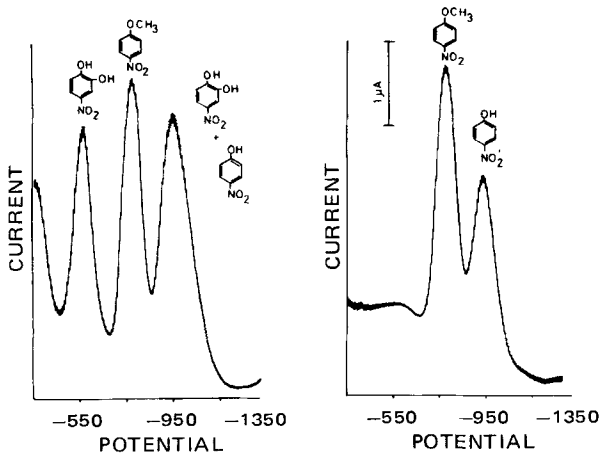

Figure 7—*Polarogram of I (10 μM), II (20 μM), and III (20 μM) in the presence of 40 μM $CuSO_4$. Key: left, before addition of magnesium ions; and right, after addition of magnesium ions.*

conjunction with calibration curves give direct measurements of III and I.

To determine II also, 200 μl of 0.1 *M* $MgCl_2$ is added to the same solution. Then this solution is bubbled again with nitrogen to achieve proper mixing and removal of the oxygen carried in during the manipulation. The newly taken polarogram (Fig. 7, right) shows peaks only for I and II, from which the concentration of II can be obtained by subtracting the base current. Theoretically, a second determination of I is possible from this second polarogram.

The accuracy of this method of determination for each substance in the presence of varying concentrations of the others is shown by the homogeneous and practically complete recovery rates given in Table I.

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Sustained-Release Applications of Montmorillonite Interaction with Amphetamine Sulfate

JAMES W. McGINITY [x] and JOHN L. LACH

Abstract □ Urinary recovery studies showed that montmorillonite significantly affects the initial therapeutic levels of amphetamine sulfate. The combination of a 1:20 drug–montmorillonite complex with pure drug in a 1:1 ratio, based on amphetamine content, resulted in recovery profiles resembling those obtained from prolonged-release dosage forms. The 1:20 complex, pure drug, and combination formulations showed comparable bioavailability after 48 hr.

Keyphrases □ Montmorillonite—effect on bioavailability of amphetamine sulfate, urinary recovery studies, applied to sustained-release dosage form □ Amphetamine sulfate—bioavailability, effect of montmorillonite, urinary recovery studies, applied to sustained-release dosage forms □ Urinary recovery studies—effect of montmorillonite on bioavailability of amphetamine sulfate, applied to sustained-release dosage form □ Dosage forms—sustained-release drug–clay complex, effect of montmorillonite on bioavailability of amphetamine sulfate □ Clays—montmorillonite, complex with amphetamine sulfate, effect on bioavailability □ Stimulants—amphetamine sulfate, effect of complexing with montmorillonite on bioavailability

The availability of a drug from a dosage form is dependent on both the rate and the completeness with which the drug is delivered to the absorption site in an absorbable form. In a recent review (1), excipients in solid dosage forms were shown to interact with various medicinals. Such drug–excipient interactions generally influence both the rate of drug absorption and the amount delivered unchanged to the general circulation.

An earlier publication (2) reported that cationic salts

Table I—Urine pH's for the Three Dosage Forms

Subject	Pure Drug		1:1 Combination, Drug–Complex		1:20 Drug–Montmorillonite Complex	
	0–10 hr	12–48 hr	0–10 hr	12–48 hr	0–10 hr	12–48 hr
1	6.25 ± 0.15	6.40 ± 0.20	6.35 ± 0.20	6.55 ± 0.25	6.25 ± 0.20	6.60 ± 0.20
2	6.35 ± 0.20	6.50 ± 0.25	6.00 ± 0.15	6.30 ± 0.25	6.20 ± 0.20	6.40 ± 0.25
3	6.05 ± 0.15	6.40 ± 0.20	5.95 ± 0.20	6.30 ± 0.35	6.20 ± 0.15	6.25 ± 0.35
4	6.20 ± 0.20	6.45 ± 0.25	6.35 ± 0.20	6.40 ± 0.25	6.15 ± 0.15	6.40 ± 0.25
5	6.65 ± 0.20	6.70 ± 0.20	6.55 ± 0.20	6.70 ± 0.25	6.89 ± 0.15	6.90 ± 0.25
6	6.50 ± 0.25	6.40 ± 0.30	6.40 ± 0.25	6.70 ± 0.25	6.35 ± 0.25	6.70 ± 0.20

of organic medicinals bind tenaciously to montmorillonite clay. Data *in vitro* demonstrated that the amount of drug released from the drug–clay complex was dependent on the amount of montmorillonite in the formulation.

