphere by vaporization, intertablet migration, and sorption by packaging materials. Nitroglycerin undergoes thermal decomposition at elevated temperature (5) and may undergo basic hydrolysis (6). However, nitroglycerin decomposition in tablets has not been demonstrated.

At 25°, the air space above nitroglycerin tablets contains 1–7 μg of nitroglycerin/liter (4), the exact figure depending on the formulation. Thus, when nitroglycerin tablets are exposed to adequate circulation of room air, measurable losses in potency occur within a few days *via* vaporization (1, 2). Intertablet migration of nitroglycerin, resulting in decreased content uniformity upon aging, is a serious problem with conventional molded tablets (1, 4). Because of the migration problem, stabilizing additives have been incorporated into tablets (1, 4). The stabilizing additive lowers the vapor pressure of nitroglycerin sufficiently to prevent most of the migration that would otherwise occur (4).

Sorption of nitroglycerin by packaging materials may also have serious consequences for tablet stability (2–5, 7, 8). Conventional tablets strip packaged in an aluminum foil–low density polyethylene laminate lost 90% of their nitroglycerin to the package (7). Sorption losses were less for stabilized tablets (2). However, none of the strip