AN ABSTRACT OF THE THESIS OF

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Title: Pharmacokinetics and Pharmacodynamics of: 1) Oral Sustained Release Acetaminophen Suspension in Children; 2) Potassium Chloride in Adults.

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James W. Ayres, PhD.

The pharmacokinetics of a novel acetaminophen sustained release suspension (ASRS), were evaluated in children and adults. ASRS consists of sustained release acetaminophen beads (3 mm diameter) dispersed in a grape jelly. Beads were coated with a multi-laminar sandwich, polymeric, release rate controlling sandwich membrane. Saliva samples were collected for 24 hours, post-dosing, and acetaminophen concentration determined using HPLC. In 10 adults administered a dose of 30 mg/kg ASRS, mean pharmacokinetic parameters are Tp= 2.8 hours, Cmax= 12.0 ug/ml and MRT (infinity)= 7.7 hours, indicating, by comparison to immediate release, that sustained release of acetaminophen from the suspension is obtained *in vivo*. A similar study was conducted in 12 healthy children, age 2 through 11 years. In 9 children (75%), mean saliva concentrations reach a peak value of 5 ug/ml at 4 hours and then decline to 2.5 ug/ml at 12 hours. In 3 children, mean peak saliva concentrations of 10 ug/ml occur at 1 hour and then decline at a rate expected following administration of immediate release acetaminophen. It is believed that incomplete sustained release effect was obtained in these 3 children because the

sustained release beads were chewed prior to swallowing. Deconvolution of acetaminophen concentration over time profiles revealed that percent acetaminophen absorbed *in vivo*, by children, is highly correlated to *in vitro* dissolution. Rate of *in vivo* absorption in adults is more rapid than predicted by *in vitro* dissolution. In a separate pharmacodynamic study, body temperature change in 10 febrile (101^0F-104^0F) children, was monitored for 24 hours following administration of a single, 30 mg/kg dose of ASRS. Mean temperature declined for a period of 12 hours with 2^0F and 3.3^0F decrements observed at 2 and 12 hours respectively. ASRS was readily taken by all children and adults enrolled in the studies. The results indicate that a single dose of 30 mg/kg ASRS, given every 12 hours, may be as effective in reducing fever, as three 10 mg/kg doses of immediate release acetaminophen given every 4 hours.

PHARMACOKINETICS AND PHARMACODYNAMICS OF: 1) ORAL SUSTAINED RELEASE ACETAMINOPHEN SUSPENSION IN CHILDREN; 2) POTASSIUM CHLORIDE IN ADULTS.

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This thesis is dedicated to my parents who made this possible with their support.

I would also like to thank Dr. Ayres for all the effort in finding financial support for my education, helping me improve my writing and for giving me the encouragement that I needed to finally get this done.

To all the people in the lab: could'nt have done it without you. Hey, if science isn't fun why bother?

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

PHARMACOKINETICS AND PHARMACODYNAMICS OF: 1) ORAL SUSTAINED RELEASE ACETAMINOPHEN SUSPENSION IN CHILDREN; 2) POTASSIUM CHLORIDE IN ADULTS.

INTRODUCTION

Acetaminophen is a safe and effective drug that is widely used to reduce fever and pain. Following administration of a conventional oral tablet, acetaminophen is absorbed rapidly and completely with peak blood concentrations occurring within 1 hour. After 1 hour, acetaminophen blood concentrations decline rapidly with a half-life of 1.5-3.0 hours. Because analgesia and antipyresis are correlated with blood concentrations, acetaminophen must be dosed every 4-6 hours to maintain fever and pain reduction. The frequency of dose administration may be reduced if absorption of acetaminophen from the gastrointestinal tract takes place over a longer period of time compared to immediate release. One method of extending the time of acetaminophen absorption is to reduce the rate at which acetaminophen leaves the dosage form and becomes available for absorption from the lumen of the gastrointestinal tract.

Polymers have been widely used by the pharmaceutical industry to reduce the rate at which drugs leave a dosage form. The strategy employed herein is to apply a coating of polymers to acetaminophen beads. Release rate of acetaminophen from coated beads is reduced by the polymer coating since the diffusion paths through the membrane are tortuous and of limited number compared to the paths that are available to an uncoated bead surface exposed to solution. The release rate from beads can be controlled by polymer selection and thickness of the polymer coating applied. Temperature at which polymers are applied and the addition of excipients may also influence the release rate by

changing the number of diffusion paths. Other factors may also be considered in the formulation of a sustained release dosage form.

According to accepted dosing protocols, 700 mg of acetaminophen is required every four hours to maintain effective control of fever or pain in a 70 kg man. If frequency of administration is reduced to a single dose every 12 hours then the dose required would be 2100 mg. Formulation of a solid oral dosage form containing 2100 mg of active drug would be impossible for many people to swallow, thus sustained release acetaminophen must be formulated as small multiple solid doses or in a form that is easy to swallow in large amounts. A suspension was selected for formulation of acetaminophen sustained release since suspensions are easy to swallow. Suspensions are also ideal for administration to children since children frequently are unable to swallow large tablets.

Chapter 1 of the thesis describes the manufacture and *in vitro* characterization, by *in vitro* dissolution, of 6 experimental sustained release acetaminophen suspension formulations. Based on the *in vitro* dissolution results, one formulation was identified as having release rate characteristics that may provide sustained therapeutic blood concentrations of acetaminophen for a period of 12 hours. 10 healthy adults were administered 30 mg/kg of the formulation. The results show that mean concentrations of acetaminophen are greater then 2 ug/ml for a 12 hour period post dosing, suggesting that the formulation, when dosed at 30 mg/kg, may be an effective anti-pyretic in adults.

In chapter 2, 30 mg/kg of the formulation was administered to 10 febrile (101^0C-104^0C) children. The results presented in chapter 2 show that the mean reduction in fever was significantly greater than the fever reduction that is expected if no acetaminophen was given. In a separate experiment the pharmacokinetics of the dosage form, using the same methods as in chapter 1,

was evaluated in 10 healthy children administered 30 mg/kg of the formulation. Pharmacokinetic evaluation in children shows that mean concentrations of acetaminophen in saliva, in children, are greater than 2 ug/ml during the 12 hour period post dosing, confirming that the dosage form is expected to be an effective antipyretic.

In chapter 3, a correlation relationship between *in vitro* dissolution and *in vivo* absorption of KCl sustained release is developed and used to simulate *in vivo* responses that are expected following the administration of KCL formulations that differ with respect to *in vitro* dissolution profiles. The analysis performed in chapter 3 is useful as a "model" approach to correlating *in vitro* dissolution results with *in vivo* drug absorption.

CHAPTER I

DEVELOPMENT OF SUSTAINED RELEASE ACETAMINOPHEN SUSPENSION

ABSTRACT

This research describes the formulation and evaluation of a sustained release suspension that is stable with respect to release rate of drug *in vitro,* during significant periods of storage in a hydrating suspension medium. The dosage form consists of acetaminophen loaded beads (3mm) coated with three layers of coating polymers ("sandwich coating"). Coated beads are suspended in a low pH, high viscosity, commercial grape jelly. The *in vitro* release rate of acetaminophen from the sustained release suspension stabilized after an equilibration period of 15 days of refrigerated storage of the coated beads in the suspension medium. A candidate formulation that demonstrated *in vitro* dissolution rates consistent with the objective of providing sustained therapeutic concentrations *in vivo* was evaluated in 10 healthy adults. Pharmacokinetic evaluation of acetaminophen concentrations in saliva over a period of 24 hours post dosing demonstrate high relative bioavailability from the dosage form and peak concentration occuring about 2 hours later than for a comparable dose of immediate release formulation of acetaminophen. Deconvolution of the observed *in vivo* saliva concentration data reveals that the sustained release suspension releases acetaminophen much more rapidly then predicted by *in vitro* dissolution. The data demonstrate that sustained therapeutic concentrations of acetaminophen were maintained at effective concentrations for periods of at least 12 hours following administration of a single dose of acetaminophen sustained release suspension.

INTRODUCTION

Many drugs must be dosed frequently, in relatively large amounts to maintain a desired therapeutic effect. Compliance with regimens requiring frequent drug administration is often poor, especially when the patient is responsible for timely drug administration. Compliance is also undermined if the dosage form is difficult to swallow or if the experience with the dosage form is stressful and unpleasant (a bitter drug taste). Compliance is improved, and therapeutic objectives are more readily met, if the dosage formulation is easy and convenient to administer. Unfortunately, many drugs are commercially available only as an immediate release tablet or capsule which requires frequent drug administration, thus the best dosage form for a given patient may not always be available. The diversity of dosage forms (or lack of diversity) available for a drug reflects the demands of the patient population for which the drug is intended and problems inherent in the formulation of alternative dosage forms.

Many drugs are potent, have long half-lives, and are used primarily in patient populations that do not have significant problems swallowing small tablets or capsules. However, sustained release formulations of relatively low potency drugs may be large and difficult to swallow. Thus, benefits of sustained release may be outweighed by problems associated with large solid formulations. The size limitation inherent in solid sustained release formulations may be overcome if sustained release can be provided in a non-solid formulation that is readily swallowed in larger volume. Sustained release suspensions may provide the compliance benefits of sustained release while providing the ease of swallowing associated with oral suspensions.

Despite the significant therapeutic advantage of sustained release suspensions over conventional immediate release formulations, a comprehensive

sustained release suspension formulation strategy, applicable to many drugs, has not been widely available. This research describes the formulation and evaluation of a sustained release suspension that is stable with respect to release rate of drug *in vitro*, during significant periods of storage in a hydrating suspension medium. The dosage form evaluated herein consists of acetaminophen coated beads surrounded by three layers of polymers ("sandwich style") commonly used in the formulation of conventional oral sustained release products. The beads are suspended in a high viscosity, commercial grape jelly. The sustained release suspension described herein has the following characteristics: 1) Release rate stability during storage, 2) Use of FDA approved coating polymers, 3) Use of familiar polymer application technology, i.e. spray coating, 4) Taste masking of unpalatable drugs, 5) Release rate may be controlled by varying the amount of rate controlling polymers applied.

Acetaminophen was chosen as a challenging model drug since it has a short half life (1.5-3.0 hours), and has a relatively low potency (10-15 mg/kg for symptomatic relief of fever and pain). In addition the concentration of acetaminophen in the central compartment may be determined non-invasively from saliva. Formulation of acetaminophen sustained release suspension may provide substantial therapeutic benefits to children and the elderly, populations which frequently have problems swallowing large solid oral dosage forms.

MATERIAL AND METHODS

Production of acetaminophen beads

150 grams of non-parriel sugar beads (28-30 Mesh) were coated with 450 grams of USP grade acetaminophen (Mallincrodt Inc., St. Louis, MO.) dissolved in 700 ml 95% ethanol USP. Polyvinylpyrrolidinone (2% w/w), MW 40,000 and hydroxypropylcellulose (2% w/w) were added to the alcoholic solution as binders. The application of acetaminophen ethanolic solution required 2 hours at ambient temperature (~25^0 C). After application of ethanolic acetaminophen solution, drug coated beads were allowed to dry for 20 minutes at 50^0 C in order to remove residual ethanol. Beads were removed from the fluid bed and weighed and assayed to evaluate the total amount of drug loaded per total weight of beads. Acetaminophen content was expected to be 70% of total bead weight, and beads were approximately 3 mm in diameter.

Production of the sustained release suspension

Polymer film coating technology was used to produce controlled release (sustained-action) acetaminophen . The coating apparatus consisted of a spray-coating chamber, with a 7 inch Wurster column (STREA-1, Aeromatic Inc., Columbia, MD) mounted on a fluid-bed dryer (Lab-Line/P.R.L. Hi-Speed Fluid-Bed Dryer, Lab-Line Instruments, Inc., Melrose Park, IL). Ethyl cellulose (Aquacoat®, Type ECD-30, Lot J7211, FMC Corp., Philadelphia, PA., USA) and Eudragit® L30D, Lot #12-906-1280, Rhom Pharma GMBH, FRG were used as the coating materials. Two plasticizers were used: triethyl citrate (Triethyl citrate 99%, Aldrich Chemical Co., Milwaukee, WI.) and dibuty sebecate (Dibutyl Pthalate 99%, Mallinckrodt, Inc., St. Louis, Mo.). Three separate applications of polymers were made during the manufacturing process. Loading of polymers was calculated as weight percent of polymer solids, including plasticizers, per

unit weight of acetaminophen beads. The order and amount of application was varied, but in one example was 2% Aquacoat® (Ethyl Cellulose suspension), 4% Eudragit® L30D (Methacrylic acid copolymer, Type A), 2% Aquacoat. Polymer solutions were applied rapidly and then coated beads were allowed to dry in the fluid bed dryer for 30 minutes before the next application of polymer solution. All processes were undertaken at 70° C. The commercial polymer suspensions (30% solids weight) were diluted with an equal volume of de-ionized water before use. Triethyl citrate (TEC), 15% of total solids, and dibutyl phtalate (DBP), 15% of total solids, were added as plasticizers. Coated beads were then dispersed in a commercial fruit jelly (Danish Orchards Grape Spread®, Lot #OD3 9 A1, Vendelbo Konserves A/S, Denmark, 53 grams of beads plus 47 grams of jelly) and refrigerated until use. During dosing the suspension was dispensed precisely by volume with a syringe. The suspension contained 37% acetaminophen by weight.

***In vitro* dissolution**

In vitro release rate of acetaminophen was determined using the paddle method (USP XXI) at 100 rpm and 37°C. Approximately 100 mg of acetaminophen sustained release suspension was weighed and placed in 900 ml of enzyme free, simulated gastric fluid, pH 1.4 for the first 2 hours of dissolution. At 2 hours gastric fluid was decanted, and poured through a filter paper in order to collect non-dissolved material including sustained release beads. For hours 2 through 24, beads were exposed to enzyme free, simulated intestinal fluid, pH 7.4, equilibrated to 37°C. Double distilled, deionized water was used to prepare all dissolution media. 5 ml samples of dissolution media were collected, with replacement, over a period of 24 hours and analyzed spectrophotometrically for acetaminophen at 254 nm using a Beckman UV 34 spectrophotometer. No

detectable interference from jelly or excipients used in the manufacture of the sustained release suspension was observed.

Analysis of acetaminophen in saliva by HPLC

Concentrations of acetaminophen in saliva have been correlated in a 1 to 1 fashion to plasma concentrations (1). The HPLC assay method for acetaminophen is similar that has been reported previously (2). Since the principle metabolites of acetaminophen, acetaminophen gluronide and acetaminophen sulphate, elute much earlier then acetaminophen, the quantitation method is specific for acetaminophen. Saliva samples were collected in plastic vials and frozen at -20^{O} C prior to assay. An aliquot of saliva was centrifuged at 14,000 rpm for 2 minutes to pelletize particulates. 50 ul of supernatant was transferred to a micro-centrifuge tube containing 100 ul water with internal standard (2-acetamidophenol, Sigma Chemical, St. Louis, MO.) and vortexed for 30 seconds. 50 ul of the mixture was injected onto an HPLC system with a 300 mm length C-18 column (Microsorb-MV, Rainen, Woburn, MA.) with >20,000 theoretical plates. A autosampler (WISP Model 712, Waters Associates Inc., Milford, MA.) was used for all injections. A mobile phase of 20% methanol 80% water was used. Elution volumes of acetaminophen and the internal standard 2-acetamidophenol were 7.5 ml at 15.0 ml respectively. Analytes were detected with a fixed wavelength UV detector at 254 nm (Model 440, Waters Associates Inc., Milford, MA.). A integrator (Model C-R3a, Schimadzu, Japan) was used to calculate peak areas. Standard curves were prepared by spiking 1 ml of saliva with 50 ul of acetaminophen solutions. The range of standard concentrations was 0.25 ug-30 ug/ml. Coefficients of determination were greater then >0.995 on all days. All saliva samples were frozen at -20C prior to assay.

Patient enrollment

Protocols and informed consents for the pharmacokinetic study were approved by the Investigational Review Board, Oregon Health Sciences University (OHSU), Portland, OR. An investigational new drug (IND) application was filed with FDA and approved. Adults were recruited from health care providers and students at OHSU in February, 1993.

Pharmacokinetic study

10 healthy adults age 26-38 were recruited. Signed, informed written consent was obtained from each subject before the study began. Subjects were provided with a single oral dose of the sustained release suspension (30 mg/kg acetaminophen) to take at home, along with plastic vials for the collection of saliva samples. Subjects were instructed not to chew the sustained release beads. No restrictions were placed on food prior to or during the study with the exception that subjects were instructed not to eat or drink for 5 minutes prior to collection of a saliva sample. Saliva samples were collected prior to dosing and at various times during a 24 hour period.

Statistical methods

Procedure NLIN of SAS (Statistical Software, Cary, NC) was used to estimate Weibull parameters (3) associated with *in vitro* dissolution profiles. Cumulative absorption of acetaminophen from the sustained release suspension over time was determined with an analytical deconvolution algorithm PCDCON.

RESULTS AND DISCUSSION

Figure 1 shows *in vitro* release rate profiles of acetaminophen sustained release suspension formulations that differ with respect to amount of coating polymers applied. Figure 1 shows that very little, if any acetaminophen has been released or is "free" in the suspension medium since the acetaminophen released, at 15 minutes is less then 3% for all sustained release suspension formulations. This result is surprising since a large acetaminophen concentration difference is present between the bead interior and the suspension medium. The absence of acetaminophen in the surrounding dissolution medium results in sustained release acetaminophen suspensions which lack the bitter taste that is associated with conventional oral solutions. Mansell (4,5)and Sahjwalla (6) have previously observed that sandwich style sustained release acetaminophen beads, stored in low pH, low osmotic pressure aqueous medium will release acetaminophen into the surrounding medium. If the amount of acetaminophen present is sufficiently large, the solubility limit for acetaminophen in the medium will be rapidly attained. Since the interior of beads stored in jelly is 'wet' in appearance, lack of water for dissolution of acetaminophen in the bead interior cannot explain these results. Absence of acetaminophen in the suspension medium reported herein may arise from the high osmotic pressure of the suspending medium used in the formulation. Earlier results with the jelly vehicle and similar sadwich coated acetaminophen beads were also effective at masking the bitter taste (4).

Figure 1 also shows that release rate decreases as the total amount of controlled release polymers increases. Mansell (4) has previously reported similar release patterns for triple layer acetaminophen suspension, however the total amounts of polymers required by to produce *in vitro* release patterns comparable to those reported herein were approximately twice as great. Since

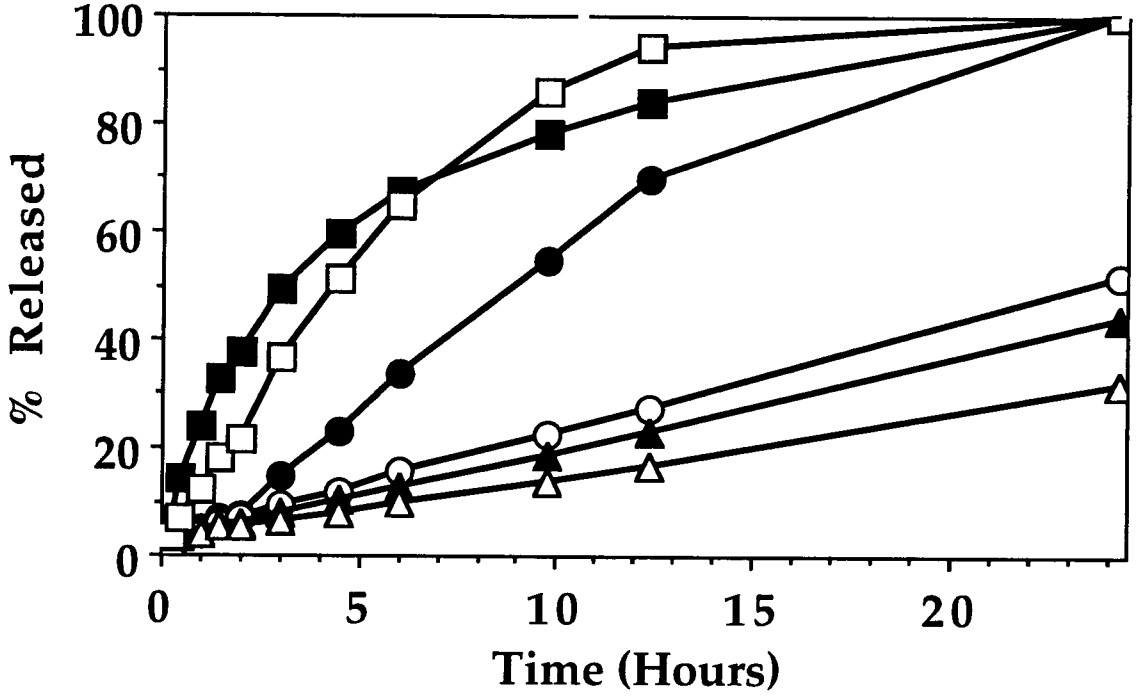

Figure I.1 *In vitro* percent release acetaminophen from sustained release acetaminophen suspension formulations that vary in amounts of coating polymers applied. The coatings are ethyl cellulose/methyl methacrylate/ ethyl cellulose. Solid square 2/2/1, open square 2/2/4, solid circle 3/5/2, open circle 3/5/4, solid triangle 3/5/6, open triangle 3/5/8. All formulations stored 3 days under refrigerated conditions (5 ^{0}C) prior to dissolution.

all formulation parameters used herein, with the exception of temperature at which polymer solutions are applied (40^0C Mansell, 70^0C here), are identical to those used previously, differences in *in vitro* release rate pattern for a given amount of polymer applied must arise from an application temperature dependent effect on the permeability of the polymeric rate controlling membrane. Hossain (7) reports similar application temperature dependent, *in vitro* release rate behavior for acetaminophen beads coated with mono-layers of ethyl cellulose (Aquacoat®). Using scanning electron microscopy, Hossain observed that as application temperatures increase, film coalescence becomes more complete and as a result, fewer pores are visible on the surface of the ethyl cellulose, rate controlling membrane. These results suggest that the *in vitro* dissolution behavior of acetaminophen beads, coated with either single or multiple layers, are similarly effected by temperature of polymer suspension application and total amount of polymers applied. The effect of total amount of coating polymers applied on *in vitro* release rate from sandwich style acetaminophen sustained release suspensions (application at 70 C^0), is summarized in figure 2.