The influence of montmorillonite in dosage forms containing diazepam (3) and tolbutamide (4) was evaluated clinically. The diazepam study showed that the clay hindered drug absorption; the opposite was true for tolbutamide. Montmorillonite reduced blood levels of dicumarol in dogs by 25% compared to blood levels following the administration of pure drug (5). However, warfarin sodium tablets containing montmorillonite released the drug much faster *in vitro* than did the best commercial tablets (6). These reports demonstrate the complexity of drug–clay interactions and indicate that the presence of montmorillonite in a dosage form may markedly influence drug effects.

The purpose of this investigation was to develop a sustained-release dosage form employing a drug–montmorillonite interaction. Amphetamine sulfate was the model drug chosen, and drug concentrations *in vivo* were followed *via* urinary recovery studies. Pure drug was compared with a 1:20 complex of drug and clay and a 1:1 combination of pure drug and complex. Previous studies *in vitro* (2) demonstrated that little drug was present in the uncomplexed form in the 1:20 formulation.

EXPERIMENTAL

Materials—Amphetamine sulfate[1], montmorillonite[2], anhydrous ether (analytical reagent), sodium hydroxide (analytical reagent), 0.5 *N* hydrochloric acid, pH 7.4 phosphate buffer, *p*-nitroaniline solution (0.69 g in 8.3 ml of concentrated hydrochloric acid, diluted to 100 ml with water and filtered), and 1.4% sodium nitrite aqueous solution were obtained commercially.

Trial Conditions—Six normal healthy subjects, one female and five males ranging in age from 23 to 26 years and weighing from 57 to 84 kg, were utilized. Subjects had no previous history of GI, liver, or kidney disease and did not require any medication on a regular basis. All subjects had undergone a physical examination for an earlier study (7) and were healthy.

The importance of maintaining an acidic urine to prevent reabsorption of amphetamine from the urine back into the blood was reported previously (8, 9). However, chemical acidosis was not induced by ammonium chloride administration for the following reasons: (*a*) huge doses of this substance are required to maintain the pH at 5, (*b*) it has been reported that up to 20% of subjects suffer emetic effects after taking ammonium chloride, (*c*) it was desired to maintain subjects in a normal physiological state, and (*d*) ammonium chloride would upset the release patterns *in vivo*, already established *in vitro*.

Breakfast, 1 hr before the test, consisted of cereal and milk. No food or liquids except water were taken during the first 4 hr following drug administration. Formulations were administered as powders with 100 ml of water and ingested at 8 am during a normal working day; thus, by being active and controlling diet and fluid intake (~200 ml of water/2 hr), a slightly acidic urine (Table I) was attained in the first 10 hr. This period was of major interest, because the maximum for urinary drug elimination would be attained during this interval.

Urine samples were collected every 2 hr for 16 hr and at 24, 36, and 48 hr. All subjects were given 15-mg doses of drug except Subject 3, who ingested 20-mg doses. The order of administration of the amphetamine formulations to the six subjects was randomized, and a washout period of at least 1 month was allowed before administering another formulation. Formulations containing the same amount of amphetamine were in three forms: pure drug, a 1:20 drug–montmorillonite complex (2), and a 1:1 combination of pure drug and complex.

Methods—The spectrophotometric assay developed and employed is similar to that of Alles and Wisegarver (10) but contains several modifications to improve sensitivity and reproducibility. Reproducibility from standard solutions of urine containing amphetamine sulfate was within ±2.5%. The colorimetric reaction used to assay amphetamine in the urine measures both amphetamine and the hydroxylated metabolite. However, in humans, less than 3% of the amphetamine is excreted as the hydroxylated metabolite (11), so interference was assumed to be minimal and fairly constant for a particular patient. In addition, the individual day-to-day variations in the blank for all patients fell within ±3.5%.

