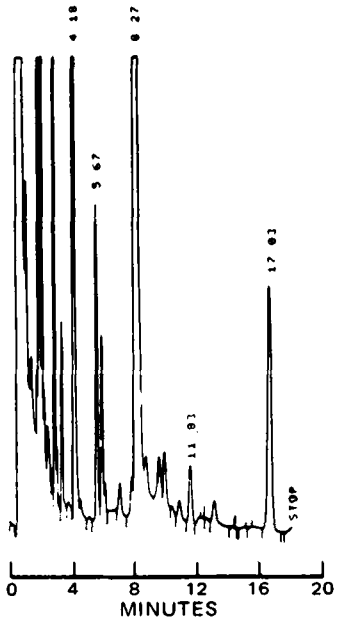

Figure 2—*Gas chromatogram of a 2-ml plasma sample extract obtained from a subject 6 hr after intake of 120 mg of isosorbide dinitrate slow-release preparation; 25 ng of isoidide mononitrate (retention time of 5.67 min) and 200 ng of isomannide dinitrate (17.03 min) were added as internal standards. Key (retention time): 4.18 min, isosorbide 2-mononitrate; 8.27 min, isosorbide 5-mononitrate; and 11.83 min, isosorbide dinitrate.*

samples, a linear relationship was found for the concentration range studied, and the intercept of the standard curve was negligible. The same was true for isosorbide 2-mononitrate and isosorbide 5-mononitrate.

As for isosorbide dinitrate (5), the recoveries of the mononitrates from plasma were quantitative. The relative standard deviations for analyses performed on different days were 7.01 for isosorbide dinitrate (11 samples, 5–20 ng), 9.13 for isosorbide 2-mononitrate (11 samples, 10–30 ng), and 10.96 for isosorbide 5-mononitrate (11 samples, 100–300 ng). The lower limits of detection were approximately 0.5 ng/ml of plasma for isosorbide dinitrate, 2 ng/ml for isosorbide 2-mononitrate, and 20 ng/ml for isosorbide 5-mononitrate.

After daily injections on the column, the glass injector adaptor was replaced weekly.

Figure 2 shows a typical plasma chromatogram 6 hr after intake of 120 mg of an isosorbide dinitrate slow-release preparation.

Several investigators reported difficulties with the quantitative mononitrate determination by conventional packed column GLC due to peak interference mainly with isosorbide 5-mononitrate (7–9). The wall-coated capillary column used here provides a suitable means of analyzing the isosorbide mononitrates with the required efficiency and specificity.

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Improved Method for Morphine Extraction from Biological Samples

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Abstract □ Methadone, morphine, or naloxone extraction from brain homogenates, plasma, and urine is described. An aqueous sample was loaded on a surgical gauze support, which was washed with extracting solvents. Aqueous samples remained on the support, and nonpolar drugs partitioned into the lipophilic extracting solvent. The procedure recovered 80–100% of nanogram levels of methadone, morphine, or naloxone from biological samples. In addition, an approximate 10-fold timesaving capacity was demonstrated compared to standard liquid–liquid extraction techniques.

Keyphrases □ Methadone—analysis, liquid–liquid extraction, brain, blood, plasma, urine □ Morphine—analysis, liquid–liquid extraction, brain, blood, plasma, urine □ Naloxone—analysis, liquid–liquid extraction, brain, blood, plasma, urine □ Liquid–liquid extraction—analysis, methadone, morphine, naloxone, brain, blood, plasma, urine □ Narcotic analgesics—methadone, morphine, naloxone, liquid–liquid extraction, brain, blood, plasma, urine

Determination of tissue narcotic drug levels frequently involves radiolabeled drug administration. Following alkalinization, the lipophilic drugs are extracted from tissue preparations using standard liquid–liquid extraction techniques (1–5). These techniques typically involve partitioning the drug between two immiscible liquids by shaking, phase separation by centrifugation, and drug analysis in the nonpolar solvent phase or in the aqueous phase after back-extraction. Improved methods for morphine and related drug extraction have not altered the basic liquid–liquid extraction technique (4, 6).