Figure 2 shows the log linear relationship between time to 63.2% release and coating level. Time to 63.2% release was estimated by non-linear estimation of parameter τ of the Weibull function (3) associated with each *in vitro* dissolution release pattern.

$$\text{Weibull function } F_t = F_\infty \left(1 - e^{-\left(\frac{t}{\tau}\right)^\beta} \right)$$

Parameters β (curvature parameter) and τ were estimated simultaneously, while F infinity (percent released at time infinity) was constrained to 100 percent of

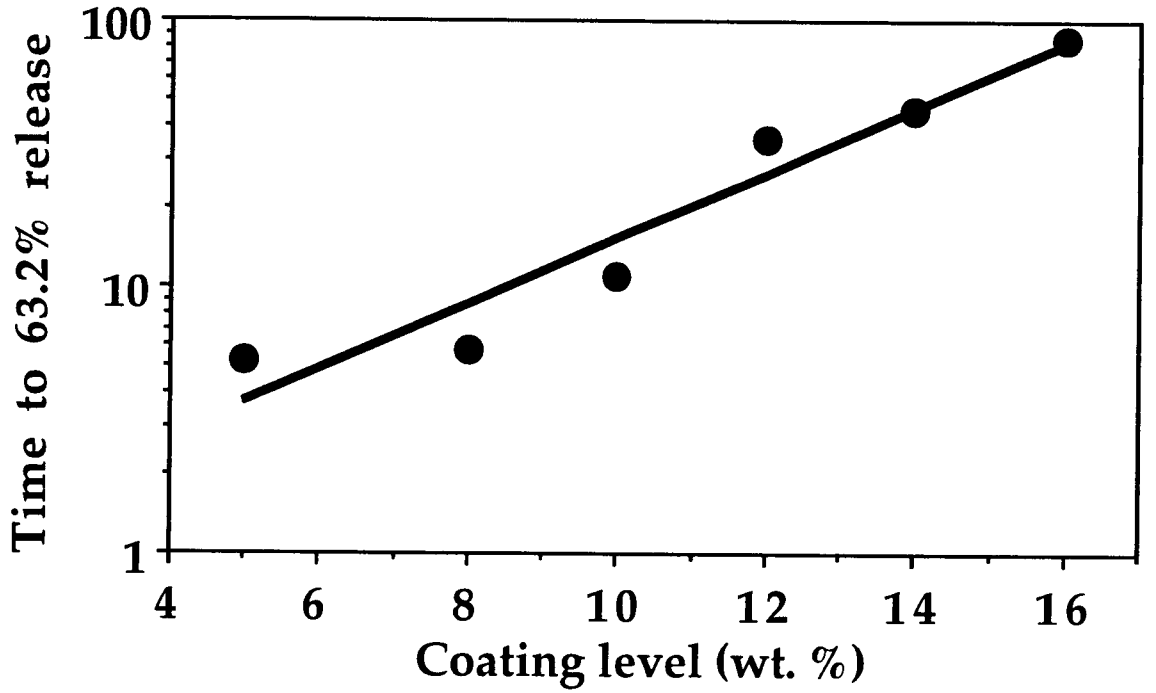

Figure I.2 Time to 63.2% release of acetaminophen vs coating level (weight percent polymeric coating solids). Open circles time to 63.2% released based on non-linear estimation of Weibull parameter b for various acetaminophen sustained release suspension formulations.

acetaminophen present in the suspension. β and τ were estimated with the SAS statistical package. The Weibull model is non-mechanistic and has been used previously to evaluate the complex *in vitro* release pattern from sustained release formulations (3). Differences between observed percent released and mathematically estimated percent released were within 2 % for all formulations at all times with the exception of the 3/5/2 formulation whose bi-phasic release pattern was not adequately described by the Weibull function. The degree of correlation between total amount of polymer applied and time to 63.2% release (correlation coefficient =0.93) indicates that the relationship may be used to predict *in vitro* dissolution rate characteristics of many formulations of this type within the range described by trial formulations. However, the bi-phasic pattern of the 3/5/2 formulation which may be due to rupture of the outer coat (discussed below) indicates there are limiting cases where the relationship in figure 2 does not apply. More sophisticated predictive models using the curvature parameter β may be developed. Predictive models based on a relatively small descriptive set of trial formulations and application of convolution methods may enable the rapid identification of formulations with precisely defined pharmacokinetic characteristics.

The *in vitro* dissolution behavior of some sandwich style, acetaminophen sustained release suspension formulations arises from the specific interaction of dissimilar polymers (ethyl cellulose and methyl methacrylate) present in the release rate controlling membrane. Thus, unusual *in vitro* dissolution patterns may be produced with the sandwich style coating strategy.

The 3/5/2 (solid circles in figure 1) shows a distinct bi-phasic release pattern. During exposure to simulated gastric fluid, pH 1.4, for the first 2 hours of *in vitro* dissolution, the 3/5/2 releases acetaminophen at a slightly greater rate then 3/5/4, 3/5/6 and 3/5/8 formulations. This slightly greater release rate,

during the first 2 hours, compared to 3/5/4, 3/5/6 and 3/5/8 formulations is consistent with the expectation that larger amounts of applied polymers will produce consistently slower release rates *in vitro*. However, when the 3/5/2 formulation is exposed to simulated intestinal fluid, pH 7.4, the rate of acetaminophen release increases dramatically and greatly exceeds the rate that is predicted by extrapolation of change in release rates of 3/5/8, 3/5/6 and 3/5/4 formulations. In fact, the rate of acetaminophen release from the 3/5/2 formulation from 2 through 12 hours is closer too, though still less then, the release rate observed for 2/2/1 and 2/2/4 formulations. The rapid rate of acetaminophen release from the 3/5/2 formulation, following exposure to simulated intestinal fluid, suggests that swelling of the middle layer of methyl methacrylate in response to pH 7.4, drastically changes the permeability of the release rate controlling membrane. Mansell (5) observed that the addition of an exterior layer of methyl methacrylate had no effect on the release rate pattern from mono-layer ethyl cellulose formulations in simulated intestinal fluid, pH 7.4. This result indicates that an exterior coating of methyl methacrylate when applied to ethyl cellulose, dissolves very rapidly at pH 7.4 and makes no contribution to the reduction in rate of drug release *in vitro*. Mansell also showed that if the amount of methly methacrylate is 3 fold greater then the amount of ethyl cellulose present in the exterior layer, a rapid and complete loss of both middle and exterior layers will occur following exposure to intestinal fluid, with the result that both coats will have a negligible effect on reduction of *in vitro* release rate. Since the release rate of acetaminophen from the 3/5/2 formulation is significantly less then either the 2/2/1 or 2/2/4 formulation, it is likely that complete loss of both the middle and exterior layer did not occur. The observation that a complex release rate pattern may be produced by a homogeneous sandwich coat formulation (i.e. not a mixture of several different

controlled release parts, example enteric coated plus sustained release) may be useful in optimizing some drug therapies.

Changes in *in vitro* acetaminophen release patterns for the 2/2/4 formulation were assessed over a 65 day period of refrigerated storage (5^0 C) to determine if this formulation would be suitable for evaluation *in vivo*. Since enrollment of patients in clinical efficacy and pharmacokinetic studies may occur over a period of 1 to 2 months, a candidate formulation for *in vivo* evaluation must demonstrate a high degree of release rate stability following storage. Figure 3 shows that release rate from the 2/2/4 sustained release suspension decreases slightly during the period of 3 to 15 days storage but that release rate pattern changes little during 15 through 65 days storage. Sahajwalla (6) found that significant change in *in vitro* release rate pattern of multi-laminar, sandwich style acetaminophen sustained release beads, during storage in aqueous suspension medium, did not occur from 2 weeks through 1 year of storage. Similar rate were obtained by Mansell (5) although for shorter storage time. Thus, it is likely that the sustained release acetaminophen suspension that is described herein will be stable for periods much longer than 65 days. Chemical stability of acetaminophen in the sustained release suspension was not evaluated here but has been shown to be high in aqueous solution (8). No detectable levels of p-aminophenol, the major hydrolysis product of acetaminophen were detected by HPLC.

The 2/2/4 formulation was selected for *in vivo* evaluation in humans since the formulation produces *in vitro* release rate patterns that are consistent with the objective of providing sustained therapeutic concentrations of acetaminophen in blood for a period of 12 hours following a single dose (9), is release rate stable and has a pleasant, non-bitter taste that is readily acceptable.

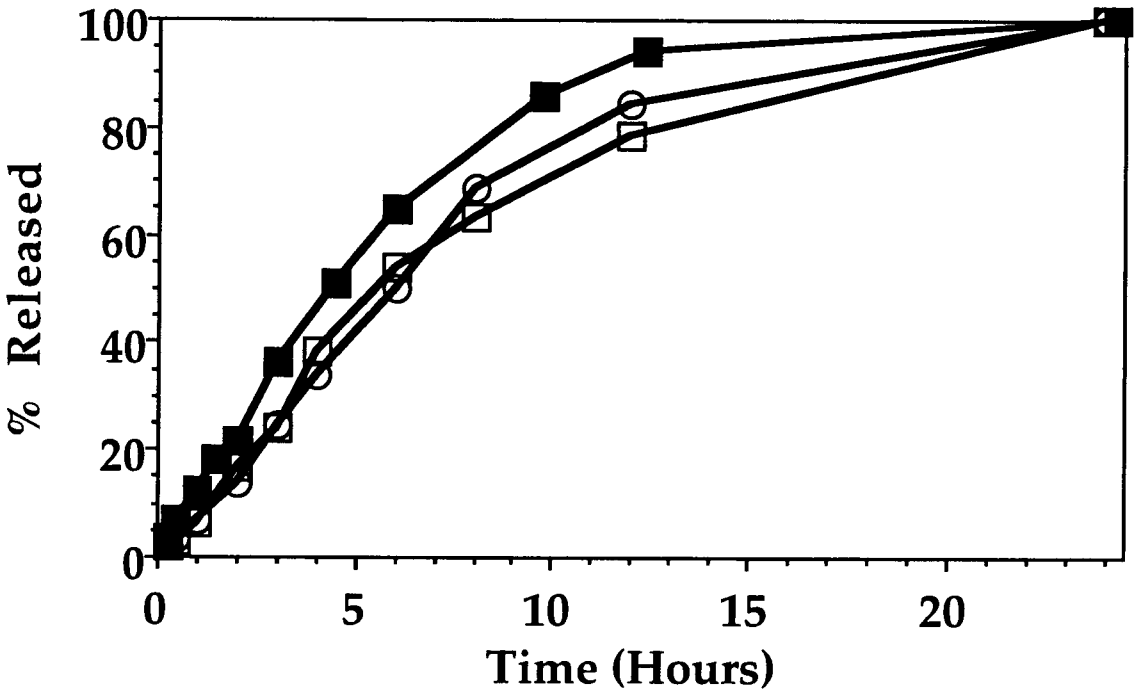

Figure I.3 Effect of refrigerated storage (5^0C) on *in vitro* release rate from 2/2/4 (ethyl cellulose/methyl methacrylate/ methyl cellulose) acetaminophen sustained release suspension. Solid squares 3 days storage, open circles 15 days storage, open squares 65 days storage.

The 2/2/4 formulation was evaluated in 10 healthy adults (ages 26-37, 1 Female, 9 Males) dosed at 30 mg/kg. Figure 4 shows the individual and mean saliva concentrations of acetaminophen in these adults over a 24 hour period. The lowest concentration at 12 hours was 2 ug/ml and the mean is 4 ug/ml. Hossain (10) has suggested that 2 ug/ml will be effective to reduce fever and that 5 ug/ml will be analgesic; thus the sustained release acetaminophen suspension may be an effective antipyretic for periods of 12 hours post administration. Individual profiles are consistent with sustained release of acetaminophen from the dosage form. Table 1 shows individual and mean non-compartmental pharmacokinetic parameters. Mean MRT (24 hours) of 6.4 hours for adults dosed with sustained release suspension of acetaminophen is longer then the 4.1 hours reported for healthy adults following administration of a 1000 mg dose of immediate release acetaminophen tablets (1). This comparison indicates that sustained release of drug is provided by the suspension. Mean time to peak concentration also occurs later for sustained release suspension (2.8 hours) than for immediate release (1000 mg dose, Cmax = 0.8 hours).

A rigorous estimate of relative bioavailability of the sustained release suspension could not be made since neither an intravenous nor an immediate release formulation were administered. Across study pharmacokinetic comparisons must be viewed with caution, but may be useful in certain cases where a drug has previously been well characterized. Thus, a comparison to literature values for AUC reported for adults following administration of immediate release formulations of acetaminophen may be useful, since inter-study variation in reported pharmacokinetic parameters for adults is relatively small (1). Such comparisons assume a 1:1 relationship between saliva and plasma drug concentrations, which has been shown to exist, on average, for acetaminophen. Following the administration of 16 mg/kg of acetaminophen as

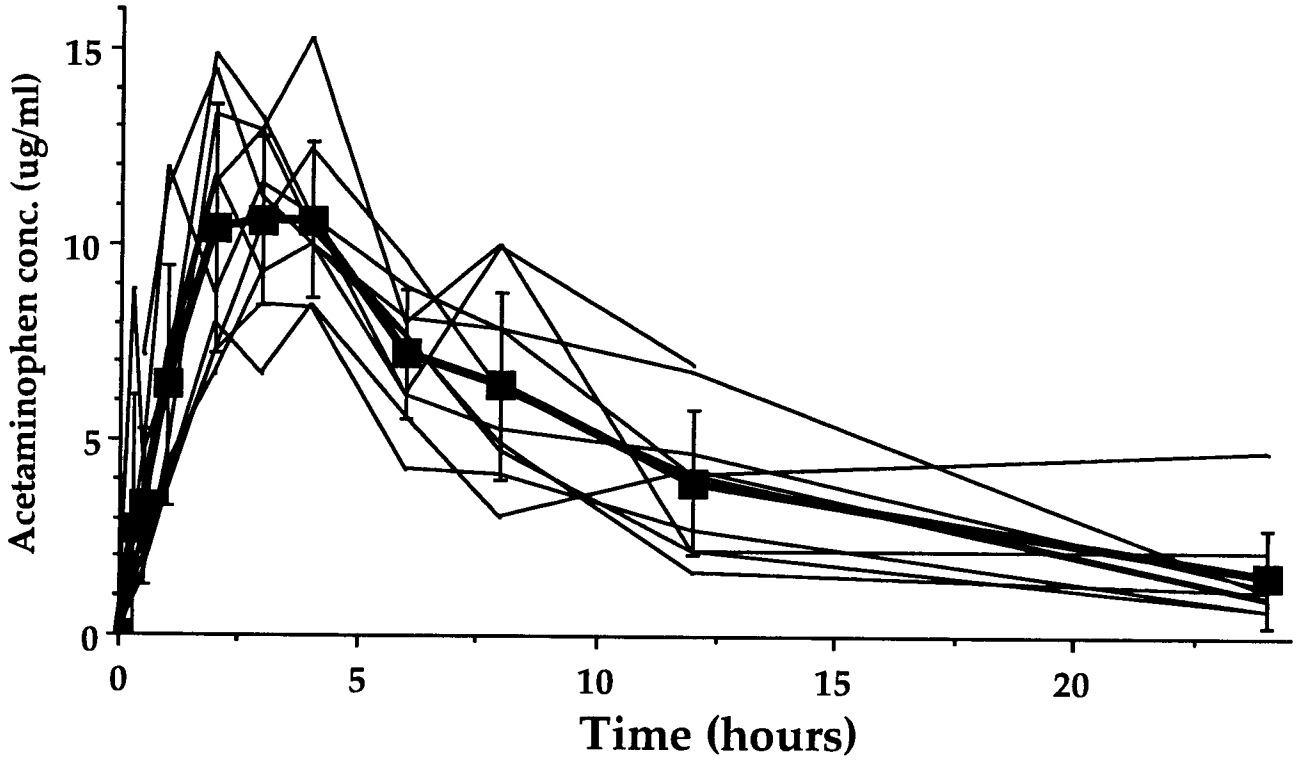

Figure I.4 Individual subjects acetaminophen in saliva profiles following administration of 2/2/4 acetaminophen sustained release suspension. Mean concentration of acetaminophen solid squares. Error bars indicate SEM (of mean) at each time point.

Table I.1 Non-compartmental pharmacokinetic parameters of adults.

Subject	1	2	3	4	5	6	7	8	9	10	Average (SEM)
Tp(hours)	2.0	3.0	4.0	4.0	4.0	4.0	1.0	2.0	2.0	2.0	2.8 (1.1)
Cmax (ug/ml)	11.7	8.4	12.5	8.5	15.2	10.0	11.5	13.3	14.8	14.4	12.0 (2.5)
AUC 24 + (ug*hr/ml)	144.3	78.3	116.4	91.5	129.8	85.1	120.6	96.5	154.8		113.0 (26.9)
AUC 12 (ug*hr/ml)	91.1	58.2	86.2	60.3	103.8	68.7	84.9	79.6	107.6	108.6	84.9 (18.5)
AUC infinity (ug*hr/ml)	162.1	80.8	119.9	95.2	137.8	89.3	125.3	99.0	159.1		118.7 (29.9)
MRT 24 (hours)	10.3	4.1	6.4	5.4	7.0	4.4	6.6	4.4	8.6		6.4 (2.1)
MRT 12 (hours)	5.5	5.3	5.7	5.5	5.3	5.1	5.2	4.9	5.5	5.6	5.4 (0.3)
MRT infinity * (hours)	9.2	7.4	7.7	8.2	7.4	7.1	7.7	6.5	7.8		7.7 (0.8)

24 hour saliva concentration for subject #10 unavailable, AUC 24 hours, AUC infinity, MRT 24 hours and MRT infinity not calculated.

+ AUC infinity calculated as AUC infinity= AUC 24 hours + C(24 hours)/Kel, where Kel=0.267, where AUC 24 hours is calculated by trapazoidal rule.

$$^{*}\ \text{MRT infinity} = \left[\int_{0\ hours}^{24\ hours} t * C(t) \ + \ \left(\text{C(24 hours)} \Big/ \text{Kel}(1+\text{Kel}) \right) \right] \Big/ \text{AUC infinity}$$

where the integral is approximated using the trapazoidal rule and Kel=0.267.

a immediate release formulation, Sahajawalla and Ayres (6) report a steady state, multiple dosing AUC (to infinity) of 58.8 ug*hr/ml. If linearity is assumed (4,6) a dose of 30 mg/kg will produce an AUC of 110.3 ug*hr/ml. Based on these assumptions, a relative bioavailability of 106% is estimated for subjects dosed with 30 mg/kg of the sustained release suspension. This value for mean relative bioavailability for subjects dosed at 30 mg/kg is a little higher then the 84 and 91% relative bioavailability reported for two acetaminophen sustained release formulations evaluated in healthy adult males (12) and the value of 90% reported by Mansell (4). These results all suggest that hepatic and gastrointestinal biotransformation of acetaminophen prior to systemic delivery are not greatly increased by decreasing the rate at which acetaminophen is released by a dosage form, which is consistent with Sahajawalla (6) but different than Borin (10).