Steam Distillation Procedure—Ten milliliters of 5 *N* sodium hydroxide and 50 ml of double-distilled water were added to 50 ml of urine in an all-glass distillation apparatus and steam distilled. The distillate, 50–60 ml, was collected in a flask containing 2 ml of 1 *N* HCl immersed in an ice bath. The distillate was transferred to a separator and made alkaline with 0.5 ml of 5 *N* sodium hydroxide, and 50 ml of ether was added. The flask was shaken for 5 min, and the aqueous layer was separated and extracted with two additional aliquots of ether.

To the final ether extract, 10 ml of 0.5 *N* HCl was added to convert the extracted amphetamine to the acid salt. This flask was shaken for 5 min, and the aqueous acidic layer was transferred to the next flask where the shaking procedure was repeated. The aqueous portion was finally shaken with the first ether extract, which contained most of the drug. This extraction was repeated with another 10 ml of acid followed by 5 ml of distilled water to wash the ether layer. The extracts and washings were combined, and the pH was adjusted to 7.5 with dilute sodium hydroxide solution. The volume was adjusted to 50 ml with distilled water.

Colorimetric Determination—Two milliliters of pH 7.4 phosphate buffer was added to 10 ml of the 50-ml extract. At this point, the pH of the reaction mixture was 7.4 ± 0.1. Then 1 ml of the diazo reagent (10) was added. The solution was mixed and placed in a water bath at 37° for 30 min to allow the reaction to go to completion. The test tube was removed, and 0.5 ml of 5 *N* sodium hydroxide was added. The tube was lightly agitated and allowed to stand for 30 min. Urine collected from the patient during the day, prior to drug administration, was extracted and treated in the same manner and used as the reference sample.

The difference in absorbance between the sample and the blank was determined at 520 nm, and the concentration of amphetamine in the urine was calculated from a standard curve. The standard curve was prepared by adding a known quantity of drug to the urine and plotting absorbance *versus* quantity of drug. The drug solution replaced the distilled water, which was added to the urine prior to the steam distillation procedure. All readings were duplicated, and the average was plotted. An excellent linear correlation between ab-

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[2] Veegum Regular, R. T. Vanderbilt Co., New York, N.Y.

Table II—Peak Urinary Excretion Rates of Amphetamine and Times of Peak Rates from 15-mg Doses of Amphetamine Sulfate

Subject	Peak Urinary Excretion Rate, mg/hr			Time of Peak, hr		
	Pure Drug	1:1 Combination of Pure Drug and 1:20 Complex	1:20 Complex	Pure Drug	1:1 Combination of Pure Drug and 1:20 Complex	1:20 Complex
1	1.19	0.66	0.97	2.0	6.0	7.1
2	0.74	0.57	0.74	1.3	3.8	5.0
3[a]	0.77	0.60	0.71	1.4	3.8	6.3
4	0.65	0.50	0.55	2.1	4.8	7.0
5	0.53	0.48	0.46	1.0	3.0	5.1
6	0.72	0.58	0.58	3.0	3.4	5.2
Mean	0.767	0.565	0.668	1.800	4.133	5.950
SD	0.224	0.066	0.181	0.724	1.093	0.973

[a] The 20-mg dose of amphetamine sulfate was administered.

sorbance and drug concentration was found, and the absorptivity value was 6250.

RESULTS AND DISCUSSION

The effect of montmorillonite in modifying the levels of orally administered cationic drugs was verified *in vivo*. For Subject 1, plots of the time course of drug excretion of pure drug, the 1:20 drug–montmorillonite complex, and the 1:1 combination of pure drug and complex are shown in Fig. 1. The smooth curves were fit by eye and drawn by hand. The peak excretion rates of amphetamine from the pure drug and the 1:20 complex occurred at 2.0 and 7.1 hr, respectivtly. This time difference of 5.1 hr was due primarily to the presence of montmorillonite in the sample, assuming all physiological conditions to be constant in both cases. Since the clay does not hydrate in acidic media, the influence of the montmorillonite on the gastric emptying rate would be minimal.