A rapid, efficient extraction technique for tissue morphine and related drug determination is presented here. Extraction of tritiated methadone, morphine, and naloxone from brain, plasma, and urine samples is described. A polar support (gauze sponges) held an aqueous tissue ho-

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Table I—Methadone, Morphine, and Naloxone Recovery from Brain, Plasma, and Urine Samples

Drug	Tissue	Drug Added, ng	Percent Recovered	Solvent
Methadone	Brain	15–85	91.0 ± 2.3[a]	Ether
	Plasma	2–19	91.4 ± 4.9	Ether
	Urine	185	98.0 ± 0.8	Ether
Morphine	Brain	2–15	98.0 ± 3.3	Ethanol–chloroform (10:90)
	Plasma	1–18	94.3 ± 3.1	Ethanol–chloroform (10:90)
	Urine	0.5–1	80.3 ± 0.8	Ethanol–chloroform (10:90)
Naloxone	Brain	4–55	104.4 ± 1.8	Chloroform
	Plasma	2–230	95.6 ± 3.4	Chloroform
	Urine	2.5–70	83.2 ± 1.8	Chloroform

[a] Mean ± *SEM*, n = 6–9.

mogenate or a sample containing radioactive drug. A nonpolar, water-immiscible solvent was passed through the column, collected, and evaporated, and the drug concentration was determined.

EXPERIMENTAL

Drugs and Reagents—All solvents were reagent grade. The drugs used included morphine sulfate, methadone hydrochloride, naloxone hydrochloride, ^{3}H-methadone hydrobromide (161.0 mCi/mmole), ^{3}H-morphine (23 Ci/mmole), and ^{3}H-naloxone (4.54 mCi/mmole). All drug concentrations were expressed in terms of the base.

Extraction Columns—These columns were similar to commercially available[1] ones but were made from inexpensive, common laboratory materials. The columns consisted of 12-ml disposable syringe barrels into which one folded and tightly rolled piece of surgical gauze (10.2 × 10.2-cm, 8-ply gauze sponge) was placed. A 21-gauge needle fitted with a piece of polyethylene tubing (PE90) was attached to a three-way stopcock, which, in turn, was attached to the extraction column (syringe tip) and was used to regulate the effluent flow.

TLC—Radioactivity extracted from brain homogenates, plasma, or urine was identified as unchanged drug (*e.g.*, methadone, morphine, or naloxone) using TLC. Tritiated drug radioactive purity was verified by TLC. After extraction, excess solvent from selected samples was evaporated using nitrogen; the resulting concentrated samples were applied to individual plastic TLC plates coated with silica gel along with appropriate standards.

The solvent systems for methadone, morphine, and naloxone were ethyl acetate–butanol–ethanol–ammonium hydroxide (70:10:15:1), ethanol–acetic acid–water (60:30:10), and ethyl acetate–hexane–ethanol–ammonium hydroxide (60:25:14:1), respectively. The radioactivity in each area was determined by cutting the chromatogram into 1-cm sections and placing them in scintillation vials containing scintillation cocktail.

Biological Tissues and Fluids—Brains and blood samples were obtained from male Swiss-Webster mice killed with carbon dioxide. Blood was removed by cardiac puncture using a heparinized syringe, and plasma was obtained following centrifugation of blood samples. Brains were removed, weighed, and homogenized in four volumes of cold, distilled water using a motor-driven polytef pestle in a Potter homogenizer. Human urine was used in all cases.

Method of Extraction—The following procedure was used to extract morphine or naloxone from mouse brain homogenates. A brain homogenate aliquot (0.2 ml) was diluted with an equal volume of glycine buffer (0.5 *M*, pH 9.0) and added to the extraction column. The column capacity was 2 ml. Two minutes after the buffered homogenate was added to the column, 10 ml of solvent was added. The effluent was collected in a scintillation vial after a 2-min equilibration period. The column was washed twice with 5 ml of solvent and allowed a 2-min equilibration with each wash.

The combined effluent (20 ml) was evaporated to dryness in a water bath, and 15 ml of scintillation cocktail was added. Radioactivity was measured by liquid scintillation spectrometry, and an internal standard (^{3}H-toluene) was used to determine counting efficiency. The procedure for methadone extraction from brain homogenates was identical except that sufficient 0.1 *N* NaOH was added to the brain homogenate to adjust the pH to 10.5 prior to extraction.

[1] Jetubes, Manhattan Instruments, Santa Monica, Calif.