The rate of absorption of systemically available acetaminophen following administration of the sustained release suspension was evaluated by deconvolution (13). An elimination rate constant of 0.267 h^{-1} (6) was used as a weighing (impulse response) function for all subjects since individual elimination rate constants could not be estimated from the data due to administration of a sustained release product. Thus, the deconvolution data are not to be viewed as "exact", but rather represent trends, which should be quite useful, on the average. Figure 5 shows the estimated cumulative percent of systemically available acetaminophen absorbed over time for the 10 subjects enrolled in the study. On the average, 60% of bioavailable acetaminophen is absorbed in a nearly linear fashion for the first 4 hours, with the remaining 40% being absorbed slowly over the period of 4 through 24 hours.

A comparison of estimated mean percent absorbed *in vivo* and percent released *in vitro* is shown in figure 6. Figure 6 shows that absorption behavior of the dosage form in adults differs from the behavior predicted by *in vitro*

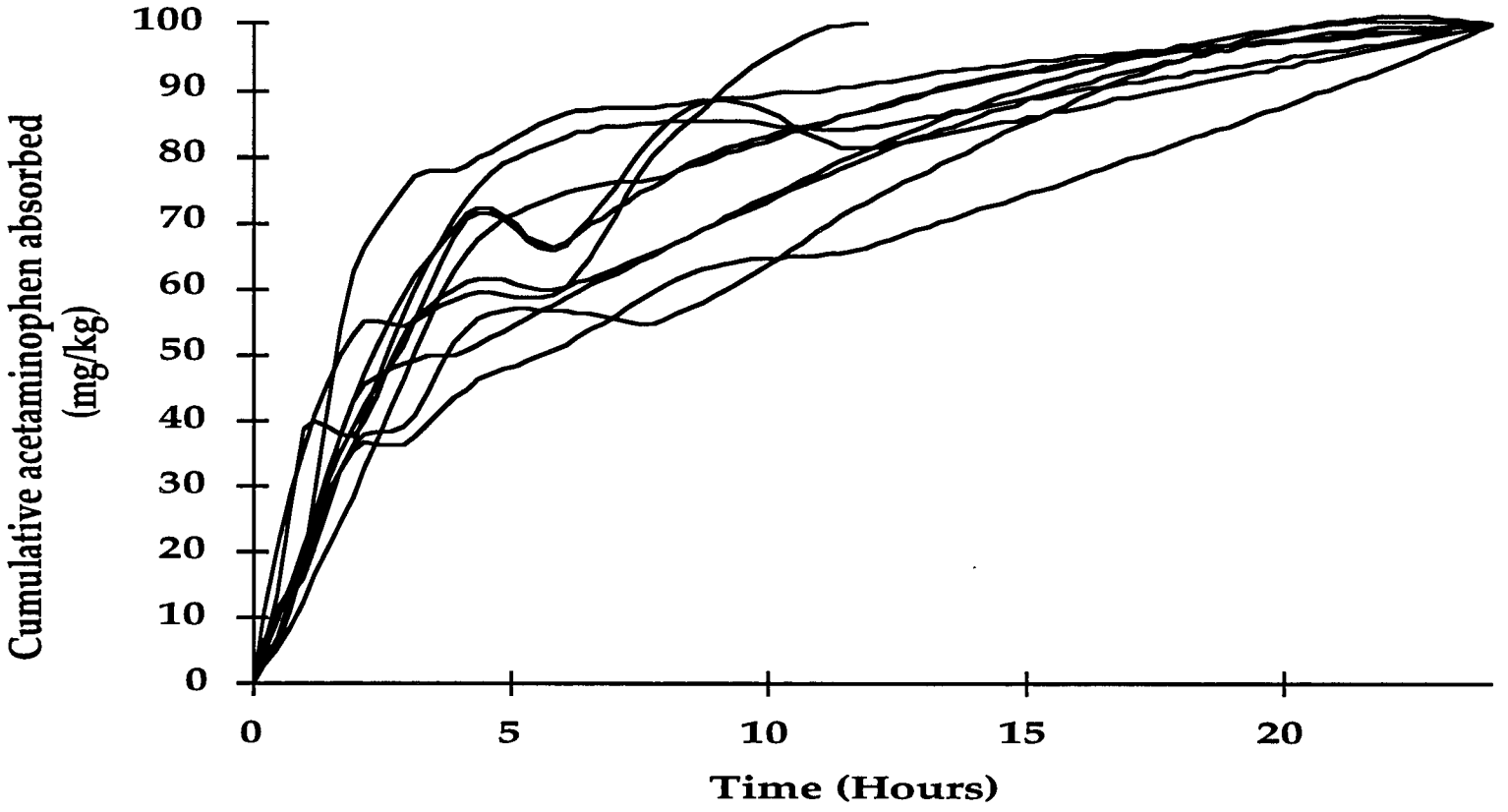

Figure I.5 *In vivo* percent bioavailable acetaminophen absorbed determined by deconvolution. Each lines is one subjects individual absorption rate profile.

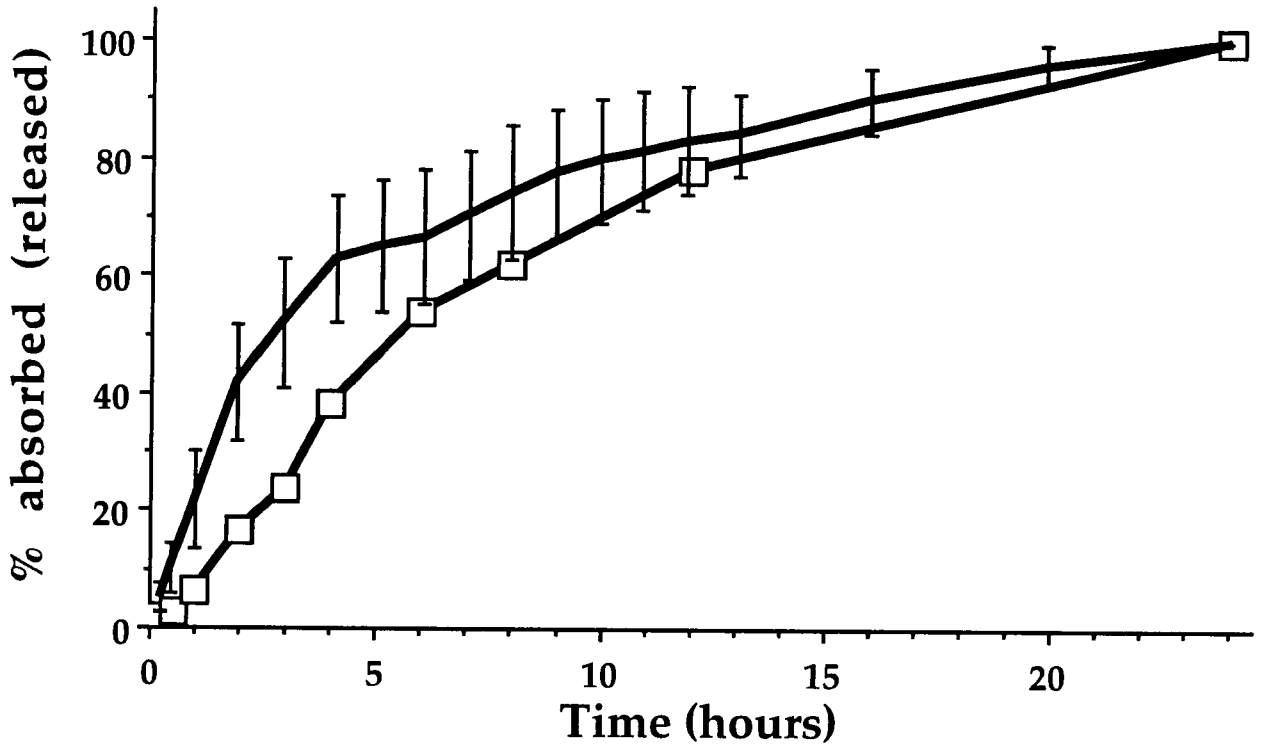

Figure I.6 In vivo percent bioavailable acetaminophen absorbed vs percent acetaminophen released from 2/2/4 sustained release acetaminophen suspension. In vitro dissolution is for a formulation stored 65 days under refrigerated conditions prior to dissolution: open squares. In vivo percent bioavailable acetaminophen absorbed, mean of 10 subjects: solid line with error bars (SEM).

dissolution. A time scaling approach (14) is sometimes useful, since *in vivo* absorption profiles that appear to be different than *in vitro* dissolution profiles may become superimposable after this mathematical treatment. Time scaling did not ameliorate the lack of correspondence between *in vivo* absorption and *in vitro* dissolution. The lack of correlation is attributable to the very rapid absorption of acetaminophen that occurs during the period of 0 through 4 hours. It is possible that *in vivo* absorption rate observed here was influenced by surfactants or enzymes in the gastrointestinal tract which were not employed in the *in vitro* dissolution conditions utilized.

Pharmacokinetics and ability to reduce fever of the sustained release suspension described herein has recently been evaluated in healthy and febrile children (Manuscript in preparation, Kalns and Ayres, 1993). In children the rate of absorption following administration of the sustained release suspension is much slower, for the first 3 hours, than observed in adults. Relative bioavailability (inter-study comparisons) of the sustained release suspension in children was found to be approximately 85%. The sustained release suspension was found to reduce fever in children for prolonged times, suggesting that the sustained release suspension will be an effective antipyretic in both children and adults.

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CHAPTER II

EVALUATION OF ANTIPYRESIS AND PHARMACOKINETICS OF SUSTAINED RELEASE ACETAMINOPHEN SUSPENSION IN CHILDREN

ABSTRACT

The pharmacokinetics and pharmacodynamics of a novel sustained release acetaminophen suspension were evaluated in children, age 2 thorough 11 years. The sustained release suspension consists of sandwich coated acetaminophen beads (3 mm diameter) dispersed in a grape jelly. Beads are coated with a multi-laminar, polymeric, release rate controlling membrane. Pharmacokinetics were determined in 12 healthy children administered a single 30 mg/kg dose of sustained release suspension. Saliva samples were collected for 24 hours, post-dosing, and acetaminophen concentrations determined using HPLC. In 9 children, mean saliva concentrations reach a peak value of 5 ug/ml at 4 hours and then decline to 2.5 ug/ml at 12 hours. Pharmacokinetics of this group are consistent with sustained release of acetaminophen from the dosage form *in vivo*, since acetaminophen concentrations in saliva peak later and decline more slowly compared to immediate release (peak at 0.5-1.0 hour, half-life following peak 1.5-3.0 hours). Estimated relative bioavailabilIty of the sustained release suspension is approximately 90%. In 3 children, mean peak saliva concentrations of 10 ug/ml occur at 1 hour and then decline at a rate similar to the rate following administration of immediate release acetaminophen. It is believed that incomplete sustained release effect was obtained in 3 children because the sustained release beads were chewed prior to swallowing. In a separate pharmacodynamic study, body temperature change in 10 febrile (101-

104^0F) children was monitored for 24 hours following administration of a single, 30 mg/kg dose of sustained release acetaminophen suspension. Mean temperature declined for a period of 12 hours with 2^0F and 3.3^0F decrements observed at 2 and 12 hours respectively. The sustained release suspension was readily accepted by all children enrolled in the studies. The results indicate that a single dose of 30 mg/kg sustained release acetaminophen suspension, given every 12 hours, may be as effective in reducing fever as three 10 mg/kg doses of immediate release acetaminophen given every 4 hours.

INTRODUCTION

The onset of fever is one of the most frequent reasons that people will seek medical attention for their children. The concern and anxiety that is associated with fever (1,2) is not misplaced since fever almost always has a distinct infectious etiology that may require medical intervention. While fever indicates the presence of an active disease process, elevated body temperature is benign (3). Despite the benign nature of elevated body temperature, parents with febrile children will frequently attempt to reduce their child's fever with drugs. Pharmacologic interventions specifically directed towards reducing fever without effecting the underlying infectious agent may slow resolution of the disease process (4,5) and thus may be considered unwarranted (6). However, the clinical goal of resolving the infection must be balanced against the overall improvement in quality of life that may be achieved by reducing fever. Fever control may reduce malaise and enable the fever suffer to carry out the functions of daily life i.e.playing, sleeping. In addition, the rapid and profound reduction in body temperature possible through the use of antipyretic drugs reduces fever anxiety (1) and promotes the feeling that a positive, demonstrable intervention is being made. Since most acute infections will resolve with or without specific interventions to reduce fever, fever reduction can be made safely with little effect on outcome. Because of the perception that fever reduction is beneficial it is likely that Americans will continue to administer antipyretic drugs to their children. The selection of an antipyretic to administer is based on several criteria.

Antipyretics should be safe, effective, have few side effects and be easy and convenient to dose. Among the drugs that are widely used to reduce

fever, acetaminophen has few side effects (7), is extremely safe (8) and effective when dosed correctly (9). Unfortunately, acetaminophen has a half life of approximately 2 hours which requires that doses be administered every 4-6 hours to maintain effective plasma concentrations (10). In addition, solid oral formulations of the drug are relatively large (500mg) and may be difficult for some adults to swallow and are inappropriate for children who require relatively small doses. To address the needs of adults who find swallowing difficult, and children, the pharmaceutical industry has developed oral solutions of acetaminophen. Oral solutions may be easier to swallow then solid oral formulations and may be dosed precisely. However, because of the intensely bitter taste of acetaminophen, the experience with an oral solution may be unpleasant and stressful. Oral solutions, like solid dosage forms, must be administered frequently which may be inconvenient. Formulating acetaminophen as a sustained release suspension may eliminate or significantly reduce many of the inconvenient and unpleasant aspects of currently available formulations .

The goal of this research was to evaluate the performance of a sustained release acetaminophen suspension in terms of pharmacokinetics, ability to reduce fever, and apparent ease of administration. Children, age 2-11 years were selected as a population that may benefit from an improvement in acetaminophen formulation. Results indicate that a single dose of the sustained release suspension is readily swallowed by children, produces saliva concentrations of acetaminophen consistent with sustained release of drug, and reduces fever.

MATERIALS AND METHODS

Production of acetaminophen beads

150 grams of non-parriel sugar beads (28-30 Mesh) were coated with 450 grams of USP grade acetaminophen (Mallinkrodt Inc., St. Louis, MO.) dissolved in 700 ml 95% ethanol USP. Polyvinylpyrrolidinone, MW 40,000 and hydroxypropylcellulose were added to the alcoholic solution as binders. The application of acetaminophen ethanolic solution required 2 hours at ambient temperature (~25^0 C). After application of ethanolic acetaminophen solution, drug coated beads are allowed to dry for 20 minutes at 50^0 C in order to remove residual ethanol. Beads were removed from the fluid bed and weighed and assayed to evaluate the total amount of drug loaded per total weight of beads. Acetaminophen content was expected to be 70% of total bead weight, and beads were approximately 3 mm in diameter.

Production of the sustained release suspension

Polymer film coating technology was used to produce controlled release (sustained-action) acetaminophen . The coating apparatus consisted of a spray-coating chamber, with a 7 inch Wurster column (STREA-1, Aeromatic Inc., Columbia, MD) mounted on a fluid-bed dryer (Lab-Line/P.R.L. Hi-Speed Fluid-Bed Dryer, Lab-Line Instruments, Inc., Melrose Park, IL). Ethyl cellulose (Aquacoat®, Type ECD-30, Lot J7211, FMC Corp., Philadelphia, PA., USA) and Eudragit® L30D, Lot #12-906-1280, Rhom Pharma GMBH, FRG were used as the coating materials. Two plasticizers were used: triethyl citrate (triethyl citrate 99%, Aldrich Chemical Co., Milwaukee, WI.) and dibuty sebecate (dibutyl pthalate 99%, Mallinckrodt, Inc., St. Louis, Mo.). Three separate applications of polymers were made during the manufacturing process. Loading of polymers was calculated as weight percent of polymer solids, including plasticizers, per

unit weight of acetaminophen beads. The order of application was 2% Aquacoat® (Ethyl Cellulose suspension), 4% Eudragit® L30D (methacrylic acid copolymer, Type A), 2% Aquacoat. Polymer solutions were applied rapidly and then coated beads were allowed to dry in the fluid bed dryer for 30 minutes before the next application of polymer solution. All processes were undertaken at 70^{o} C. The commercial polymer suspensions (30% solids weight) were diluted with an equal volume of de-ionized water before use. Triethyl citrate (TEC), 15% of total solids, and dibutyl phtalate (DBP), 15% of total solids, were added as plastacizers. Coated beads were then dispensed in a commercial fruit jelly (Danish Orchards Grape Spread®, Lot #OD3 9 A1, Vendelbo Konserves A/S, Denmark, 53 grams of beads plus 47 grams of jelly) and refrigerated until use. During dosing the suspension was dispensed precisely by volume with a syringe. The suspension contained 37% acetaminophen by weight.

Analysis of acetaminophen in saliva by HPLC

Concentrations of acetaminophen in saliva have been correlated in a 1 to 1 fashion to plasma concentrations (1). The HPLC assay method for acetaminophen is similar that has been reported previously (2). Since the principle metabolites of acetaminophen, acetaminophen gluronide and acetaminophen sulphate, elute much earlier then acetaminophen, the quantitation method is specific for acetaminophen. Saliva samples were collected in plastic vials and frozen at -20^{o} C prior to assay. An aliquot of saliva was centrifuged at 14,000 rpm for 2 minutes to pelletize particulates. 50ul of supernatant was transferred to a micro-centrifuge tube containing 100ul water with internal standard (2-acetamidophenol, Sigma Chemical, St. Louis, MO.) and vortexed for 30 seconds. 50ul of the mixture is injected onto an HPLC system with a 300mm length C-18 column (Microsorb-MV, Rainen, Woburn, MA.) with >20,000 theoretical plates. A autosampler (WISP Model 712, Waters Associates

Inc., Milford, MA.) was used for all injections. A mobile phase of 20% methanol 80% water was used. Acetaminophen elutes at 7.5 ml and the internal standard 2-acetamidophenol at 15.0 ml. Analytes were detected with a fixed wavelength UV detector at 254 nm (Model 440, Waters Associates Inc., Milford, MA.). A integrator (Model C-R3a, Schimadzu, Japan) was used to calculate peak areas. Standard curves were prepared by spiking 1ml of saliva with 50ul of acetaminophen solutions. The range of standard concentrations was 0.25ug-30ug/ml. Coefficient of determination were greater then >0.995 on all days. All saliva samples were frozen at -20C prior to assay.

Patient enrollment

Protocols and informed consents for the fever response study and pharmacokinetic study were approved by the Investigational Review Board, Oregon Health Sciences University, Portland, OR. An investigational new drug (IND) application was filed with FDA and approved. Children for both studies were recruited during the period of January and February, 1993.

Fever response study

Children, 2 through 11 years of age with oral temperatures of 101^{o} to 104^{o} F were recruited from patients who came to the Outpatient pediatric clinic of Dohrnbecker Children's Hospital, Portland, Oregon. Children who were concurrently taking drugs whose metabolism may be effected by the acetaminophen (phenytoin, phenobarbital, etc.), or had a reported history of antipyretic drug use within the 2 hours prior to presentation in the clinic, or had a medical history of hepatitis or other liver disease, or reported a previous adverse experience with acetaminophen were excluded. The study was described by a pharmacist both verbally and in writing. Signed, informed written consent was obtained from each subjects parent before the study began. Patients received a single oral dose of the sustained release suspension (30mg/kg

acetaminophen) while in the clinic. Parents were instructed regarding the use of a digital thermometer which was provided, and how temperatures were to be recorded on a form (also provided). No monetary incentive was offered for participation in the study. Oral temperatures were recorded prior to dosing with the suspension and 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 hours post dose.

Pharmacokinetic study

Healthy children, ages 2 through 11 were recruited. Signed, informed written consent was obtained from each subjects parent before the study began. Parents were provided with a single oral dose of the sustained release suspension (30mg/kg acetaminophen) to give to their child at home, along with plastic vials for the collection of saliva samples. All parents and patients were carefully coached to swallow the dose gently without chewing or crushing the beads in the jelly. No restrictions were placed on food prior to or during the study with the exception that parents were instructed not to allow their children to eat or drink for 5 minutes prior to collection of a saliva sample. Saliva samples were collected prior to dosing and 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 hours after dosing.

RESULTS AND DISCUSSION

The novel sustained release suspension consists of acetaminophen loaded beads (3mm) coated with three layers of coating polymers ("sandwich coating"). Coated beads are suspended in a low pH, high viscosity, commercial grape jelly. Polymers are applied to acetaminophen beads in the order of ethyl cellulose (Aquacoat®, Type ECD-30), methyl methacrylate (Eudragit® L30D, Methacrylic acid copolymer, Type A), ethyl cellulose (Aquacoat®, Type ECD-30). Triple coated beads suspended in high osmotic pressure jellies have been previously been shown to have a high degree of in vitro release rate stabilty during periods of up to 1 year storage (13). Because of the relatively low pH and high osmotic presure of the suspending jelly, very little water flux accross the release rate controlling membranes occurs and as a consequence, detectable amounts of acetaminophen are not found in the jelly. This means that the bitter taste of acetaminophen is masked. In addition, unique bi-phasic *in vitro* dissolution patterns are described by some triple layer formulations.

