clotting time data on fresh blood samples containing various amounts of sodium bicarbonate. The amount of sodium bicarbonate added to the samples ranged from 12.5 to 125 mEq/liter of blood, assuming an average adult blood volume of 65 ml/kg. At added bicarbonate concentrations of 1–2 mEq/kg, significant changes in clotting were observed. Both the prothrombin time and the thrombin clotting time were elevated (Samples 1 and 2, Table I). Increasing the bicarbonate concentration beyond 2 mEq/kg inhibited clotting markedly.

DISCUSSION

Sodium bicarbonate is used widely as a therapeutic agent for acid–base imbalance (10–12). The usual dose of sodium bicarbonate ranges from 1 to 2 mEq/kg and normally is administered intravenously as a 7.5% hypertonic solution or as a 1.5% isotonic solution. Sodium bicarbonate has been regarded as a safe and effective therapeutic agent and is relatively free of serious side effects. With the exception of overdose-induced seizures or tetany secondary to hypocalcemia and hyperalkalosis, no known adverse reactions have been reported (13, 14).

Results from the present investigation demonstrate that sodium bicarbonate inhibits the conversion of fibrinogen to fibrin. Although the mechanism of clot inhibition is unclear, it is well known that some neutral salts retard coagulation and that others accelerate the conversion of fibrinogen to fibrin (2–9). Both cations and anions may bind to the fibrinogen or fibrin molecules during clot formation, and, depending on the individual chemical species, they can either accelerate or inhibit coagulation. The degree of clot inhibition also is affected greatly by the concentration and ionic strength of the salt.

Although data from pH determinations do not correlate well with observed changes in the prothrombin time and the thrombin clotting time, the effect of increasing pH on clotting cannot be ignored. Previous studies revealed that the structure and properties of fibrin clots are modified greatly by variations in pH and ionic strength during coagulation. However, even at constant pH and ionic strength, certain ions and neutral molecules at low concentration greatly affect the structure of the fibrin clot and its formation rate.

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Polydimethylsiloxane Pellets for Sustained Delivery of Morphine in Mice

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Received February 11, 1980, from the *Departments of Pharmacology and Pharmaceutics, Drug Dynamics Institute, College of Pharmacy, University of Texas at Austin, Austin, TX 78712.* Accepted for publication March 13, 1980.

Abstract □ A new dosage form was designed whereby a polymeric silicone elastomer pellet provided sustained delivery of morphine to mice over 11 days. These pellets, which can be made easily and inexpensively with a standard tablet mold, gradually released morphine sulfate into the implanted mice. Maximal morphine-induced physical dependence, measured by jumping during naloxone-induced withdrawal, was observed 3–5 days after implantation. At this time, slightly less than 50% of the morphine sulfate had been released. Drug release continued through Day 11 and was accompanied by a physical dependence of decreased magnitude compared to that observed on Day 3 or 5.

Keyphrases □ Morphine—sustained release from polydimethylsiloxane pellets, mice □ Drug delivery systems—sustained release of morphine from polydimethylsiloxane pellets, mice □ Sustained-release systems—delivery of morphine from polydimethylsiloxane pellets, mice

As a representative of the narcotic analgesic drugs, morphine has been used to induce tolerance and physical dependence in experimental animals (1). Its relatively short half-life in small animals requires frequent injections of morphine to sustain drug levels over an extended period.

BACKGROUND

Attempts have been made to deliver morphine to experimental animals without these injection routines but with the production of maximum tolerance and dependence. For example, Collier *et al.* (2) used an oily sustained-release preparation. Hui and Roberts (3) adsorbed morphine sulfate onto molecular sieves. Goode (4) used an implanted silicone rubber tubing reservoir of morphine solution. Teiger (5) used continuous intraperitoneal infusion. The usefulness of several of these methods is marginal as judged by the lack of utilization by fellow researchers. According to this criterion, employment of implanted tableted pellets that slowly release their morphine content appears to be the most accepted method to date.

The most commonly used morphine implant is the microcrystalline cellulose pellet described by Gibson and Tingstad (6). However, this pellet has several disadvantages as a drug delivery system for morphine. One disadvantage is that drug delivery in the animal ceases after 3 days, with only half of the drug being released from the pellet for absorption; the pellet tends to become encapsulated by membranous tissue, thereby diminishing morphine bioavailability (7, 8). Another disadvantage is that the pellet quickly becomes soft and mushy, so complete removal of the implant from the animal is difficult. This result is due to the fact that the pellet contains ~50% microcrystalline cellulose, a tablet disintegrant. Failure to remove all of the implanted pellet gives erroneously high values

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for the percent of morphine absorbed. However, the greatest disadvantage is the need for expensive equipment and pharmaceutical expertise to produce these tableted pellets.