A similar procedure was used to extract drugs from plasma and urine samples. Aliquots (0.05 ml) of plasma or urine containing morphine or naloxone were diluted with nine volumes of glycine buffer, and the total volume added to the extraction column was 0.5 ml in each case. The upper limit of plasma or urine that was applied to the column was 1.0 ml; extraction efficiency was not reduced by applying smaller volumes. The pH of plasma or urine samples containing methadone was adjusted to 10.5 after nine volumes of glycine buffer were added. The extraction solvents were ether for methadone, ethanol–chloroform (10:90) for morphine, and chloroform for naloxone.

Determination of Percent Recovery—The specific activity of tritiated methadone, morphine, and naloxone was known. The purity was determined using TLC and appropriate standards. A measured radiolabeled drug amount was added to a sample (brain homogenate, plasma, or urine), the sample pH was adjusted, and drug was extracted. The radioactivity was measured after solvent evaporation in certain samples, and the identity of radioactivity was confirmed by TLC in others. The percent recovery was calculated, and corrections were made for the purity of added radioactivity as well as the amount of radioactivity lost in the transfer of 0.4-ml aliquots to the extraction columns.

Drug Extraction from Tissues after Subcutaneous Administration—Tritiated methadone, morphine, or naloxone was administered subcutaneously to mice to measure the extraction of metabolites and unchanged drug(s) from brain and blood. Mice were killed with carbon dioxide 20 min after drug administration, and whole brains and blood samples were obtained. Brains were homogenized, plasma was obtained, and radioactivity was extracted as already described. The percent of total radioactivity corresponding to unchanged methadone, morphine, or naloxone was determined using TLC.

RESULTS AND DISCUSSION

Table I shows the percent tritiated methadone, morphine, and naloxone recovery from brain homogenate, plasma, or urine. In each case, the extracted radioactivity was chromatographically identical to authentic methadone, morphine, or naloxone. These results demonstrate highly efficient extraction of the three drugs from mouse brain homogenates even at very low drug concentrations. The concentrations used represented values typical of pharmacological studies.

The recovery of methadone, morphine, or naloxone from brain samples in other studies using standard liquid–liquid extraction techniques was between 66.0 and 96.2% (1, 4, 6, 7). The highest recoveries required three consecutive liquid–liquid extractions. A recent study (4) reported 90% morphine recovery (0.125–0.5 μg added) from biological tissues using standard liquid–liquid extraction methods and siliconized glassware. The present extraction procedure provided efficient recovery of morphine (98.0%) from brain homogenates at low morphine concentrations (1–20 ng added) without special precautions such as siliconized glassware.

The present procedure also provided efficient methadone recovery from plasma and urine samples (91.4 and 98.0%, respectively). Extraction of morphine and naloxone from plasma samples was also efficient (94.3 and 95.6%, respectively). Morphine and naloxone recovery from urine samples (80.3 and 83.2%, respectively) was significantly lower than morphine and naloxone recovery from plasma but was acceptable. The buffered urine sample pH was not significantly lower than the buffered plasma pH, so reduced urine morphine and naloxone extraction efficiency may have been related to the procedure. Urine alkalinization (7) and extraction with a polar solvent might improve the efficiency.

Subcutaneous methadone, morphine, or naloxone was administered to mice to measure selective extraction of parent drugs and metabolites from brain and plasma. The total radioactivity extracted from brain was

chromatographically identical to methadone (80.5%), morphine (95.5%), and naloxone (100%). Total extracted plasma radioactivity represented methadone (76.9%), morphine (75.5%), or naloxone (91.8%). Parent drug extraction selectivity in each case was acceptable.

Timesaving is the greatest advantage of this method. Extraction of 20 tissue samples required 10–15 min; 1.5–2 hr was required with standard liquid–liquid extraction. Since brain and plasma drug concentrations are routinely required in pharmacological studies, the utility of the present method is obvious. Theoretically, the present procedure could be substituted for any organic base or acid solvent extraction from biological fluids and tissues.

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Effect of Water-Soluble Carriers on Morphine Sulfate Release from a Silicone Polymer

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Received August 14, 1978, from the *Drug Dynamics Institute, College of Pharmacy, University of Texas, Austin, TX 78712.* Accepted for publication November 2, 1978.