Depending on the relative amounts of methyl methacrylate and the exterior coat of ethyl cellulose, some triple coat formulations have been observed to release acetaminophen slowly at low pH, and upon exposure to high pH, release drug at a much more rapid rate. However the rate of rapid release can be controlled and thus can be much slower then the "burst" release that is often evident in enteric coated products. Bi-phasic release patterns may be desirable for some drug therapies. Bi-phasic release patterns can also be achieved by the mixing of sustained release particles that release drug at dissimilar rates. However, mixed formulations suffer from manufacturing problems associated with segregation of particles that differ

with respect to size and surface charge. Since triple coated beads are relatively homogeneous , segragation during manufacture is less likely to be a problem compared to mixed formulations.

Pharmacokinetics of the sustained release formulation were evaluated in 10 healthy, Caucasian children dosed with 30mg/kg of acetaminophen sustained release suspension. The age, weight and sex of these subjects is found in table 1. Figure 1 shows the concentration of acetaminophen in saliva over time for individuals. Samples from 6 of 10 children in this group showed very high saliva concentrations of acetaminophen (30-100 ug/ml) during the first 0.5 hour of the study. It is likely that some children were unable to swallow the dosage form without chewing and breaking some of the sustained release beads incorporated in the jelly which released drug which may have become trapped in the oral cavity. Residual acetaminophen attributable to bead breakage was cleared from the oral cavity within the first hour, in an apparent first order process, with a t1/2 of approximately 15 minutes. In these children, 0.25 and 0.5 hour concentrations were fit to a mono-exponential decay expression. The expected value of the decay expression was subtracted from the observed acetaminophen concentration in saliva for 0.25, 0.5, 1 and 2 hour. Data obtained after correcting for early high values are shown in figure 1 and were used in all subsequent calculations.

Figure 1 shows that sustained release of drug was achieved in most children, and that some children display pharmacokinetics which are consistent with some immediate release as well. Table 2 shows non-compartmental pharmacokinetic parameters for the sustained release acetaminophen suspension in healthy children. Based on MRT and Cmax for individuals, a group that absorbed acetaminophen rapidly (n=3) and a group that absorbed acetaminophen slowly (n=7) were identified. Differences in

Table II.1 Demographic data of children in pharmacokinetic study.

subject	age	wt (kg)	sex
1	6	21.4	F
2	4	16.7	M
3	11	42.9	F
4	7	21.4	F
5	9	34.8	M
6	5	20.0	M
7	2	14.8	F
8	10	33.3	M
9	7	26.7	M
10	5	20.5	M

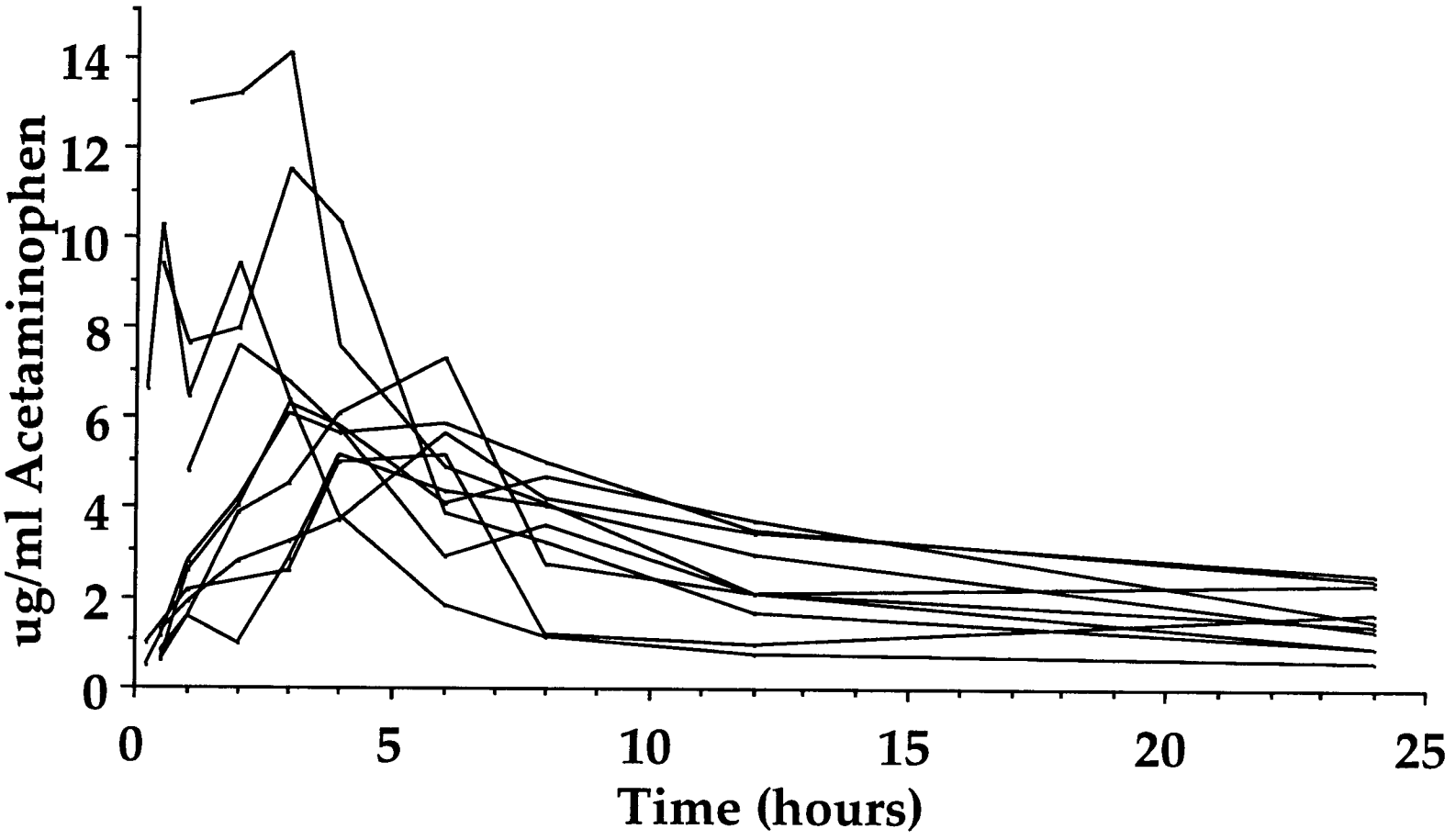

Figure II.1 Concentration of acetaminophen in saliva for subjects in pharmacokinetic study (n=10).

Table II.2 Non-compartmental pharmacokinetic parameters of sustained release formulation in healthy children.

Subject	Tp (hr.)	C max (ug/ml)	AUC (ug*h/ml)	MRT (hr.)
1	6	5.18	46.09	10.05
2	4	5.16	64.82	10.14
3	6	5.60	79.38	11.24
4	2	6.70	74.80	9.74
6	3	14.00	95.38	6.40
7	3	6.23	81.29	9.69
8	3	6.03	89.61	10.30
9	6	7.27	66.00	9.10
11	2	10.26	47.73	5.54
12	3	11.44	81.55	6.57
Average (SEM)	3.8 (1.6)	7.8 (3.0)	72.7 (16.5)	8.9 (2.0)

AUC and MRT calculated to 24 hours using linear trapezoidal rule. Time to peak (Tp) and Cmax observed values.

rates of absorption may arise from chewing of beads prior to swallowing. Difference in MRT and Cmax between these two groups are statistically significant ($p<.05$) in spite of the small sample sizes involved. Of the three children with early release of drug, 2 showed >2ug/ml of acetaminophen in saliva 12 hours post dosing, a level greater then expected if all drug in the dosage form had been released immediately. Thus, even in cases in which significant immediate release occurred, a sustained release was in part preserved, providing a relatively high concentration of acetaminophen for 12 hours. Figure 2 shows the mean acetaminophen concentration in saliva for the two identifiable subgroups receiving the sustained release product. The mean drug concentration of the sustained release group shows a gradual increase to reach a peak concentration of ~5.5 ug/ml at 4 hours with a gradual decline to ~3.5 ug/ml at 12 hours. Hossain and Ayres (14) have suggested that acetaminophen may be expected to provide an anti-febrile effect so long as the drug concentration exceeds 2ug/ml.

The cumulative amount of acetaminophen input into the systemic circulation following administration of the sustained release formulation was calculated by deconvolution (15) and is shown from time zero to 24 hours for each individual in figure 3. A mono-exponential impulse response function (weighing function), based on population pharmacokinetic parameter estimates from febrile children dosed with 12.5mg/kg immediate release oral formulation was used (16). A value of 1.03 l-1 and 0.42 h^{-1} were used for F/Vd and Kel respectively. The sustained adsorption group (n=3) exhibit a rapid input for the first hour, followed by a gradual positive input for the following 24 hours, indicating that even in cases where a large portion of available acetaminophen was input rapidly, a sustained release was still achieved. The sustained absorption group (n=7) exhibited a rapid input for the first hour,

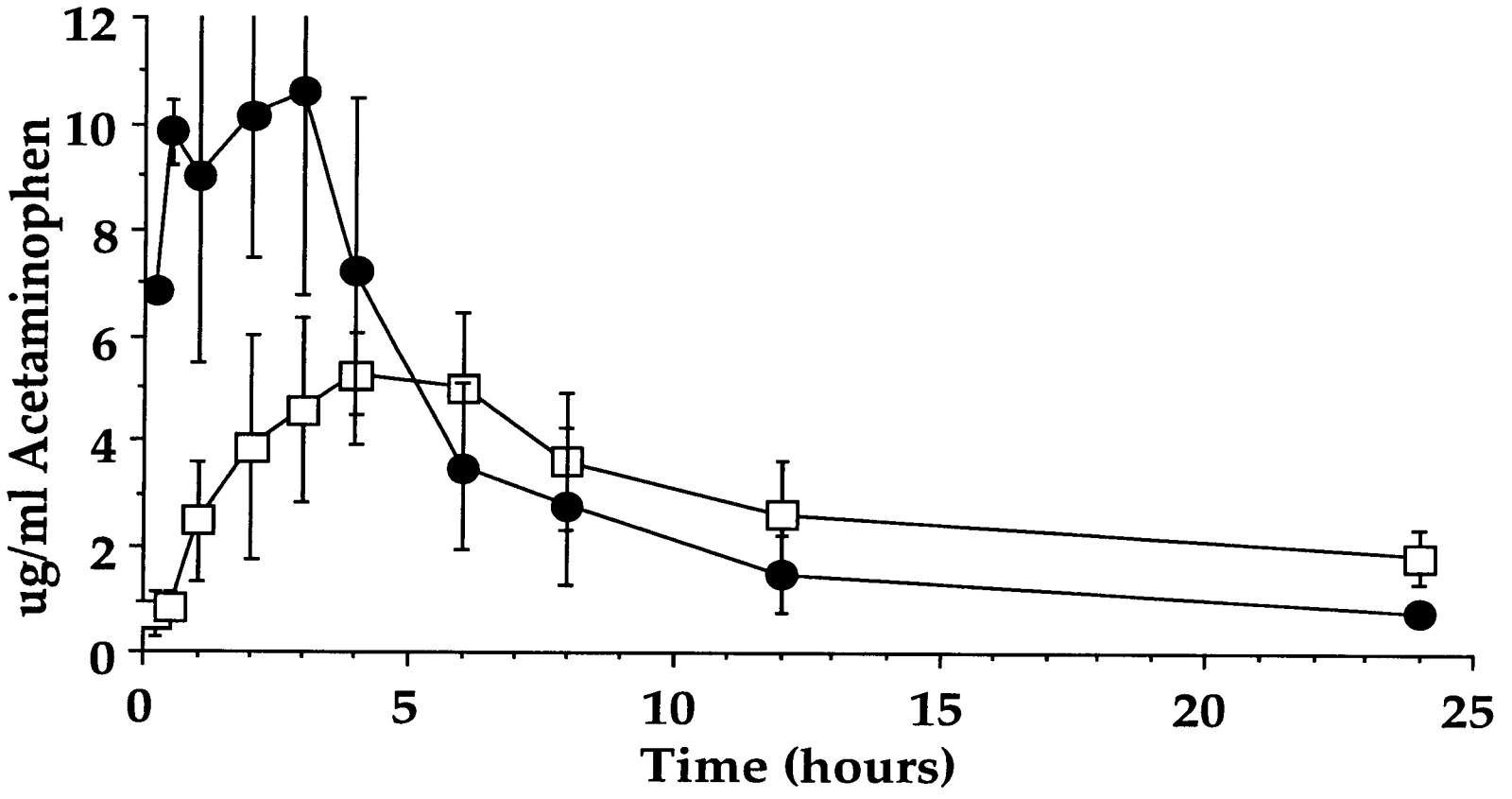

Figure II.2 Mean concentrations of acetaminophen in saliva following administration of acetaminophen sustained release in healthy children. Solid circles mean of 3 subjects with early high values (Subjects 6, 11, 12), open squares mean values of 7 subjects without early high peak values (Subjects 1,2,3,4,7,8,9,11). Error bars SEM of mean.

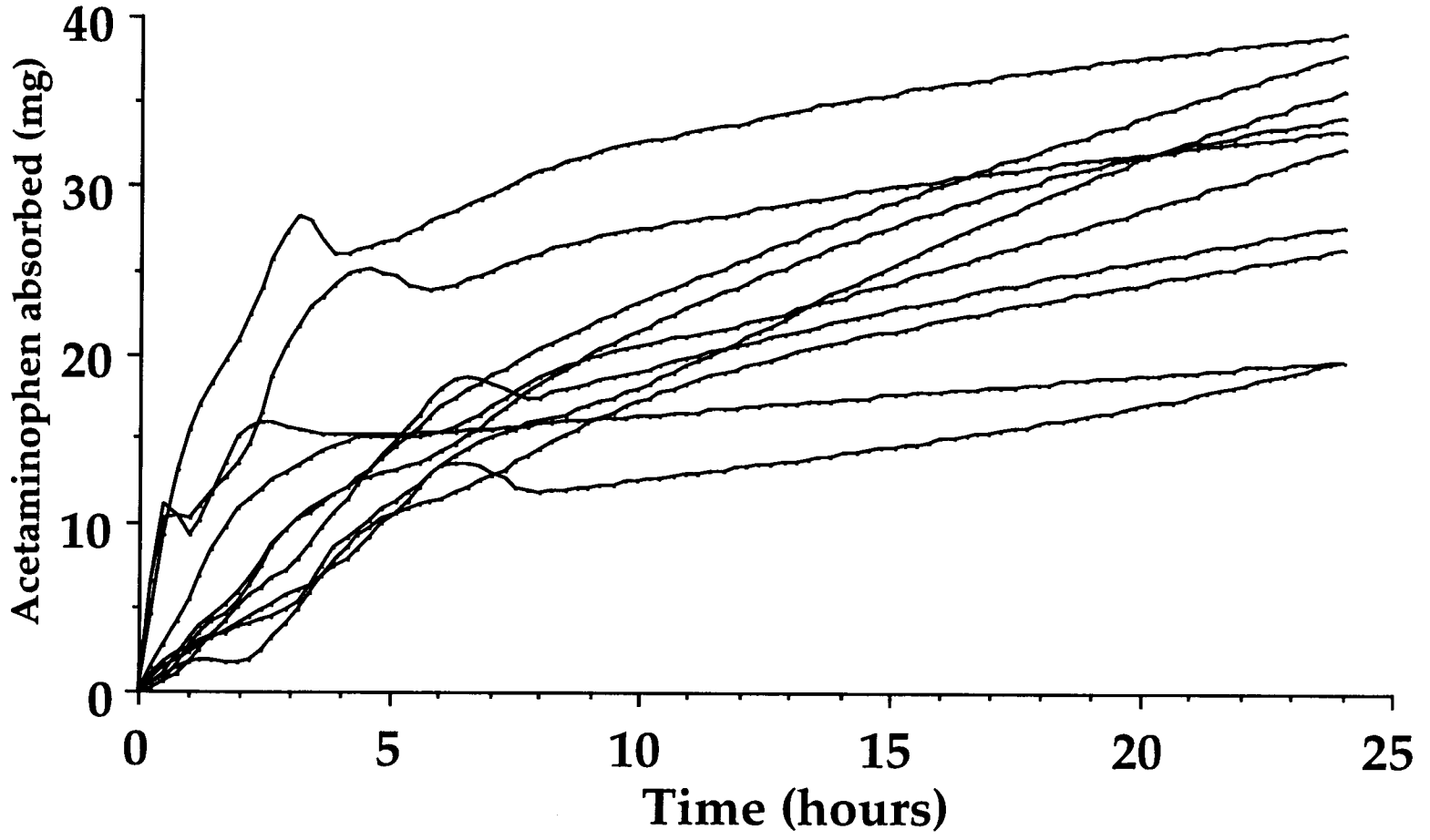

Figure II.3 Cumulative acetaminophen absorbed from sustained release dosage form in healthy children. Population pharamcokinetics used as an impulse response function (weighing function). See text.

followed by a gradual positive input for the following 24 hours, indicating that even in cases where a large portion of available acetaminophen was input rapidly, a sustained release was still achieved. The slow absorption group (n=7) shows a nearly constant rate of input for the first 6 hours followed by a gradually decline in input rate with significant input of drug occurring for at least 12 hours.

Use of deconvolution methods enables a direct comparison between *in vivo* absorption rate of bioavailable acetaminophen and release of acetaminophen from the sustained release suspension *in vitro*. Individuals *in vivo* cumulative absorption was normalized by dividing cumulative amount absorbed at a given time by the cumulative amount absorbed at 24 hours. This normalization method assumes that all absorption of bioavailable acetaminophen *in vivo* is 100% at 24 hours, which may not be entirely accurate but is a close approximation in this case. A comparison between mean normalized percent released *in vivo* for children exhibiting slow rate of acetaminophen input, and percent released *in vitro* is shown in figure 4. A very close correlation between release rate *in vitro* and *in vivo* absorption is found for the first 6 hours. After 6 hours the dosage form appears to release drug at a slower rate *in vivo* then *in vitro*. Correlation was not attempted for the 3 subjects with rapid absorption since it is believed they crushed some drug beads and correlation is not expected with in vitro dissolution where crushing was not avoided.