McGinity and Mehta (9) recently reported a method for morphine delivery in rats utilizing a silicone rubber matrix formulated with algin, a water-soluble carrier. This method has been modified for use in mice, and the present study evaluates the ability of this pellet system to render mice physically dependent on morphine.

EXPERIMENTAL

Male experimental CD-1 albino mice[1], 20–35 g, were permitted to acclimate to the animal housing and testing facility (adjacent rooms) for at least 3 days prior to experimentation. Food and water were available *ad libitum*. The animal quarters were maintained on a 12-hr light–dark cycle (lights on at 7 am and off at 7 pm). All testing was done between 8 am and 6 pm.

The drugs and chemicals used were morphine sulfate[2], algin[3], polydimethylsiloxane[4], silicone oil[5], and stannous octate[6].

The pellets were prepared by the addition of silicone oil to the polydimethylsiloxane elastomer in a ratio of 1:1, followed by homogeneous mixing with the powders (morphine sulfate and algin). The method of preparation was described previously (10). When the pellets hardened, edges were trimmed carefully and the pellets were removed from the mold. The cylindrical pellets were 6 mm in diameter and 3 mm in height. Ten percent of the pellets were selected at random and tested for batch uniformity and morphine content. The morphine content in each pellet (both for batch uniformity and for each experimental pellet) was determined spectrophotometrically at 286 nm, and the morphine concentration was calculated using a standard curve.

Light ether anesthesia was used to permit the implantation of two morphine pellets in each mouse (pilot studies indicated two pellets would induce a satisfactory level of physical dependence). The pellets were implanted subcutaneously along the midline of the dorsal neck region posterior to the ears. Placebo pellets (pellets with lactose substituted for morphine) were implanted similarly.

Fifty mice were implanted, and groups of eight were challenged with naloxone at 5 mg/kg on Days 1, 3, 5, 7, 9, and 11 following implantation (each group was used for only one challenge). Each animal was placed in a large, clear glass (4-liter capacity) container and observed for spontaneous jumping off the floor. This behavior was videotaped and subsequently quantified by viewing of the videotapes. Counts for the number of spontaneous jumps were recorded for 5-min intervals up to 30 min after the naloxone challenge. Statistical analysis was done using analysis of variance and *post hoc* tests for differences among means.

RESULTS AND DISCUSSION

Figure 1 shows the concentration of morphine remaining in the pellet over time. Twenty-four hours after implantation, 81% of the morphine remained in the pellet, whereas 56% remained 3 and 5 days after surgery. The concentration of morphine then decreased to 35–40% 7–11 days after implantation.

The spontaneous jumping behavior also is shown in Fig. 1. Approximately 25 spontaneous jumps were shown over a 20-min period following naloxone challenge on Day 1. The peak withdrawal jumping behavior was observed at 3 and 5 days after implantation (207 and 206 jumps, respectively). After Day 5, jumping behavior decreased (Days 7 and 9) to approximately 135 jumps, falling to a level not significantly different from that of placebo-implanted mice on Day 11 (90 jumps).

A morphine-containing silicone rubber implant was reported previously for use in rats (9). This pellet was modified and reformulated for use in mice in the present study. The time course of development of physical dependence agrees well with that obtained by Way *et al.* (11) and Isom *et al.* (12). The former investigators used microcrystalline cellulose pellets, first described by Gibson and Tingstad (6), which contained 75 mg of morphine base; the latter investigators used silicone pellets containing 300 mg of morphine sulfate and mineral oil. The silicone pellet described in the present report contained 32 mg of morphine sulfate and algin, thus providing the implantation of 64 mg of drug/mouse. The major advantage in the use of these sustained-release drug