Abstract □ The influence of gelatin, sodium lauryl sulfate, lactose, and sodium alginate on morphine sulfate diffusion from cylindrical silicone polymer pellets was examined in isotonic pH 7.4 phosphate buffer. These water-soluble carriers caused the pellets to swell in aqueous media. Sodium alginate exerted the greatest influence on drug release. The morphine sulfate diffusion rate from the cylindrical pellets increased as the matrix alginate content increased up to 20%. Water-soluble carrier incorporation into silicone polymeric matrixes permits controlled release of water-soluble drugs that otherwise would be released extremely slowly from the polymer. Drug diffusion from the silicone matrix containing sodium alginate followed second-order kinetics. The release mechanism probably involves the creation of pores or pathways through the matrix secondary to the swelling.

Keyphrases □ Morphine sulfate—release from silicone polymer, effect of water-soluble carriers □ Drug delivery systems—morphine sulfate, release from silicone polymer, effect of water-soluble carriers □ Silicone polymers—release of morphine sulfate, effect of water-soluble carriers □ Dosage forms, controlled release—morphine sulfate, release from silicone polymer, effect of water-soluble carriers

The drug release rate from an inert matrix is dependent on solute solubility in the matrix and diffusivity as well as on parameters independent of the particular drug (1). Previous silicone polymer work has been concerned largely with developing long acting steroidal delivery systems and investigating factors controlling drug release from silicone devices (2–9).

The divergent *in vitro* silicone polymer release patterns of four progesterone-type steroids with similar diffusion coefficients were attributed to differences in the polymer steroid solubilities (5). The release of salts and water-soluble drugs from silicone polymers has received little attention. Since polymer drug solubility is an important drug release determinant, water-soluble drug diffusion from a silicone polymer would be expected to be extremely slow.

The present study examined the influence of water-soluble carriers on the *in vitro* morphine sulfate release from a polysiloxane polymer. Recent studies (10, 11) demonstrated that polysiloxane rubber implants containing morphine sulfate and a water-soluble carrier are excellent drug delivery systems for inducing morphine dependence in rats.

EXPERIMENTAL

The following items were used: simethicone liquid[1], polydimethylsiloxane polymer and silica filler[2], stannous octoate[3], morphine sulfate, polysorbate 80[4], sodium alginate[5], sodium chloride, 0.1 *N* HCl, lactose, sodium lauryl sulfate, monobasic sodium phosphate, dibasic sodium phosphate, and microcrystalline cellulose[6].

Pellets were prepared by the addition of simethicone fluid to polydimethylsiloxane elastomer (1:1), followed by homogeneous mixing with the powders to be added. The pellets contained 25% (w/w) morphine sulfate. The drug and water-soluble carrier (lactose, sodium lauryl sulfate, gelatin, or sodium alginate) were passed through a 100-mesh screen prior to mixing with the silicone polymer. To polymerize the mixture, stannous octoate catalyst, 25 mg/g of mixture, was added and dispersed uniformly. Next, the mixture was added rapidly to a plastic tablet mold, and the pellets were allowed to cure for 24 hr.

The cylindrical pellets were 5.5 mm in diameter and 3.5 mm in thickness. The mean weights varied from 78 to 88 mg, depending on the amount of powder embedded in the pellet. Surface area measurements were made with a micrometer. Homogeneity studies were carried out by cutting individual pellets into small pieces and extracting them in 100 ml of purified water for 48 hr. Recoveries of 99 ± 2% were obtained (n = 6).

Drug release from the pellets was studied in screw-capped vials, 1.5 cm diameter × 10.5 cm length, containing 15 ml of phosphate buffer (0.13 *N*, pH 7.4). These vials were maintained at 37° and rotated end-over-end at 15 rpm. Aliquots of 5 ml were withdrawn from the vials at various times and assayed by UV spectrophotometry for morphine. To maintain sink conditions, 5-ml volumes of fresh medium were added to each vial after

[1] Medical fluid 360, 200 cps, Dow Corning Corp., Midland, Mich.
[2] Silastic 382 medical grade elastomer, Dow Corning Corp., Midland, Mich.
[3] Catalyst M, Dow Corning Corp., Midland, Mich.
[4] Tween 80, City Chemical Corp., New York, N.Y.
[5] Kelco Gell LV, Kelco Co., Clark, N.J.
[6] Avicel, FMC Corp., Marcus Hook, Pa.

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