A time scaling approach (17) was applied to refine the correlation between *in vitro* release and *in vivo* absorption. A time scaling parameter for *in vitro* dissolution data was calculated by first obtaining a non-linear estimate of Weibull parameters associated with *in vivo* absorption rate:

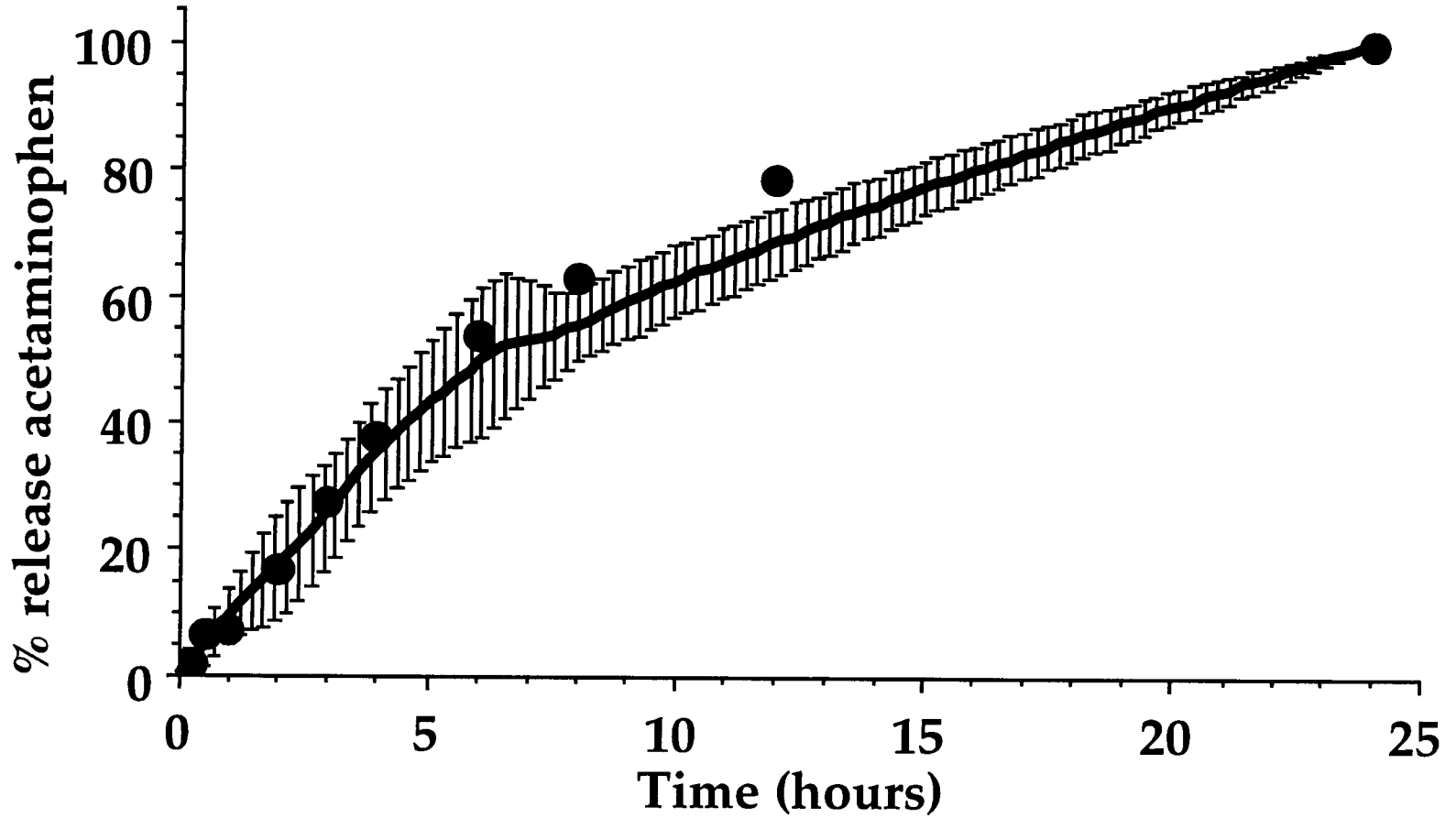

Figure II.4 In vitro % released compared to in vivo % released. Solid circles in vitro % released, solid line in vivo % released. SEM for in vivo % released shown.

$$F_t = F_\infty \left(1 - e^{-\left(\frac{t}{\tau}\right)^\beta} \right)$$

Where β and τ are parameters estimated by non-linear curve fitting procedures and t is time. The resulting fit is within 2% of the mean *in vivo* percent absorbed at all times. *In vitro* mean percent dissolution was fit to a Weibull model using the *in vivo* parameters estimates for β and τ with the addition of an estimated time scaling term a:

$$F_t = F_{\infty,\ \text{in vivo}} \left(1 - e^{-\left(\frac{a*t}{\tau_{\text{in vivo}}}\right)^{\beta_{\text{in vivo}}}} \right)$$

The value of a was estimated to be 1.2. A very similar approach using the Weibull mathematical model of dissolution and time scaling has been reported previously18. Figure 5 shows that following introduction of a time scaling parameter (a=1.2), *in vitro* dissolution is within 1% of *in vitro* dissolution release percent for all times up to 12 hours. The very close correlation between the time scaled *in vitro* release rate and *in vivo* absorption rate suggests that an FDA, level A correlation relationship is present (19). The improved correlation from application of time scaling may simply result from adding one more correction term to the mathematical relationships employed rather than being an effect of time. However, these

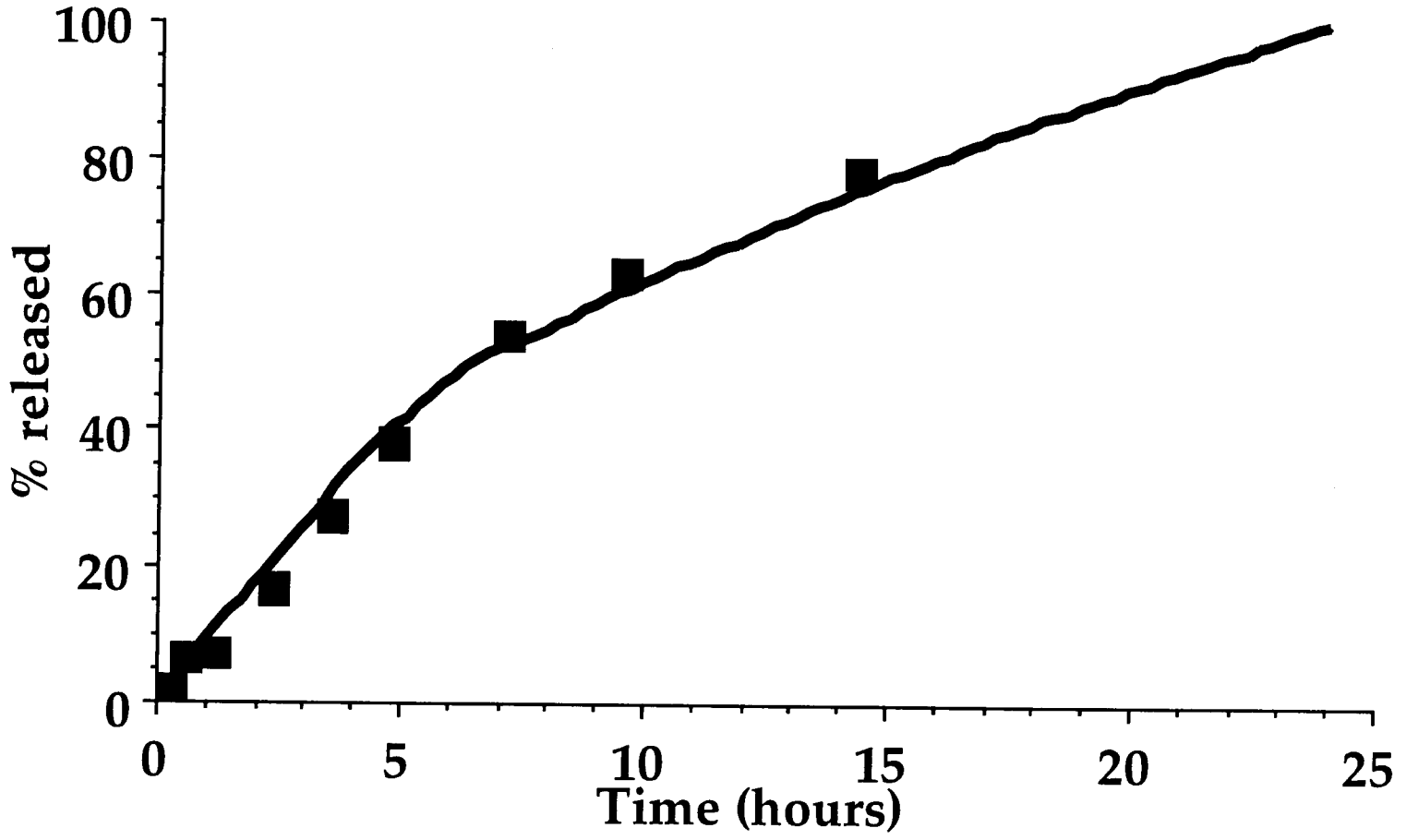

Figure II.5 In vitro, in vivo correlation after applying an in vitro time scaling factor. Solid squares are normalized in vitro % release with time scaling (factor=1.2); solid line is normalized in vivo % absorbed.

findings are still useful since *in vivo* performance of dosage forms of this type may be predicted precisely by *in vitro* dissolution, using the relationships described.

A rigorous estimate of relative bioavailabilty of the sustained release suspension could not be made since an intravenous or an immediate release formulation were not administered. A comparison to literature values for AUC reported for children following administration of immediate release formulations of acetaminophen may be useful, since inter-study variation in reported pharmacokinetic parameters for children is relatively small (20). Such comparisons assume a 1:1 relationship between saliva and plasma drug concentrations, which has been shown to exist, on average, for acetaminophen. Following the administration of 12.5mg/kg of acetaminophen as a immediate release formulation, Brown et al. (15) report a AUC (to infinity) of 35.4 ug*hr/ml. If linearity is assumed (21,22) a dose of 30 mg/kg will produce an AUC of 85.0 ug*hr/ml. A relative bioavailabilty can be estimated for the sustained release suspension, based on the preceding estimate of immediate release AUC, of 85%. This value for relative bioavilability is in good agreement with the 84 and 91% relative bioavailability reported for two acetaminophen sustained release formulations evaluated in healthy adult males (16), compared to acetaminophen solution in the same subjects. This would indicate that hepatic and gastrointestinal biotransformation of acetaminophen prior to systemic delivery are not greatly increased by decreasing the rate at which acetaminophen is released by a dosage form, which is consistent with Sahajawalla (20) but different then Borin (21).

The pharmacokinetic investigation of the dosage form in healthy children confirmed the hypothesis that the sustained release formulation is

able to produce a sustained concentration of acetaminophen in saliva for a period of 12 hours, and that the concentrations produced by the dosage form may effectively control fever. The ability of the dosage form to reduce fever in children was evaluated in a population of acutely ill children age 2 through 11. Demographic data of children enrolled, concurrent drugs and diagnosis are presented in table 3.

The sustained release suspension was readily accepted by children and parents. Flavor, texture and appearance were generally accepted as was the instruction to swallow without chewing or crushing the beads. Results from the earlier study suggest that most subjects were able to comply with these instructions. Of 12 children enrolled, 1 child immediately vomited the suspension shortly after administration, and one child refused to take the dose. Cause of vomiting was not attributed to the suspension, but rather to the general condition of the patient.

Oral temperatures collected for each subject and mean values at each observation time are given in table 4. Subjects 1,4,5 and 6 showed a large effect (>2 F^0) by 1 hour, while subject 7 showed little response to treatment. It is interesting to note that subjects 1,4 and 6 did not receive concomitant antibiotic therapy (Table 3). Other subjects exhibited a gradual decreasing fever during the study period. Figure 6 shows oral temperature for each patient over the 24 hour period following administration of the sustained release acetaminophen formulation. Mean values given in table 4 show an overall decreasing trend, however the relatively large variance associated with the mean temperature at each time does not imply that a statistically significant reduction in oral temperature is achieved. This result is not surprising since the response of fever to antipyretic drugs is variable, oral temperature at time of enrollment varied over a wide range, and the number

Table II.3 Demographic data, diagnosis and concurrent medications of children participating in fever control study.

subject	age	sex	weight (kg)	Temp (F^0)	Diagnosis	Concurrent Medication
1	4	M	17.2	101	viral	none
2	4.5	F	15	101.1	Strep phar.	Penicillin
3	3	M	15.2	103.3	Strep phar.	Penicillin
4	1.5	M	12	103.5	viral	none
5	2	F	11.8	102.2	OM	Amoxicillin
6	6	F	18.6	103.9	viral	none
7	7	M	50	103.6	Strep	Cephelexin
8	7	M	19.1	103	Sinusitis	Amoxicillin
9	4	F	15.1	104.9	OM	Amoxicillin
10	7	M	33.6	102.7	OM	Amoxicillin

Diagnosis codes: viral= general viral syndrome, Strep phar.= Presumed streptococcal pharyngitis (culture results not known), OM= Otitis Media. Strep= culture positive streptococcal pharyngitis. Subject 10 was a treatment failure on Penicillin VK, hence the use of Cephelexin in treatment. All antibiotics were given orally.

Table II.4 Body temperature response to administration of a sustained release suspension of acetaminophen.

Time (hr.) Subject	0	0.5	1	2	3	4	6	8	12	24
1	101	100.1	98.6	99	99.5	99.2	100.4	100.3	99.5	98.7
2	101.1	101	99.9	99.2	99.4	98.9	99	99	99	99
3	103.3	103.2	102.2	102	101.8	100.8	100.2	99.5	99.8	99.2
4	103.5	100.2	101.5			102	99.4	99	99	99
5	102.2	99.1	98.6	97.9	98.6			98.9	97.9	98.6
6	103.9	103.8	100	100	99	100	100	100.1	98.9	98.9
7	103.6	104	102.6	103.2	103.8	104.3	102.9	102.9	101	103.8
8	103	102.6	102.1	101.9	102.2	101	100.5	99.1		98.1
9	104.9	104.7		104	104.2	103.4	102.7	102.5		101.5
10	102.7		102.3		102.3	102.9	101.3	99.7	99.1	99.1
Average	102.7	102.1	100.9	100.9	101.2	101.4	100.7	100.1	99.28	99.59
SEM	1.294	2.017	1.609	2.185	2.123	1.89	1.353	1.454	0.888	1.73

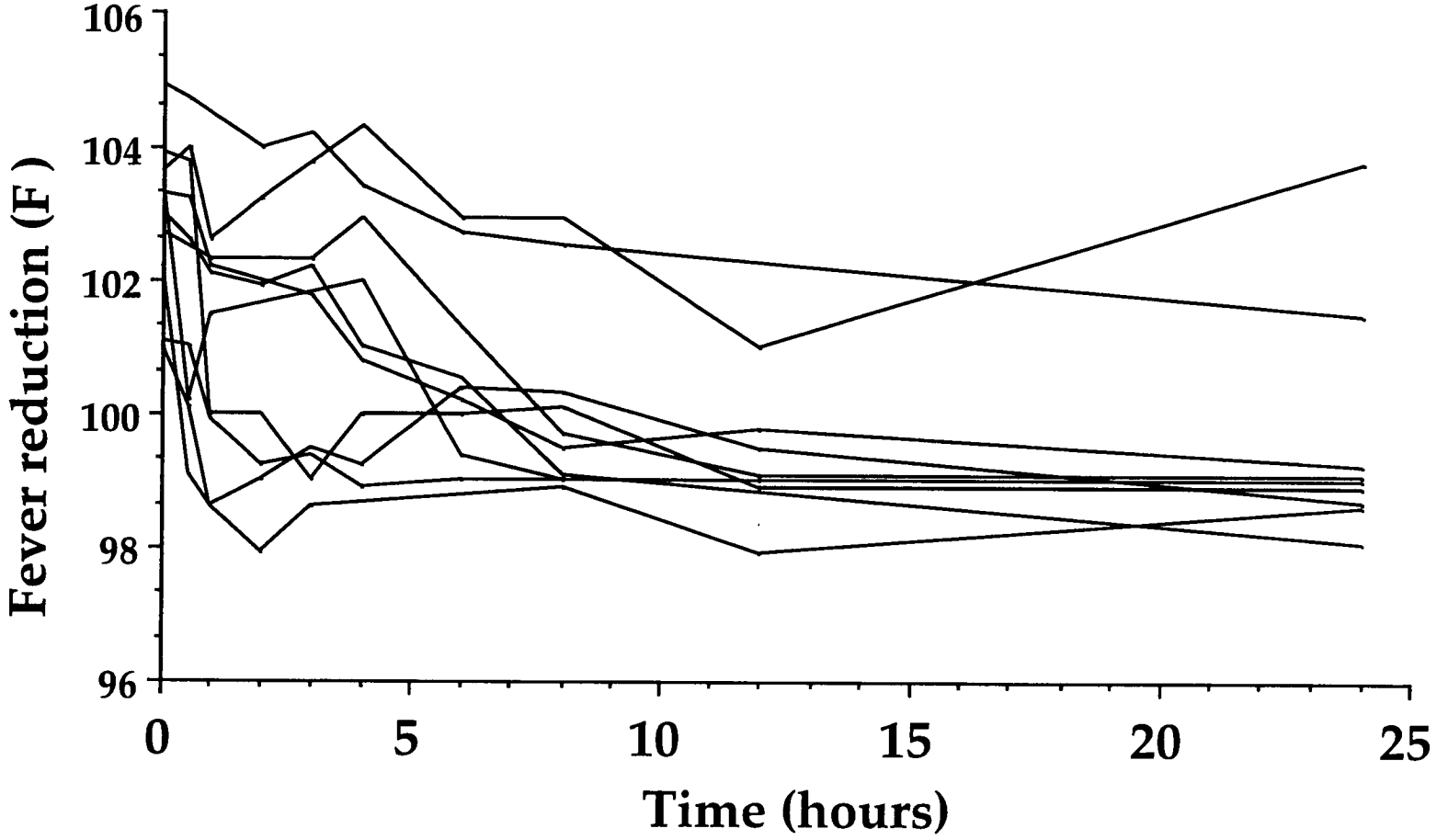

Figure II.6 Oral temperature of febrile children administered sustained release acetaminophen suspension.

of subjects enrolled in this study was small. Oral temperature at time of enrollment may be normalized by subtracting the temperature at time of enrollment from each subsequent oral measurement taken over the 24 hour study period. The resulting plots of fever reduction over time can be seen in figure 7. Table 5 shows that mean fever reduction standard deviation at each time is reduced compared to standard deviation of the mean oral temperature given in table 4. One sided student t-tests of mean fever reduction show that a statistically significant fever reduction ($p<0.05$) is achieved at 8, 12 and 24 hours relative to the temperature at time of enrollment. Temperature in some patients may have decreased even if no treatment had been administered since the underlying infection may in some individuals be nearing resolution at time of enrollment, and thus mean fever is expected to decline without treatment.

Literature reports of temperature reduction in children following the administration of placebo generally indicates that fever response to non-treatment is negligible. However, Walson et al. (23) report that following administration of placebo, febrile children show a steady decrease in mean fever, with a total reduction of 1.1 F in 8 hours. Wilson et al. (24) report that placebo did not produce a reduction greater then 0.2 C0 at any time during a 6 hour study period. Kauffman et al. (25) report that placebo treated children showed a small increase (<0.5 C0) in mean body temperature over an 8 hour study period. Attributing the statistically significant fever reduction in this study to the treatment, to temperature a time zero is made with caution. It is concluded that the sustained release suspension formulation has a helps to reduce fever in children, a claim that may require testing in more patients to be validated with greater confidence.

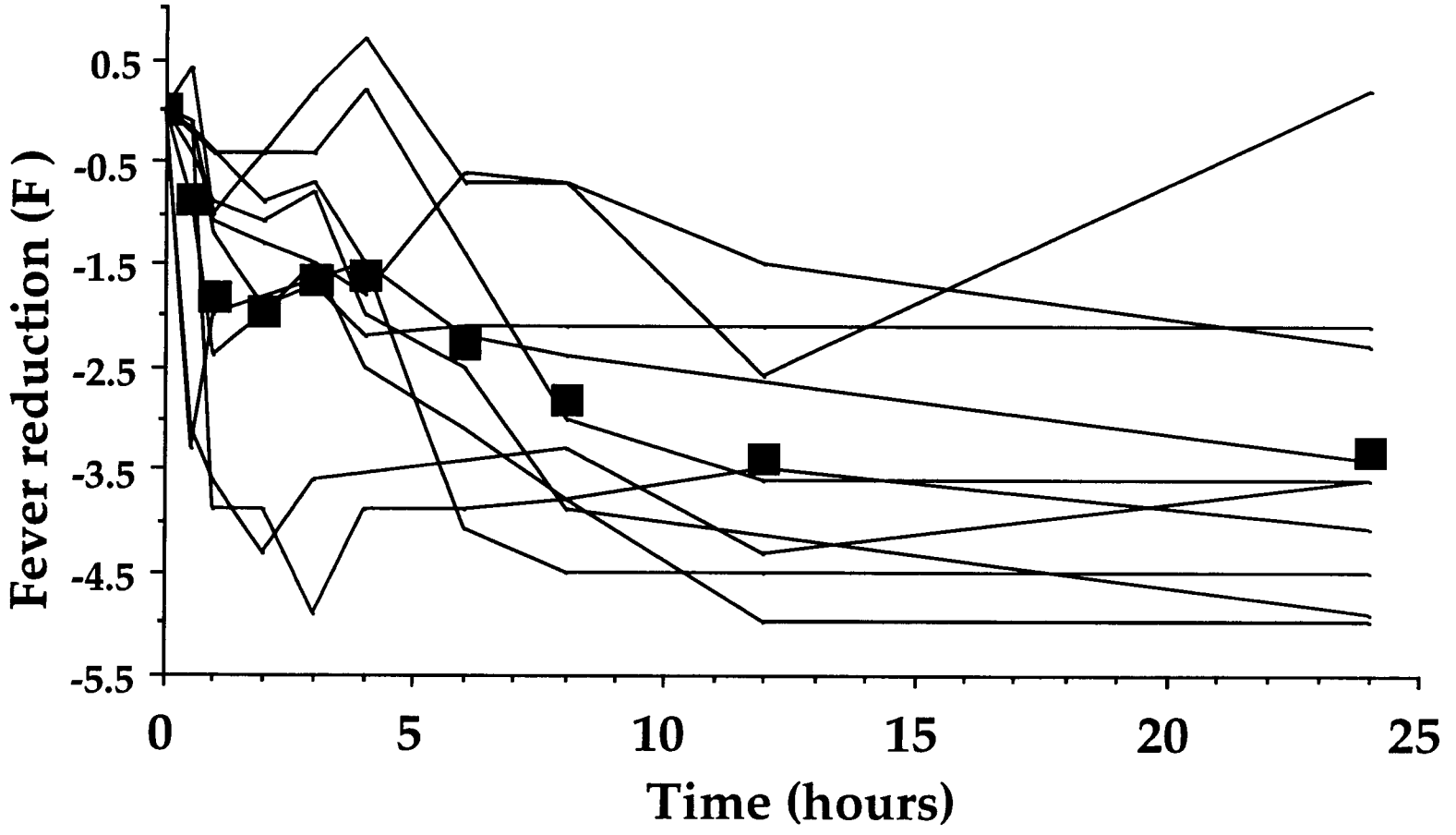

Figure II.7 Fever reduction of febrile children administered sustained release acetaminophen suspension. Solid squares are the mean fever reduction.