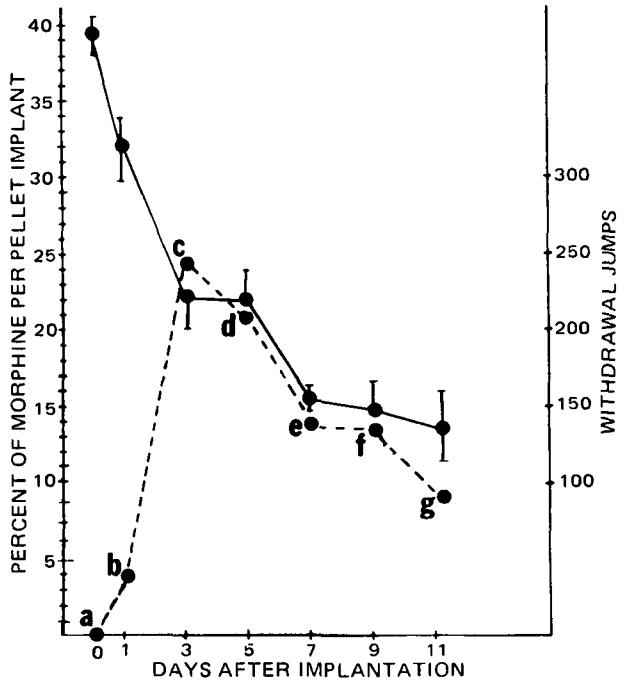

Figure 1—*Percent of morphine remaining in the polydimethylsiloxane pellet (—) (left axis) and the number of jumps observed during withdrawal induced by naloxone (- -) (right axis) plotted against the number of days the pellets were implanted. Symbols a–g represent the mean number of withdrawal jumps per 20 min after naloxone injection. The following symbols were not statistically significantly different from each other ($p < 0.05$): a and b; c and d; d, e, and f; and e, f, and g.*

delivery systems is the rapid induction of physical dependence without time-consuming repetitive injections. However, the microcrystalline cellulose pellets are manufactured *via* tablet presses available to relatively few investigators interested in this area of study.

The silicone pellet containing mineral oil (12) required approximately five times the amount of morphine used in the silicone pellet developed in these laboratories. The former pellet also produced a relatively low level of naloxone-induced jumping (*e.g.*, a peak withdrawal jumping score of 42 over 30 min compared to 247 jumps in 30 min in this study using 4 and 5 mg of naloxone/kg, respectively). The continuous intraperitoneal infusion reported by Teiger (5) allows reliable, controlled, and easily measurable quantities of morphine to be delivered to an experimental animal. However, the extensive surgery, long recovery, and the possible limitation for use in rats or larger animals are distinct disadvantages of this system.

An advantage of the polydimethylsiloxane pellets reported in this study is the use of algin, which causes the pellet to swell in the presence of tissue fluid. As the swelling occurs, the slow release of morphine sulfate within the pellet occurs. The incorporation of algin at the 10% level appears to be optimum for the incorporation and release of morphine sulfate at a concentration of 40% of the pellet weight. The manufacture of these pellets is extremely simple and inexpensive. In addition, only minor surgery and a very brief recovery period are required, thus permitting any investigator the opportunity of developing a sustained-release drug delivery system for morphine with which to study events associated with chronic morphine administration (*e.g.*, tolerance, physical dependence, and morphine-induced alterations of neurotransmitter dynamics).

The quantification of the level of physical dependence on morphine utilizing vertical jumping (as in the present study) *versus* platform jumping was studied by Takemori and Sprague (13). They reported no differences between the two methods. The vertical jumping is a simple method of assessing physical dependence induced by morphine, and the use of videotape greatly enhances the ability to quantitate this behavior accurately.

[1] Charles River, Wilmington, Mass.
[2] Mallinckrodt, St. Louis, Mo.
[3] Kelco Co., Clark, N.J.
[4] Silastic 382 elastomer, Dow Corning Corp., Midland, Mich.
[5] Medical fluid 360, Dow Corning Corp., Midland, Mich.
[6] Catalyst M, Dow Corning Corp., Midland, Mich.

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Determination of Procainamide and *N*-Acetylprocainamide in Biological Fluids by High-Pressure Liquid Chromatography

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Abstract □ A modification of a high-pressure liquid chromatographic method for the simultaneous determination of procainamide and *N*-acetylprocainamide in plasma is described. The deficiencies in the specificity of the existing method were overcome by replacing the cation-exchange column and the mobile phase. The recovery and reproducibility of both procainamide and *N*-acetylprocainamide from human, dog, and rat plasma and urine spiked with either compound were excellent in the concentration range of 0.05–10 μg/ml for plasma and 0.5–20 μg/ml for urine. The comparison of this method with a specific extraction method for sets of plasma samples from human subjects and rats receiving *N*-acetylprocainamide and procainamide, respectively, showed no statistically significant difference.