In vitro studies (2) demonstrated that, under acidic conditions, the uncomplexed moiety rapidly passed into solution, but drug complexed to the clay was bound so strongly that little was released in the medium. This slow release at low pH explains the initial low excretion rates for the 1:20 complex (Fig. 1). However, with the passage of the 1:20 complex down the GI tract, it appeared that drug was released from the complex for absorption. This phenomenon of increased bioavailability in alkaline media could not be illustrated *in vitro* due to problems in methodology; *i.e.*, when the drug–montmorillonite samples were added to alkaline buffer media, hydration of the montmorillonite occurred and the drug was released. If the drug was not removed, then readsorption of the drug onto the clay would occur. However, since the body is a perfect sink, the concentration of drug in the blood is negligible in comparison to the intestinal concentration. Thus, as the formulation passed from the stomach into the small intestine at a higher pH, more drug was released and absorbed before readsorption of the active substance back onto the clay could occur.

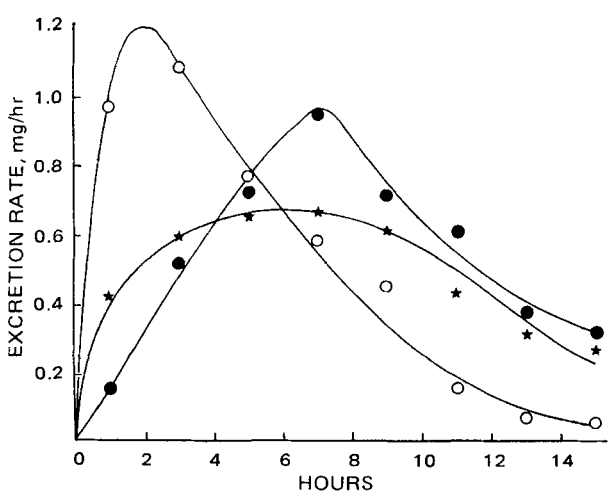

Figure 1—*Urine amphetamine levels following a single oral dose of amphetamine sulfate for Subject 1. Key: ○, 15 mg of drug alone; ★, 7.5 mg of drug–7.5 mg of drug as 1:20 complex; and ●, 15 mg of drug as 1:20 complex.*

Since it was shown *in vitro* that sodium chloride elutes drug from the drug–montmorillonite complex, and because of the high content of Na^+ ions in the intestinal juice, an increase in the concentration of free drug in solution also would be expected. Intestinal juice secreted at the rate of 0.7–3 liters/24 hr is rich in sodium ions (~140 mEq/liter). Because fresh liquid is continually being secreted, the elution rate of drug from the complex due to electrolyte effects would be expected to be greater *in vivo* than that found *in vitro*. The vacant sites on the clay left by the released drug would be occupied by the secreted cations and aid the absorption process. This would make readsorption of the amphetamine back onto the montmorillonite much more difficult and less likely to occur.

At the higher pH of the small intestine, amphetamine sulfate reverts to amphetamine, which is a nonpolar liquid. In such a physical state, this lipid-soluble substance may readily partition into the intestinal wall and be absorbed.

For Subject 1, the recovery curve (Fig. 1) for the combination sample containing 50% amphetamine sulfate and 50% as the 1:20 complex is typical of prolonged-action dosage forms. The peak excretion rate for the pure drug was 1.19 mg/hr, whereas the peak for the combination sample was only 0.66 mg/hr. By eliminating the peak levels seen with the pure drug, the incidence and intensity of possible side effects of the drug are reduced when amphetamine sulfate is administered as a combination of pure and complexed drug.

The peak excretion rates of amphetamine for each subject following the administration of the three test formulations appear in Table II. The time periods for the maxima to occur are also included. In all cases, the times for the peak excretion rates for the combination samples fell between the peak excretion rates for the pure drug and the 1:20 complex. The time period separating the peaks of these latter two samples varied from 2.2 hr for Subject 6 to 5.1 hr for Subject 1.