Table II.5 Fever reduction for subjects enrolled in fever response study. AUC(12 Hour) and AUC(24 hour) for temperature vs. time curve calculated by linear trapezoidal rule.

Subject Time (hours)	1	2	3	4	5	6	7	8	9	10	Average (SEM)
0.5	0.9	0.1	0.1	3.3	3.1	0.1	0.4	0.4	0.2		0.9 (1.37)
1	2.4	1.2	1.1	2	3.6	3.9	1	0.9		0.4	1.8 (1.24)
2	2	1.9	1.3		4.3	3.9	0.4	1.1	0.9		2.0 (1.41)
3	1.5	1.7	1.5		3.6	4.9	-0.2	0.8	0.7	0.4	1.7 (1.62)
4	1.8	2.2	2.5	1.5		3.9	-0.7	2	1.5	0.2	1.6 (1.38)
6	0.6	2.1	3.1	4.1		3.9	0.7	2.5	2.2	1.4	2.3(1.26)
8	0.7	2.1	3.8	4.5	3.3	3.8	0.7	3.9	2.4	3	2.8 (1.33)
12	1.5	2.1	3.5	4.5	4.3	5	2.6			3.6	3.4 (1.23)
24	2.3	2.1	4.1	4.5	3.6	5	-0.2	4.9	3.4	3.6	3.3 (1.58)
AUC (12 hr.)	15	23	32	36	31	47	8.4	22	16	19	25 (11.5)
AUC (24 hr.)	38	48	78	90	78	107	23	52	36	63	61 (26.7)

The febrile response to treatment with acetaminophen immediate release dosage forms is characterized by dose dependence (19), anti-clockwise hysteresis. Acetaminophen antipyresis also does not depend on age or etiology of infection (26,27). Peak reduction in fever occurs 3-4 hours post dosing, and 2-3 hours later then the observed peak plasma concentration. Rate of increase in body temperature following peak reduction is slower then the initial decline in temperature.

The relationship between saliva concentration and fever reduction in this study is shown in figure 8. Figure 8 shows that fever reduction occurs later (12 hours) then peak saliva concentrations (4 hours) and that rate of decrease in body temperature is slower in general then that seen with immediate release formulations. A 1 to 1 mapping of fever reduction to acetaminophen concentration in saliva is shown in figure 9.

The anti-clockwise hysteresis described by the mapping of the sustained release acetaminophen suspension is very similar in shape to the hysteresis map, following immediate release acetaminophen (21), described by Hossain (13). A notable difference is that the loop described in 12 hours by the sustained release suspension is described in 3-4 hours by immediate release formulations. It is quite surprising to note that at the 'end' time of the hysteresis loops, for immediate release dosage forms a drug concentration of 2 ug/ml is associated with a 1.1^0 temperature decrease but for the sustained release suspension, 2ug/ml is associated with 1.8^0 temperature decrease. After equilibrium (with a deep tissue compartment) , one would expect the same effect from equal drug concentrations. Examination of this effect is underway since it has significant clinical implication if confirmed. However the finding may simply be due to different subject populations or study design.

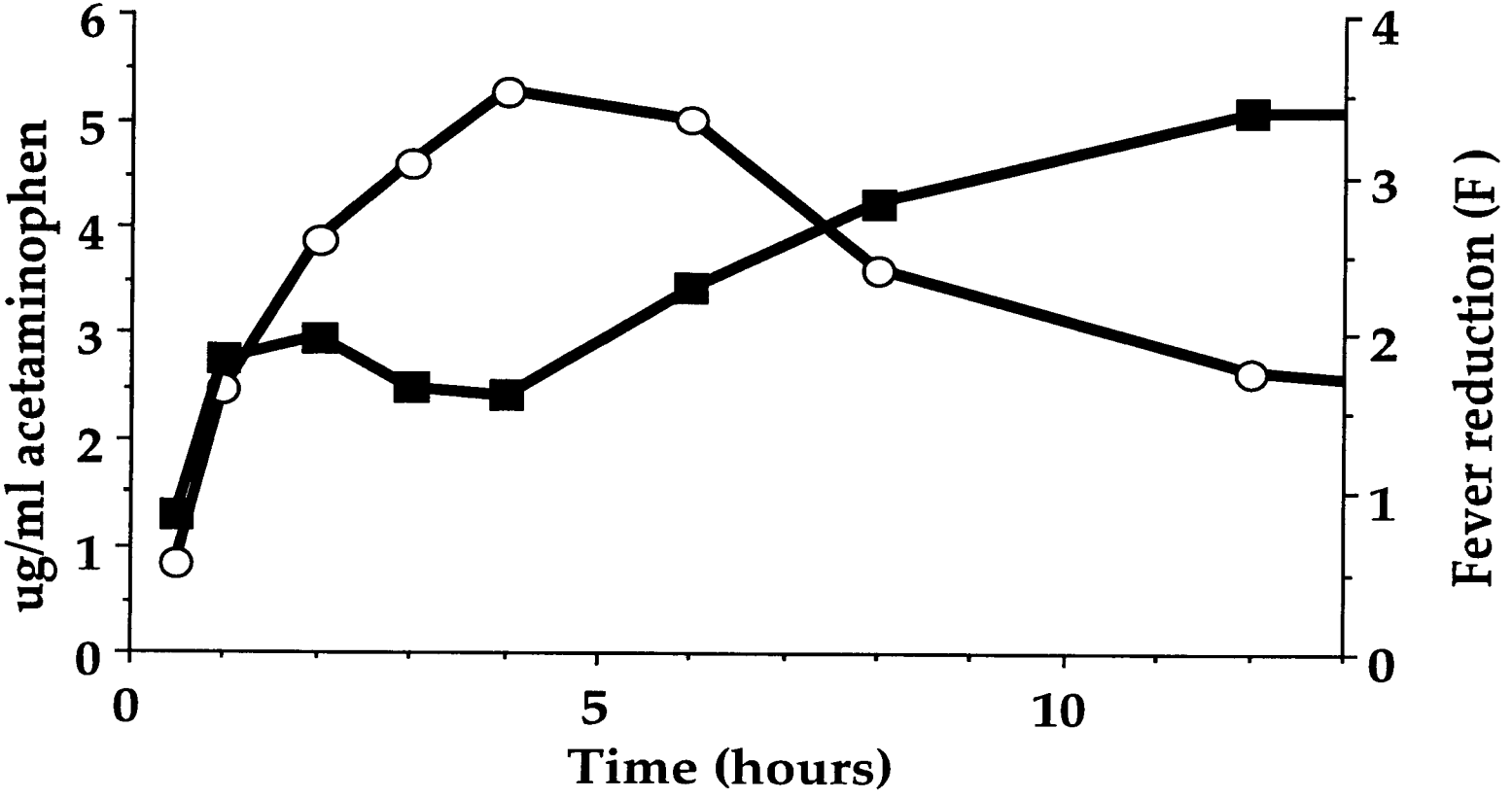

Figure II.8 Mean fever reduction and mean acetaminophen concentration in saliva. Observed temperature and mean acetaminophen concentration over time were observed in different groups of children. Open circles, mean acetaminophen concentration in saliva of children who absorb acetaminophen slowly(n=9), closed squares mean fever reduction (n=10).

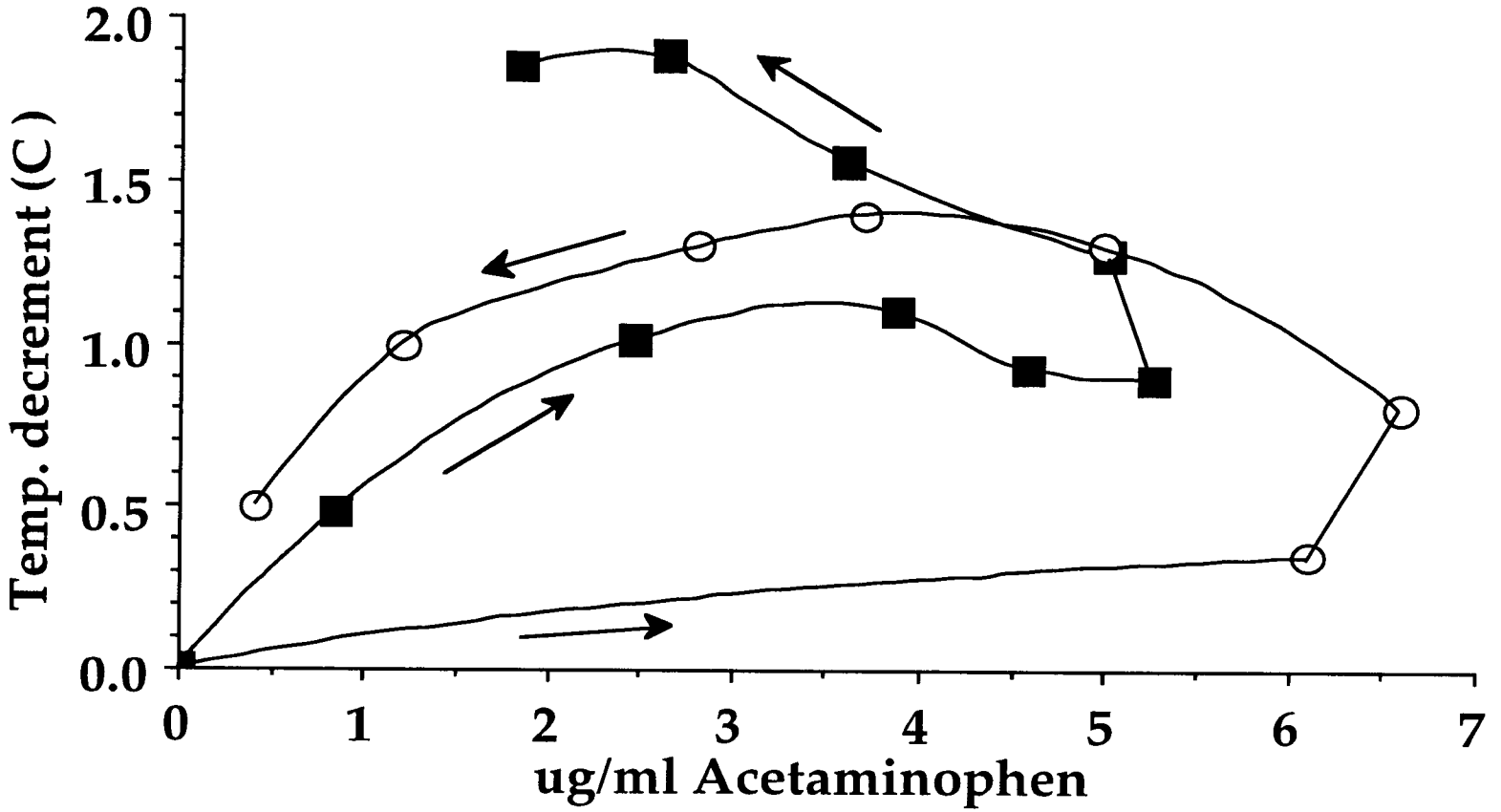

Figure II.9 Comparison of hysteresis of sustained release suspension (solid squares) and immediate release (Walson, et al., 1989). Arrows indicate time course of transit. Peak acetaminophen occurs at 1 hour and 4 hours for immediate and sustained release respectively. Time end points 8 and 24 hours for immediate and sustained release respectively.

Several explanations for the hysterisis loop of antipyresis have been offered. The pharmacologic action of acetaminophen is to reduce synthesis of PGE2 a prime mediator of thermoregulation (28). Since the primary organ of thermoregulation is the hypothalamus, a rate limiting step in antipyresis may be the rate at which effective concentrations of drug develop at this site (29). In support of this model is the observation that acetaminophen time course in cerebrospinal fluid (CSF) suggests a course similar to analgesic effect (30). This means that a map of acetaminophen concentration in CSF vs analgesia would show no looping, but rather a straight line. Since analgesia and antipyresis show a similar time course relative to acetaminophen concentration in blood, antipyresis hysterisis may also collapse if a CSF concentration map is constructed. The model assumes that the hypothalamus is in equilibrium with CSF with respect to acetaminophen, and further that CSF-tissue partitioning is the most important route of acetaminophen entry to hypothalamic tissue. These assumptions are as yet unproven. Wu et al. (31) have recently observed that acetaminophen inhibits transcription of prostaglandin synthase at clinically relevant concentrations in human umbilical endothelial tissue culture. If acetaminophen acts in a similar manner in hypothalamic tissue, then the antipyresis hysterisis is a result of lag times associated with catabolism of mRNA coding for the prostaglandin synthase and clearance of expressed enzyme from the hypothalamus. Half lives of mRNA coding for prostaglandin synthase have been reported to be less then 10 minutes in a variety of different rat tissues (32). In an earlier report, Wu et al. (33) report that the degradation kinetics of prostaglandin synthase in whole human umbilical endothelial cells are biphasic, with a rapid degradation half life of 10-20 minutes followed by a much slower elimination half life of 2 hours. The relatively slow increase in

body temperature following peak antipyresis is explained by a lag time required for sufficient quantities of enzyme to be expressed. Our results do not support the validity of one model over another, however our results do suggest that the time to reach peak temperature reduction may be effected by the rate at which acetaminophen is absorbed. This may be somewhat unexpected based on immediate release dosage forms as one might expect a slower input to have no influence on peak effect time as drug distribution could be as fast with slow input as with fast input, i.e. distribution should be able to occur as rapidly as slow absorption even if distribution is slower then rapid absorption.

The sustained release formulation evaluated here-in in a fever model may also be an effective long acting analgesic. Acetaminophen sustained release formulations have been evaluated in several different pain models. Nielsen et al. (34) report that a single dose of sustained release acetaminophen produces a significant and prolonged analgesia, in a laser pain model, for a period of 12 hours and that this formulation is as effective as dosing every 6 hours with the same amount of immediate release acetaminophen. In addition, the time to maximum analgesic effect, following administration of the sustained release formulation is similar to immediate release. The pharmacokinetics reported for their formulation indicate a more rapid release rate then the sustained release formulation that we reported here. Under very similar experimental conditions, Nielsen et al. (35) reports that a formulation which releases acetaminophen at rates nearly identical to rates reported here, does not produce significant analgesia when compared to placebo. In the same study, an equal dose of acetaminophen formulated as an immediate release preparation does produce significant analgesia, though peak blood concentrations of the two formulations are very similar. This

observation implies that rate of input may determine not only the time of onset of analgesia, but may also determine if significant efficacy is achieved.

The results obtained in this study suggest that fever may be controlled in an acutely ill pediatric population by dosing with the sustained release suspension. Immediate release drug may be included with the sustained release drug in this dosage form. The fever response indicates that fever is significantly reduced for a period of 12 hours and may be particularly useful for fever control during the night when parents may find repeated dosing with an immediate release formulation inconvenient. The formulation strategy employed here may also be applied to other drugs that require large doses, have a bitter taste, and are used in populations that find traditional dosage forms difficult to swallow.

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CHAPTER III

EXPECTED BIOAVAILABILITY OF KCL SUSTAINED RELEASE DOSAGE FORMS COMPUTED BY NUMERICAL SIMULATION

ABSTRACT

The use of *in vitro* dissolution to predict bioequivalence of formulations that differ with respect to *in vitro* release rate and *in vivo* absorption rate is valid if the mathematical relationship between *in vitro* release rate and *in vivo* absorption is known. An experimental dosage form was compared to a marketed standard for *in vitro* dissolution rate and bioavailability in 27 human subjects. The following steps were used to describe a range of *in vitro* dissolution profiles associated with bioequivalence: 1) Estimation of apparent whole body elimination rate of K^+ following dosage form administration. 2) Deconvolution of mean urinary excretion rate to estimate *in vivo* absorption rate 3) Relationship of *in vivo* absorption rate with *in vitro* dissolution rate 4) Computer simulation of expected mean urinary excretion rate profiles following administration of dosage forms with different *in vitro* dissolution profiles. Formulations are bioequivalent if bioavailability parameters are not significantly different ($p<0.1$) from bioavailability parameters associated with an innovators formulation. *In vitro* dissolution profiles associated with anticipated *in vivo* bioequivalence were used to describe upper and lower bounds for % release over time (1 hour 0.2-12.0%, 2 hour 5.4- 39.0%, 4 hour 61.2- 80.2%, 8 hour 68.0-89.0%). The modeling method used here may be generally applied when an *in vitro* drug evaluation is demonstrated to be closely related to an *in vivo* drug response.

INTRODUCTION

In vitro dissolution often correlates with and may be predictive of *in vivo* absorption of drugs from both immediate release and sustained release oral formulations. The use of *in vitro* dissolution to predict the bioequivalence of formulations that differ with respect to *in vitro* dissolution is valid if the mathematical relationship between *in vitro* release rate and *in vivo* absorption rate is known. In this research, two sustained release KCl formulations (generic sustained release, Biocraft Laboratories, Inc., Patterson, NJ and Micro-K, A. H. Robbins, Richmond, VA) were compared with respect to *in vivo* urinary excretion rate of K^+ and *in vitro* release rate of K^+. Comparison of pharmacokinetic parameters (Cumulative urinary excretion of K^+ in 24 hours, time to peak urinary excretion rate, and peak urinary excretion rate), calculated for each formulation, indicate no significant difference ($p<0.1$) in bioavailability of the two formulations. The products are also bioequivalent if one defines bioequivalence as less then 20% difference in each of the three parameters identified above. Under currently acceptable statistical comparisons, a 20% difference is the maximum difference allowed and a smaller difference may be required for products to be bioequivalent when there is a small variance associated with the parameters of interest. *In vitro* cumulative release rates up to 8 hours are statistically significantly different for the two products, however. The observation that formulations which differ statistically significantly with respect to *in vitro* release rate are bioequivalent suggests that similar formulations, which describe a range of *in vitro* dissolution profiles, may also be bioequivalent. A range of dissolution profiles, associated with expected bioequivalence, may be used to guide development of new formulations or as a basis for making quality control decisions.

Formulations which differ significantly with respect to *in vitro* dissolution profiles can be evaluated for bioavailability in test subjects. Such bioavailability studies are expensive and time consuming, and extensive evaluation of many similar formulations in human subjects is not reasonable. Alternatively, *in vivo* response (plasma concentration vs time, urinary excretion rate vs. time, or physiological effect vs. time) may be related mathematically to *in vitro* dissolution rates. In this study the mathematical relationship identified is used to simulate an expected *in vivo* response after administration of a formulation that releases drug at rates different from the reference formulation *in vitro.* Expected bioavailability parameters associated with simulated data are compared to the reference formulation, and a range of *in vitro* dissolution profiles associated with bioequivalence can be described. The validity of predictions made by the mathematical model is dependent on several assumptions:

1) Rate of absorption of drug from the gastrointestinal tract is dosage form limited, i.e. dependent upon release from the product.

2) Changes in rate of absorption from the gastrointestinal tract are associated with a concomitant change in *in vivo* response. In the case of K^+, a concomitant increase in urinary excretion rate is assumed to occur in response to increased absorption of K^+ from the gastrointestinal tract.

3) An apperent whole body elimination rate of drug, after absorption of drug from the gastrointestinal tract is complete, is estimable.

4) The pharmacokinetics of the drug are linear, i.e. $C(t) = \sum_{i=1}^{n} A_i e^{-k_i t}$ and that the property of superposition is applicable, and that the concentration of drug in plasma is linearly related to the observed *in vivo* response (mean urinary excretion rate).

The application of these assumptions to an observed *in vivo* response measured over time provides a basis for comparison of *in vitro* dissolution to *in vivo* absorption.

The mathematical relationship used herein to compare *in vitro* dissolution to *in vivo* absorption is:

Equation 1 $$F_{vr}(t) = \frac{[\text{Cumulative \% absorbed, in vivo(t)}]}{[\text{Cumulative \% released, in vitro(t)}]}$$

Where $F_{VR}(t)$ is the estimated fraction of drug absorbed *in vivo* at a given time relative to drug dissolved *in vitro* at the same time. Known mean urinary excretion rate data from two sustained release KCl products were used to estimate the rate of K^+ absorption *in vivo*, and % K^+ absorbed *in vivo*. The fraction of drug absorbed *in vivo* at a given time relative to drug dissolved *in vitro* at the same time function ($F_{VR}(t)$) can then be used to simulate cumulative % absorbed, *in vivo*. This relationship is described by the following equation:

Equation 2 $$\begin{bmatrix}\text{Simulated cumulative \%} \\ \text{absorbed in vivo(t)}\end{bmatrix} = F_{vr}(t) * \begin{bmatrix}\text{Simulated \% drug} \\ \text{released in vitro(t)}\end{bmatrix}$$

The simulated rate of drug absorption can then be used to estimate the expected *in vivo* response. Bioavailability parameters expected after administration of dosage forms that have different *in vitro* dissolution rates also can be estimated and compared for bioequivalence to the reference formulation (Micro-K). The relationships expressed in the preceding equations are simple, however description of *in vivo* absorption processes are mathematically complex. The

functionally complex description of *in vivo* absorption imposes an important limitation on how *in vitro* dissolution rate must be described.