Keyphrases □ Procainamide—simultaneous high-pressure liquid chromatographic determination with *N*-acetylprocainamide in biological fluids □ *N*-Acetylprocainamide—simultaneous high-pressure liquid chromatographic determination with procainamide in biological fluids □ High-pressure liquid chromatography—analysis, procainamide and *N*-acetylprocainamide in biological fluids □ Antiarrhythmic agents—procainamide, simultaneous high-pressure liquid chromatographic determination with *N*-acetylprocainamide in biological fluids

High-pressure liquid chromatographic (HPLC) procedures for the determination of procainamide and its major metabolite, *N*-acetylprocainamide, in plasma have been described (1–6). One approach requires selective extraction of the drugs from plasma with an organic solvent, followed by reextraction or evaporation of the organic solvent prior to assay (1–4). The other approach involves deproteinization of plasma with acetonitrile and direct injection of the supernate following centrifugation (5). The former procedure usually is tedious. The latter procedure, while simple and rapid, may exhibit specificity and recovery deficiencies under certain conditions.

Nation *et al.* (7) suggested that the extraction method, although tedious and time consuming, may be a prudent approach for the determination of procainamide and *N*-acetylprocainamide. The present investigators experienced similar difficulties in using the deproteinization method. The purpose of this report is to describe a modified deproteinization method which produced consistent results and eliminated the lack of specificity and recovery. This result was achieved by replacing the cation-exchange column and the mobile phase utilized previously (5, 7).

EXPERIMENTAL

Materials—*N*-Acetylprocainamide hydrochloride[1] (an investigational new drug), procainamide hydrochloride USP[2], dioxane[3], toluene[3], acetonitrile[3], TLC plates[4], heparin USP[5], and a carbonated beverage[6] were obtained from commercial sources. Dioxane, toluene, and acetonitrile were glass distilled and purchased commercially. [^{14}C]Procainamide hydrochloride (specific activity 32 μCi/mg) and [^{14}C]-*N*-acetylprocainamide hydrochloride (specific activity 23.1 μCi/mg) were obtained commercially[7]. The internal standard, *p*-amino-*N*-(2-dipropylaminoethyl)benzamide, was synthesized in these laboratories.

Apparatus and Conditions—The high-pressure liquid chromatograph[8] was equipped with a 30-cm long × 4-mm i.d. alkylphenyl column[9] and a UV detector[10] at 280 nm. The column was eluted with a mobile phase that was a 60:40 mixture of acetonitrile and phosphate buffer (0.01 *M* NaH_2PO_4 and 0.005 *M* Na_2HPO_4, pH 6.6). The final pH of the mobile phase was 7.7. The flow rate was 2.0 ml/min. The solutions containing the drugs and the internal standard were injected into the chromatograph *via* an automatic processor[11].

The TLC plates were developed in an ascending fashion in a saturated chamber containing dioxane–toluene–ammonium hydroxide (80:30:1). The radioactivity on the developed TLC plates was detected by a radiochromatographic scanner[12], and the activity was determined on a liquid scintillation counter[13].

Preparation of Standards—The stock solution of the internal standard was prepared in methanol at a concentration of 2 mg/ml. This solution was diluted with acetonitrile to give a working standard solution of 1.5 μg/ml.

The standard solutions in plasma (human, dog, and rat) were prepared by spiking known amounts of procainamide and *N*-acetylprocainamide from a stock solution containing 1 mg/ml of each drug as the

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[2] Ganes Chemicals, New York, N.Y.
[3] Burdick & Jackson Laboratories, Muskegon, Mich.
[4] Aluminum oxide 60F-254, E. Merck, Rahway, N.J.
[5] Eli Lilly and Co., Indianapolis, Ind.
[6] Coca Cola.
[7] Custom synthesized, New England Nuclear, Boston, Mass.
[8] Model ALC/GPC 204, Waters Associates, Milford, Mass.
[9] μBondapak phenyl column, Waters Associates, Milford, Mass.
[10] Model 440, Waters Associates, Milford, Mass.
[11] Model 710 WISP, Waters Associates, Milford, Mass.
[12] Model 7201, Packard Instrument Co., Downers Grove, Ill.
[13] Model 2425, Packard Instrument Co., Downers Grove, Ill.

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