Montmorillonite affected the pharmacological response of the drug from all formulations. The stimulating effect of amphetamine in the complexed form reached its maximum effect several hours after drug administration. The peak response from the pure drug occurred within the 1st hr after administration. Due to the large doses of amphetamine sulfate administered, it was considered most unlikely that the hyperexcitability experienced by these subjects was psychological. Some subjects who suffered severe headaches from the pure and

Table III—Percent of Dose Recovered in Urine after 48 hr and the Bioavailability[a] of Amphetamine from the Combination Sample and Complex Compared to the Pure Drug for the 48-hr Period

Subject	Pure Drug	Combination	1:20 Complex
1	53	48 (91)	56 (105)
2	54	57 (105)	55 (102)
3	54	51 (94)	58 (107)
4	53	50 (94)	51 (96)
5	37	40 (108)	39 (105)
6	56	55 (98)	54 (96)
Mean	51.167	50.167	52.167
SD	7.026	5.981	6.853

[a] In parentheses and expressed as percentage.

complexed drug noticed a decrease in severity or no headache at all with the combination sample. This response can be explained from the recovery curve for the combination samples, since it was unlikely that development of tolerance to the stimulant occurred.

Table III shows the amount of amphetamine recovered in the urine over 48 hr and also the bioavailability of amphetamine from the complex and combination samples. When using the Student paired t test, no significant differences were seen in the bioavailability of amphetamine from all three formulations. However, excretion rates of amphetamine from the 1:20 complex were significantly different from the pure drug during the initial 4 hr.

In conclusion, the drug–excipient interaction between cationic drugs and montmorillonite clay can be employed successfully to prolong the action of such medicinals. The peak urinary excretion rate of amphetamine from the 1:20 drug–clay complex occurred at a much later time than the pure drug, indicating delayed absorption of amphetamine from the complex. In addition, the initial excretion rate of amphetamine from the complex was significantly lower than that of the pure drug. Finally, for all subjects, the bioavailability of the amphetamine from the 1:20 complex and the amphetamine–1:20 complex combination formulations over 48 hr was not significantly different from that of the pure drug.

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Mass Screening and Confirmation of Codeine and Morphine in Urine by Radioimmunoassay–GLC

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Abstract □ A rapid, sensitive, and specific procedure is described for the mass screening and confirmation of codeine and morphine in urine specimens. The method is sensitive to 0.5-μg/ml levels of both opiates in free and/or conjugate forms. The raw urine is screened directly by radioimmunoassay, which is reactive to both free and glucuronide forms of codeine and morphine. Specimens that are screened positive are confirmed by GLC using a flame-ionization detector. The opiates are analyzed as their acetyl derivatives on two different columns, OV-25 and Poly-A 103. This multiple approach eliminates false positives caused by interfering substances or structurally similar compounds present in the urine.

Keyphrases □ Codeine—radioimmunoassay–GLC analysis, human urine □ Morphine—radioimmunoassay–GLC analysis, human urine □ Radioimmunoassay–GLC—analysis, codeine and morphine in human urine □ GLC—analyis, codeine and morphine in human urine □ Opiates—codeine and morphine, radioimmunoassay–GLC analysis in human urine □ Narcotics—codeine and morphine, radioimmunoassay–GLC analysis in human urine

The widespread use and abuse of codeine, morphine, and heroin, which is largely metabolized to morphine (1), necessitate the development of large-scale procedures for the determination of codeine and morphine in urine samples. A large urine drug testing laboratory must have a sensitive and specific method that can rapidly separate "true negative" from "presumptive positive" specimens. It is equally important to have a fundamentally different confirmatory method that can independently and accurately confirm the presence of an opiate and, at the same time, identify the individual drug. The method should also give quantitative results when needed. Since codeine and morphine are primarily excreted in the urine as conjugates in the form of glucuronides (1, 2), the methods of analysis must be able to detect them in both forms.

BACKGROUND

Most laboratories use TLC to screen urine specimens for various drugs including codeine and morphine (3, 4). The technique is relatively inexpensive but, on a large scale, has many disadvantages such as poor sensitivity, inconsistency in R_f values, variations with humidity, subjectivity in data interpretation, and the requirement of hydrolysis to detect the opiate glucuronides. Although the reagents and equipment for radioimmunoassay are expensive, the consistency of results, ability to detect glucuronide forms, increased sensitivity, greater accuracy, and semiautomation of the method make it well worth the cost involved (5–8).

Since free opiates have poor sensitivity by GLC, a number of GLC procedures have been published to separate and identify their derivatives (9–11). Most methods use only one column, which provides no assurance of avoiding false positives from interfering peaks with retention times similar to the opiate derivatives on that particular column.