An analytical deconvolution method (1) was applied to estimate rate of *in vivo* absorption of K^+ from the gastrointestinal tract from mean urinary excretion rate data. *In vivo* absorption rate determined by deconvolution is mathematically complex and continuous, hence the relationship between *in vitro* dissolution and *in vivo* absorption will also be continuous and complex. The mathematical properties of the *in vivo* absorption function, complexity and continuity, impose the requirement that *in vitro* dissolution data must be described by a continuous function. Thus, after utilizing deconvolution to obtain an *in vivo* absorption function, then the Weibull model, an empirical relationship that has been used to describe cumulative % release *in vitro*, (2,3,4) was selected in this research as a useful approach to approximate *in vitro* dissolution as a continuous function:

Equation 3
$$F_t = F_\infty \left(1 - e^{-\left(\frac{t}{\tau}\right)^\beta} \right)$$

Where F_t is the cumulative release in %, F_∞ is the cumulative % release at time infinity, τ is a time scaling parameter, and β is a parameter associated with curvature of the release rate profile. It is anticipated that other models which closely approximate dissolution data would be equally useful. In fact, a simple zero-order dissolution model is also evaluated herein to define extreme linear release boundaries of drug release patterns (See results and discussion for full description.). A great many stochastic and empirical models have been proposed and used to describe dissolution data. Unfortunately, none has been found

which consistently describes drug release from dosage forms, especially sustained release products. The Weibull model given in equation 3 may be used both to 'fit' experimental data and to simulate dissolution data.

Simulated *in vitro* dissolution rate may be applied through equation 2 to produce a simulated rate of *in vivo* absorption. From the simulated absorption rate, a corresponding mean urinary excretion over time profile may be generated. Simulated urinary excretion rate profiles may be compared to the reference formulation for bioequivalence. A range of simulated dissolution profiles associated with expected bioequivalence may be used as a guide for dosage form development and/or quality assurance standards.

MATERIALS AND METHODS

Bioavailability study

The bioavailability study was designed to determine the rate, extent and variability of absorption and urinary excretion of two potassium chloride formulations (generic sustained release, Biocraft Laboratories, Inc., Patterson, NJ and Micro-K, A. H. Robbins, Richmond, VA) following randomized, equivalent, repeated doses of 8 sustained release 10 mEq potassium chloride capsules. The design was open, non-blinded, random, repeated-dose, two-way cross over design carried out from August 15 to August 30, 1989, at the bioavailability center of Ian W. French and Associates Limited, Scarborough, Ontario, Canada. 27 normal, healthy, adult male volunteers, who provided informed consent, were institutionalized and placed on a standard diet containing 100 mEq potassium, 340 mEq sodium, 3700 calories and 5200 ml fluid per day. All subjects were maintained on this diet during the period of the study. Each subject was randomly assigned to receive either Generic sustained release or Micro-K formulation (8, 10 mEq capsules at 7 AM with 400 ml of water) and after a two day period of washout each subject was administered the formulation they did not receive initially. After a seven day washout, the study was repeated, except that subjects received the reverse order of formulations. Urine was collected for a period of 48 hours post dosing and assayed for potassium by atomic absorption (IWF Research Laboratories, Mississauga, Ontario, Canada). Baseline urinary excretion of potassium was determined during a two day period, three days after institutionalization. Only mean data, corrected for baseline, from the first series of drug administration was used in subsequent data analysis presented herein.

In vitro dissolution

In vitro dissolution (USP XXI, basket method) was performed on 12 capsules per study lot. Dissolution media consisted of 900 ml of simulated gastric fluid (without enzymes) for hours 0-1, then 900 ml of a modified simulated intestinal fluid (without enzymes and a phosphate buffer not containing potassium) for hours 1-8, all at 37 C^0. Samples were collected for assay of potassium at 1, 4, and 8 hours.

Data Analysis

Steps taken in the analysis fall into three broad categories: 1) Estimation of observed *in vivo* absorption rate (deconvolution) and estimation of parameters associated with observed *in vitro* dissolution data i.e. Weibull parameters. 2) Simulation of *in vitro* dissolution profiles and urinary excretion rates. 3) Comparison of simulated urinary excretion rate vs. time curves for bioequivalence.

Parameters associated with the Weibull model of cumulative % release *in vitro* were estimated using procedure NLIN of SAS (Statistical Analysis Software, Cary, NC). Analytical deconvolution of observed mean urinary excretion rate was performed with PCDCON (Dr. William Gillespie, University of Texas at Austin) on an IBM compatible PC. A program was written to perform all simulation steps and calculate bioavailability parameters. The program was written in THINK PASCAL 4.0 (Macintosh).

Estimation

1) **Estimation of apparent whole body elimination rate, post absorption.** A linear regression estimate of slope of ln of observed mean urinary excretion rate at 7, 10, 14 and 20 hours was made for each product. The 7

hour time point was included as absorption is over 90% complete at 7 hours (shown in results section). Rate of change in urinary excretion rate, post absorption is expressed as:

$$\text{Urinary excretion rate}(t) = A * e^{-k_{el}t}$$

where K_{el} is the apparent whole body elimination rate constant estimated by linear regression. A is a term estimated by the regression for t=7 hours.

2) **Deconvolution of observed mean urinary excretion rate.** Deconvolution analysis requires the apparent whole body elimination rate constant obtained above and observed mean urinary excretion rate data. The deconvolution analysis produces a function that describes cumulative urinary excretion rate over time. Cumulative mean urinary excretion rate has no physiological meaning, however this function is assumed to be linearly related to cumulative amount of K^+ absorbed over time.

3) **Normalization of cumulative mean urinary excretion rate to 100% at maximum cumulative mean urinary excretion rate.** Normalization to 100% results from dividing each cumulative excretion rate by the maximum cumulative urinary excretion rate calculated in step two and enables expression of the relationship in equation 1:

$$F_{vr}(t) = \frac{[\text{Cumulative \% absorbed, in vivo(t)}]}{[\text{Cumulative \% released, in vitro(t)}]}$$

Recall that cumulative urinary excretion rate is directly related to cumulative amount of K^+ absorbed from the gastrointestinal tract.

4) **Estimation of Weibull model parameters associated with observed *in vitro* cumulative release % (equation 3).** The variables β and τ are obtained using SAS procedure NLIN (Statistical Analysis Software, Cary, NC) to obtain the best fit of known dissolution data to the equation:

$$F_t = F_\infty \left(1 - e^{-\left(\frac{t}{\tau}\right)^\beta} \right)$$

5) **Computation of elements of equation 1:**

$$F_{vr}(t) = \frac{[\text{Cumulative \% absorbed, in vivo(t)}]}{[\text{Cumulative \% released, in vitro(t)}]}$$

This equation uses the results of steps 3) and 4) above applied to data from the known dissolution and bioavailability study.

6) **Simulation of *in vitro* dissolution profile using equation 3.** This is accomplished by varying parameters β, τ, and F_∞ within limits described in the text.

7) **Simulation of *in vivo* absorption rate.** This step utilizes the results of step 5 (relationship between *in vivo* absorption and *in vitro* dissolution) and step 6 (*in vitro* dissolution simulation by variation of Weibull parameters) and applying these results through equation 2:

$$\begin{bmatrix}\text{Simulated cumulative \%}\\ \text{absorbed in vivo(t)}\end{bmatrix} = F_{vr}(t) * \begin{bmatrix}\text{Simulated \% drug}\\ \text{released in vitro(t)}\end{bmatrix}$$

Recall that change in rate of absorption of K+ from the gastrointestinal tract is directly related to change in rate of urinary excretion. Thus, cumulative % excretion rate, a term that arises from deconvolution of urinary excretion rate data and has no physiological meaning, is directly related to cumulative % absorbed *in vivo*.

8) **Simulation of mean urinary excretion rate over time profile.** The estimated *in vivo* drug absorption rate, from step 7, is approximated by assuming a series of sequential 0 order input functions every 30 minutes (results from assumptions inherent in the complex mathematics of deconvolution). The apparent whole body elimination rate estimated in step 1 is used in this model. For time during which an input i occurs, change in urinary excretion rate is given by equation 5 (5):

Equation 5

$$U_i(t) = \left(\frac{k_i}{k_{el}}\right) * \left(1 - e^{-k_{el}\left(t - t_{begin,i}\right)}\right)$$

Where k_i is the input rate, $t_{begin,i}$ is the time that the ith input begins, and k_{el} is the apparent whole body elimination rate constant determined in step 1). After the ith infusion has ended, change in urinary excretion rate is expressed as equation 6 (5):

Equation 6

$$U_i(t) = \left(\frac{k_i}{k_{el}}\right) * \left(1 - e^{-k_{el}\left(t - \left(t_{end,i} - t_{begin,i}\right)\right)}\right) * e^{-k_{el}\left(t - t_{begin,i}\right)}$$

Where $t_{end,i}$ is the time that the ith infusion ends.

The contribution of all infusions are summed. Thus the simulated urinary excretion rate at time t is:

Equation 7

$$SimUrate(t) = \sum_{i=1}^{n} U_i(t)$$

The simulation software allows input to occur for up to 12 hours. If the rate of change in simulated *in vivo* absorption becomes very small (.005% of previous input) or negative, the input rate is set to 0. This means that the simulated cumulative urinary excretion rate profile produced in step 7) is approximated by 23 sequential, 0 order inputs from the gastrointestinal tract for these products.

Bioavailability parameters associated with the simulated mean urinary excretion rate vs time profile are calculated and compared with the bioequivalence intervals associated with the reference formulation (Micro-K).

RESULTS AND DISCUSSION

Table 1 shows mean cumulative urinary excretion of K^+ in the urine following administration of 80 mEq of generic sustained release product, and mean cumulative excretion of K^+ following administration of 80 mEq of Micro-K, to 27 subjects in a cross-over study design. Only the first administration is considered for analysis. Mean values in table 1 are corrected for baseline excretion of K^+ in the urine and show that most excretion of K^+ absorbed occurs within 24 hours, but that some excretion from the dosage forms occurs during the period of 24 to 48 hours. Table 2 shows mean urinary excretion rates for the period of 24 hours. Mean urinary excretion rates after 24 hours were small and varied in a random fashion, and were not included in subsequent analysis. FDA, Division of Bioequivalence guidelines for slow release KCl products recommends comparison of cumulative urinary excretion from 0 through 24 hours. Table 3 describes the statistical confidence intervals ($p<0.1$) associated with bioavailability parameters of Micro-K while Table 4 shows upper and lower bounds associated with bioequivalence to Micro-K, assuming $\pm$ 20% variation in each parameter is acceptable.

The peak urinary excretion rate obtained from averaging the excretion rates at each time for the Micro-K product (see table 2 and figure 5 , 5.38 mEq/hr. at 7 hours) was used as a bioequivalence reference value for this parameter. Note that this value is significantly less than the value obtained if mean of individuals peak values are used (generic sustained release peak rate 7.56 mEq/hr., Micro-K peak rate 7.82 mEq/hr., Table 5). This difference is expected since the former method of averaging individual time point data results in attenuation of the data relative to averaging the peak values for each individual. Table 5 shows that peak excretion rates for most individuals occur at 5 or 7 hours

Table III.1 Cumulative urinary excretion of K^+. In mEq following 80 mEq dose, first administration. Standard deviations in parenthesis.

Time (hours)	Generic SR	Micro-K
1	0.31 (1.82)	-0.31 (1.62)
2	1.60 (2.37)	1.29 (3.10)
4	8.58 (4.91)	8.15 (3.27)
6	18.66 (7.22)	18.78 (7.44)
8	31.24 (10.32)	29.53 (9.48)
12	46.49 (10.93)	46.14 (11.48)
16	53.26 (10.75)	52.93 (11.87)
24	56.55 (11.35)	55.34 (11.48)
36	64.40 (18.25)	63.57 (18.72)
48	65.55 (22.38)	63.95 (20.06)

Table III.2 Mean urinary excretion rate (mEq/hr.) at midpoint of collection interval[a]. Standard error[b] in parenthesis.

Time (hours)[a]	Generic SR	Micro-K
0.5	0.31 (1.8)	0 (1.62)
1.5	1.29 (2.1)	1.29 (2.2)
3	3.49 (1.4)	3.43 (1.3)
5	5.04 (1.7)	5.32 (1.6)
7	6.29 (2.1)	5.38 (2.0)
10	3.81 (1.1)	4.15 (1.1)
14	1.69 (1.2)	1.70 (1.2)
20	0.41 (0.6)	0.30 (0.6)

[a] Time in hours represents the midpoint of time between collection intervals (see table 1).

Mean urinary excretion rate(t)=[Cumulative(t_{i+1})-Cumulative(t_i)]/(hours in interval).

[b] Standard error for table 2 is the standard deviation for difference of mean cumulative urinary excretion reported in table 1 divided by the number of hours in the cumulative interval i.e. SEM = $\left(\sqrt{\left(s_i^2 + s_{i+1}^2\right)}\right)$ / (Hours in interval)

Table III.3 Bioavailability parameters (lower and upper bounds of confidence interval) and associated statistics.

Parameter	Micro-K mean	Generic SR mean	SD	lower bound[a]	upper bound[a]
Time to peak	6.74	6.22	2.35	5.65	7.83
Peak rate	5.38	6.29	2.58	4.16	6.60
Cumulative excreted 24 hr.	55.34	56.55	11.48	50.01	60.67

[a] Confidence intervals based on. n=27, with Micro-K data as the 'standard'. Upper, Lower bound = mean $\pm$ $\left[1.70 * \left(SD * \sqrt{2/n}\right)\right]$, where 1.70 is critical t value for a two-sided student t test where p<.1, df=26.

Table III.4 Bioavailability parameters and statistics associated with bioequivalence (lower and upper bounds of confidence interval), assuming ±20% variation is allowed in each parameter and Micro-K is the 'standard'.

Parameter	Micro-K mean	Generic SR mean	lower bound	upper bound
Time to peak	6.74	6.22	5.39	8.09
Peak rate	5.38	6.29	4.30	6.46
Cumulative excreted 24 hr.	55.34	56.55	44.27	66.41

Table III.5 Mean peak urinary excretion rate of K^+, of individuals.

Number of individuals that peaked at that time given in parenthesis.

Time of peak excretion rate (hour)	Generic SR, mean peak excretion rate (mEq/hr.)	MicroK, mean peak excretion rate (mEq/hr.)
3	5.01 (3)	7.83 (1)
5	7.81 (8)	7.81 (11)
7	8.04 (15)	8.54 (10)
10	----- (0)	7.24 (4)
14	5.85 (1)	3.67 (1)

for both formulations and that these values are significantly higher then the mean average values reported in table 2. Cumulative urinary excretion of K^+ in 24 hours and time to peak urinary excretion rate reference values are the means of individuals observed values for these parameters.

Figure 1 shows the *in vitro* dissolution profiles of Micro-K and generic sustained release products evaluated in the bioavailability study. The dissolution profiles differ significantly ($p<0.0001$) at 1 hour and 4 hours, and are similar at time points 2 and 8 hours. Weibull model estimates of individual *in vitro* dissolution data points are within 1 standard deviation (6 replicates for dissolution) at all times. The Weibull model predicts no significant release of K^+ occurs from the generic sustained release product after 8 hours, but Micro-K is predicted to continue to release K^+ at a significant rate for nearly 12 hours. Although the Weibull model prediction can be useful, it is important to point out here that the model is correct in estimating total K^+ release from the generic sustained release product at 8 hours but underestimates the actual amount dissolved at time infinity. The remaining 14% of K^+ will be released after 8 hours of dissolution, and the error in estimation results from the curvature assigned by the model in order to closely fit the very few (4) data points. Unfortunately there are no dissolution data beyond 8 hours, and only one point between 2 and 8 hours. Dissolution does continue to 100% release for the generic sustained release product since cumulative K^+ excretion essentially equals the Micro-K product at both 24 and 48 hours (Table 1).

Estimation of rate of absorption *in vivo* requires that apparent whole body elimination rate is estimable. Since subjects dietary regimen includes an amount of potassium sufficient to meet the bodies needs, the potassium absorbed from the sustained release dosage forms is rapidly eliminated. Thus, the apparent whole body elimination rate referred to herein is the rate at which

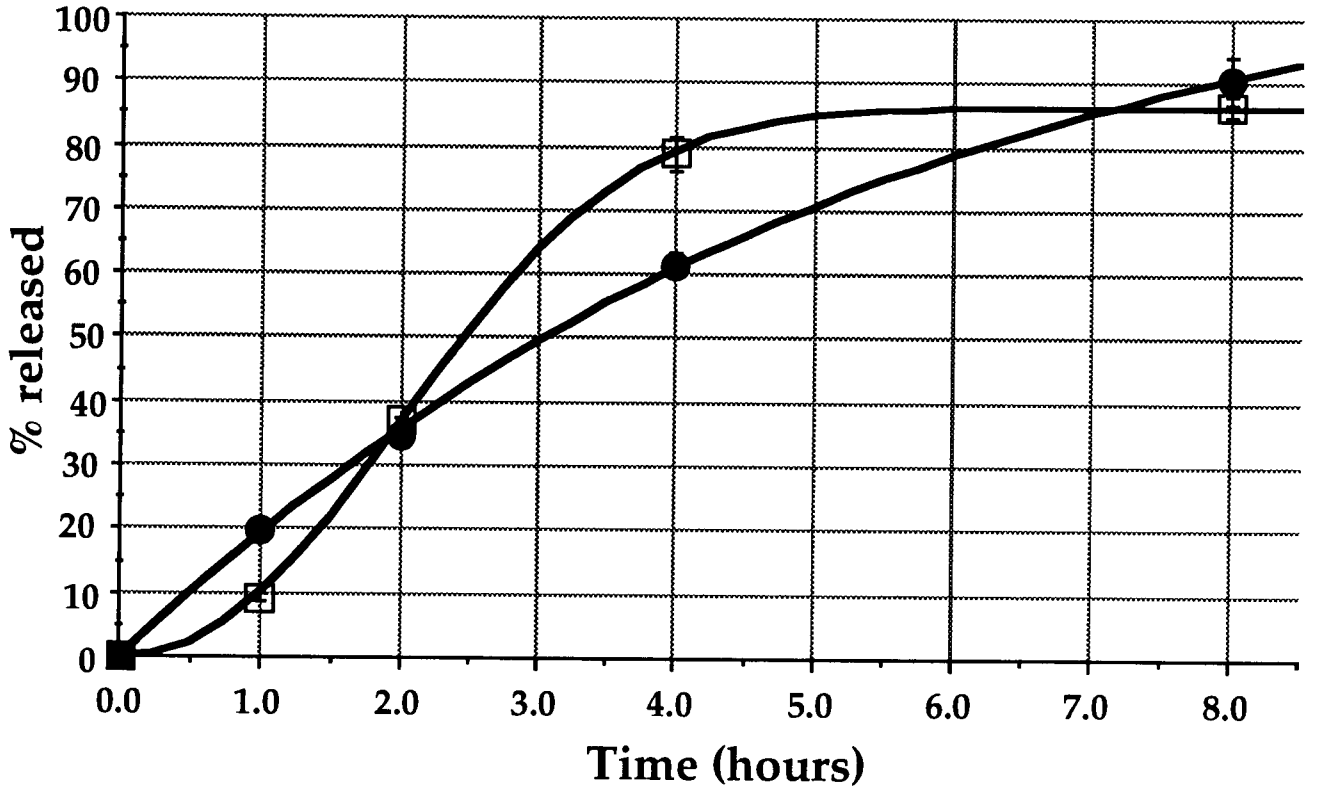

Figure III.1 Observed *in vitro* dissolution profile vs Weibull model fit (solid lines). Micro-K solid circles, Generic SR, empty boxes. Standard deviations of 6 replications shown, but are smaller then symbol for most observations

potassium is eliminated by the body when dietary intake is sufficient to maintain whole body stores at equilibrium and additional potassium has been administered in the sustained release dosage form. The slope associated with a least squares fit of log transformed mean urinary excretion rate versus time data at 7, 10, 14 and 20 (Table 2) hours was used to estimate the apparent whole body elimination rate constant (Kel) post absorption (hereafter called elimination rate constant, Kel). An elimination rate of .2125 hr^{-1} and .2283 hr^{-1} were calculated for generic sustained release and Micro-K respectively and are in good agreement with values reported in other studies (6,7). The relatively similar estimates for whole body elimination rate constants are expected since elimination rate should not depend on which formulation is administered. However, the estimate of *in vivo* percent absorbed, provided by analytical deconvolution, indicates that 15% and 8% of the absorbed dose will be absorbed during 7 though 11 hours post dosing with Micro-K and generic sustained release formulations respectively. Thus, estimates of whole body elimination rate, based in part on observed mean urinary excretion rates at 7 and 10 hours, are somewhat confounded by concomitant absorption of potassium from the gastrointestinal tract. An alternative estimate of whole body elimination rate, for each formulation, using mean urinary excretion rate at 10,14 and 20 hours for estimation, provided whole body elimination rate constant estimates that differ by 15% (0.2238 hr^{-1} vs 0.2625 hr^{-1} for generic sustained release and Micro-K respectively). Differences in estimated Kel of K^+, in previous studies using a crossover design, may vary as much as reported here (6), however when a large number of post absorption time points are available, estimates of Kel for different treatments are similar (7). Thus, despite the lack of confounding by concomitant absorption during the period of 10 through 20 hours, whole body elimination rate constant estimates based on this period may be less reliable then

estimates based on the period of 7 though 20 hours since the statistical uncertainties associated with observed low mean urinary excretion at 14 and 20 hours may be large and may provide misleading estimates of whole body elimination rate constant. As an alternative, a reference value for Kel from the literature could be applied to analysis of both generic sustained release and Micro-K mean urinary excretion data. This may be as meaningful as estimated Kel's. However, since Kel varies with dietary regimen, use of a literature value of Kel to be representative of the bioavailability study examined here is also uncertain. For these reasons, whole body elimination rate constant was estimated from the last four observed mean urinary excretion rates.

Figure 2 is the outcome of deconvolution analysis, using the whole body elimination rate constants chosen above, which mathematically converts the mean urinary excretion rate vs time data into a cumulative area over time relationship. The y-axis units in figure 2 are real mathematically but the labeled values have no directly useful physiological meaning. Figure 2 shows the "cumulative mean urinary excretion rate" (y axis) required to produce the experimentally observed urinary excretion rates. Based on deconvolution assumptions, cumulative mean urinary excretion rate is directly related to cumulative amount of K^+ absorbed. Figure 2 shows that K^+ absorption from the gastrointestinal tract for the generic sustained release product remains nearly constant for 8 hours, and then plateaus, i.e. the cumulative area for mean urinary excretion rate (y-axis) reaches an asymptote. Input from Micro-K is nearly constant from 1 to 4 hours and then slows gradually until absorption ends at 11 hours.

Deconvolution results shown in figure 2 were normalized so that at some time, all absorption of K^+ which is bioavailable, is 100% complete. This does not require that bioavailability will be 100%, but only that there is a time at which all

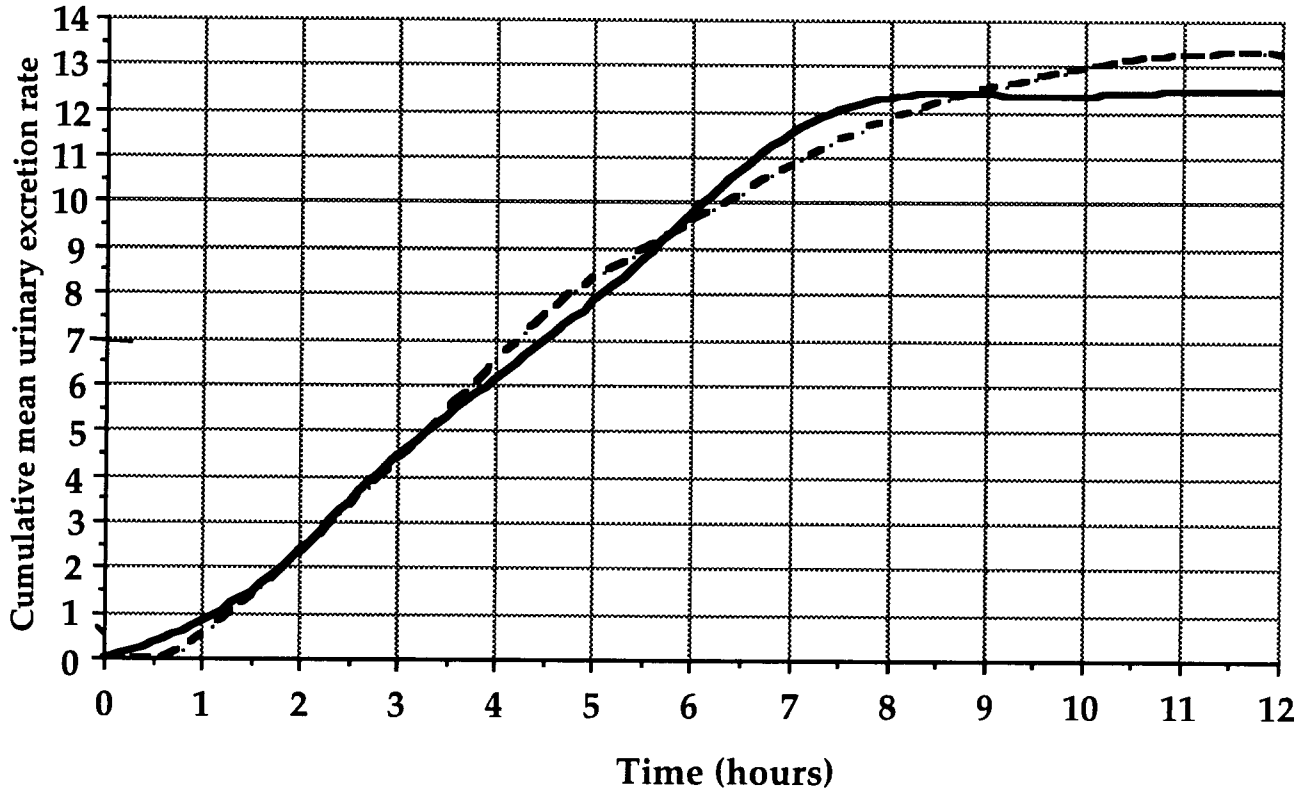

Figure III.2 "Cumulative urinary excretion rate in mEq/hr.", result of deconvolution. Dotted line Micro-K, solid line Generic SR.

drug that will be absorbed has been absorbed, and no further absorption occurs after that time. 100% completion of absorption occurs at 8 and 11 hours and has values of 12.4 and 13.3 mEq/hr. for generic sustained release and Micro-K formulations respectively. The normalized percent cumulative urinary excretion rate can be equated to the percent drug absorbed *in vivo* , and then be compared directly with observed *in vitro* cumulative percent release (figure 3).

Figure 3 shows that amount of K^+ released *in vitro* is greater then absorption of K^+ *in vivo* at the same time points for all times except 8 hours when the amount released *in vitro* is approximately equal to the amount absorbed *in vivo*. The heavy solid line describes the relationship that would be observed if *in vitro* dissolution rate exactly predicted *in vivo* absorption rate. The relationship between *in vitro* dissolution and *in vivo* absorption is continuously described by application of equation 2. This result can be seen in figure 4.

Figure 4 shows that for the generic sustained release product, *in vitro* dissolution cumulative % release closely approximates the *in vivo* % absorption at 0.5 hours (value of fraction=1.2), then declines steadily to a value of ~0.5 at 2 hours and then increases gradually to assume a final value of 1.15 at 8 hours. By contrast, the *in vitro-in vivo* relationship associated with the Micro-K product begins at 0 and increases steadily reaching an asymptote of ~1.0 at 8 hours. Figure 4 shows for example, that for the generic sustained release product, *in vivo* absorption at 2 hours is predicted to be 52% of the *in vitro* dissolution at 2 hours. For the generic sustained release formulation during the period prior to 0.5 hours, the fraction described in equation 2 is very large. These values are artifacts that arise from the division of a relatively large number (% absorbed as determined by deconvolution) by a relatively small number (% release described by the Weibull dissolution model). These large values were replaced by a simple linear interpolation of the value from 0 to 0.5 hours. The relationships depicted

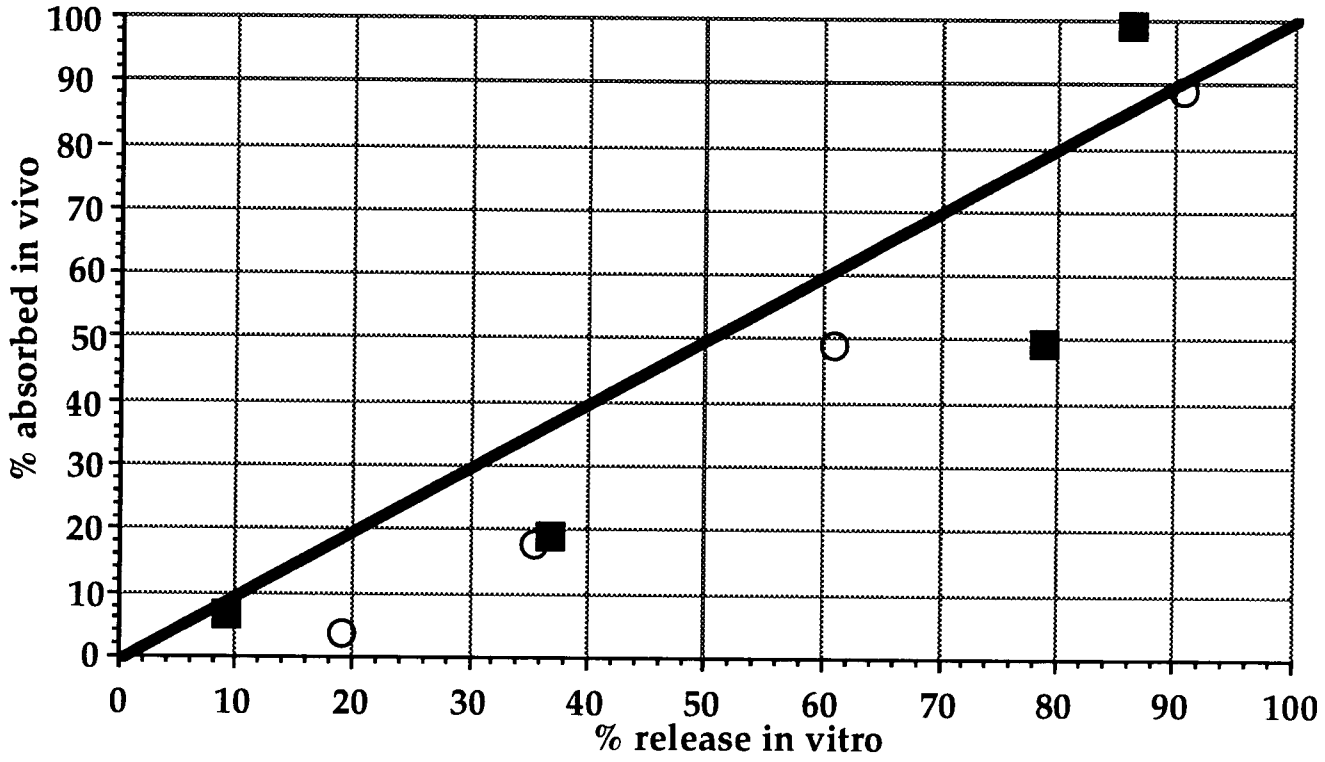

Figure III.3 *In vivo* % absorbed vs *In vitro* cumulative release (as estimated by mean urinary excretion rate), at the same time point. Solid squares are Generic SR formulation, open circles are Micro-K. Solid line represents perfect relationship between *in vitro* % cumulative release and *in vivo* % absorption.

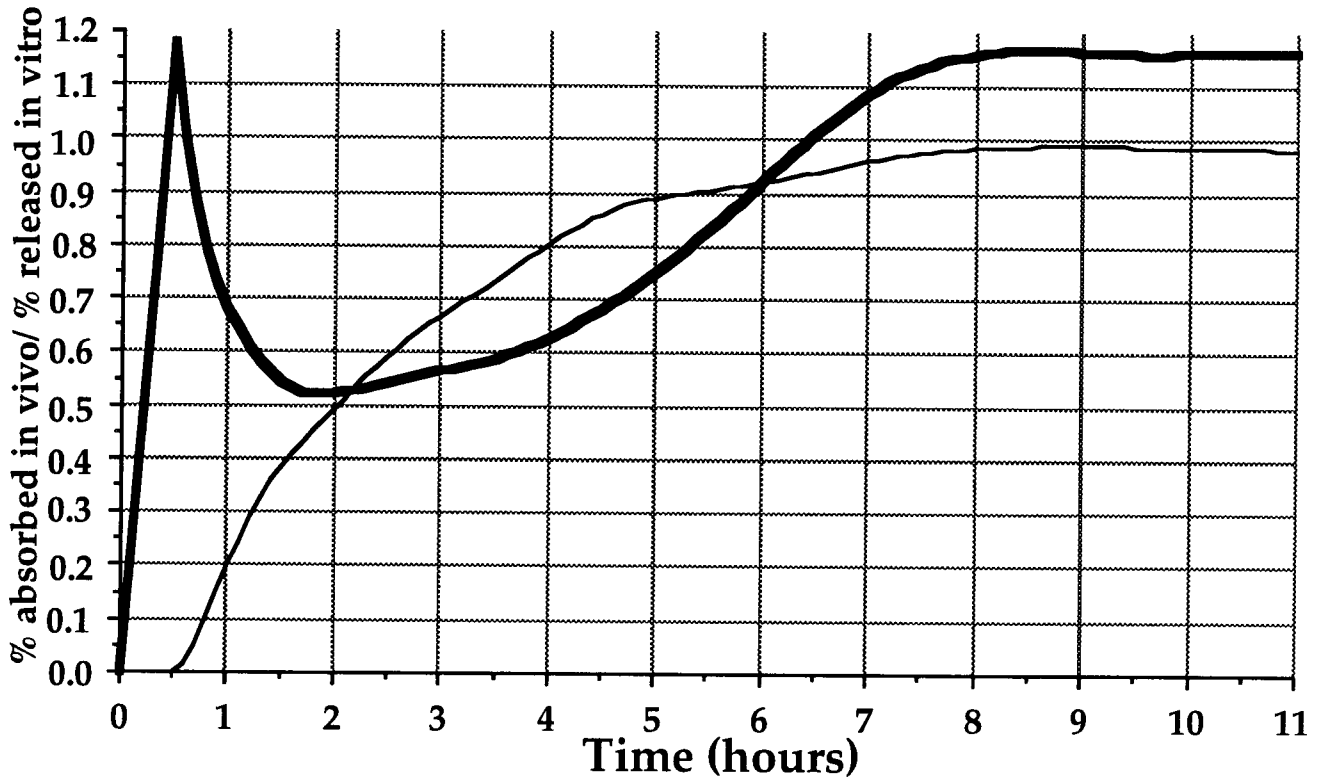

Figure III.4 Percent absorbed *in vivo*/% released *in vitro* vs time. Thin line Micro-K, thick line Generic SR.

in figure 4 and mathematically described by equation 2 may now be used to simulate *in vivo* absorption rates from simulated dissolution profiles. The inherent assumption, of course, is that while different lots of product from the same manufacturer may have different dissolution profiles, the *in vivo-in vitro* relationship is still described by the function plotted in figure 4. These absorption rates are then used to simulate mean urinary excretion rate vs time profiles.

The software developed generates a simulated mean urinary excretion function (excretion rate vs time profile) that describes the expected mean urinary excretion rate at intervals of 6 minutes for a period of 24 hours. The program also records the peak urinary excretion rate and time to peak rate. Cumulative urinary excretion rate was computed as the area under the excretion rate vs. time profile calculated as the sum of adjacent simulated urinary excretion rates divided by two and multiplied by 6 minutes for the entire 24 hour period (trapezoidal rule). Prior to application of the software to simulated *in vitro* dissolution profiles, the precision of the model was tested by comparing known dissolution data and simulated urinary excretion rates with observed urinary excretion rate data from the bioavailability study. The results of these applications are seen in figure 5.

Figure 5 shows that the deconvolution method coupled with the Weibull parameters determined by fitting the dissolution data and the newly developed simulation software very closely describes the observed mean urinary excretion rate data. Small differences between simulated and observed data during the period of 7 to 20 hours may reflect errors associated with lack of fit corresponding to the estimation of whole body elimination rate constant. Cumulative urinary excretion of K^+ in 24 hours also closely approximated observed values. For example, predicted total K^+ excreted in 24 hours for the

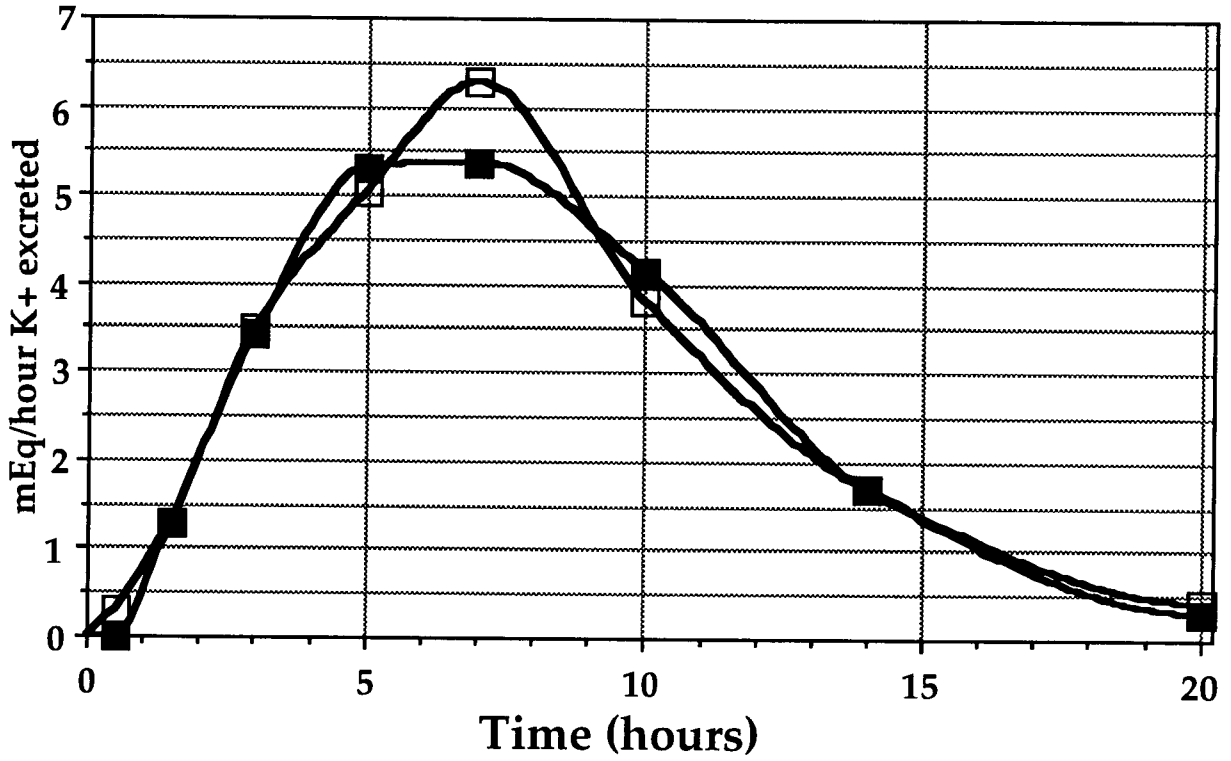

Figure III.5 Observed mean urinary excretion rate for Generic SR (open squares) and Micro-K (solid squares). Simulated urinary excretion rate shown by lines.

generic sustained release product was 56.50 mEq vs observed 56.35 mEq and for Micro-K predicted total K^+ excreted 55.82 mEq vs observed 55.34 mEq (Table 3). These close fits are expected, of course, since the deconvolution-convolution methods were developed using exactly the same *in vitro* dissolution and *in vivo* urinary excretion data as are now shown to produce the lines of good fit in figure 5, i.e., the computer programs and mathematical models are working correctly.

Description of a range of *in vitro* dissolution profiles associated with bioequivalence was achieved by simulating *in vitro* dissolution profiles by varying Weibull parameters β (curvature of *in vitro* dissolution profile) and F_∞ (maximum release at infinite time) and evaluating the associated urinary excretion profiles for bioequivalence by comparison to values in table 4. In addition, whole body elimination rate constant was also varied (.19-.22 hr^{-1}) to evaluate the effect of errors in estimation of whole body elimination rate constant. Approximately 1000 simulations were performed to evaluate the parameter space associated with bioequivalence. Figure 6 shows the extreme limits of dissolution profiles estimated to be associated with bioequivalence with the Micro-K product. The generic sustained release product evaluated for bioavailability falls within limits at all times. The upper and lower limits for acceptable dissolution, at times 1,2,4, and 8 hours for the Weibull dissolution model is found in table 6. Figure 7 shows the expected mean *in vivo* urinary excretion rates which correspond to dissolution profiles shown in figure 5. Note that while the curves in figure 7 are quite different, the extremes differ by less then 20% in all three bioavailability parameters of interest when compared to the values observed.

A linear model to simulate zero order *in vitro* dissolution was also applied. Linear models of in vitro dissolution (i.e. dissolution described by a 0 order process with 100% release) were unable to describe any mean *in vivo* urinary

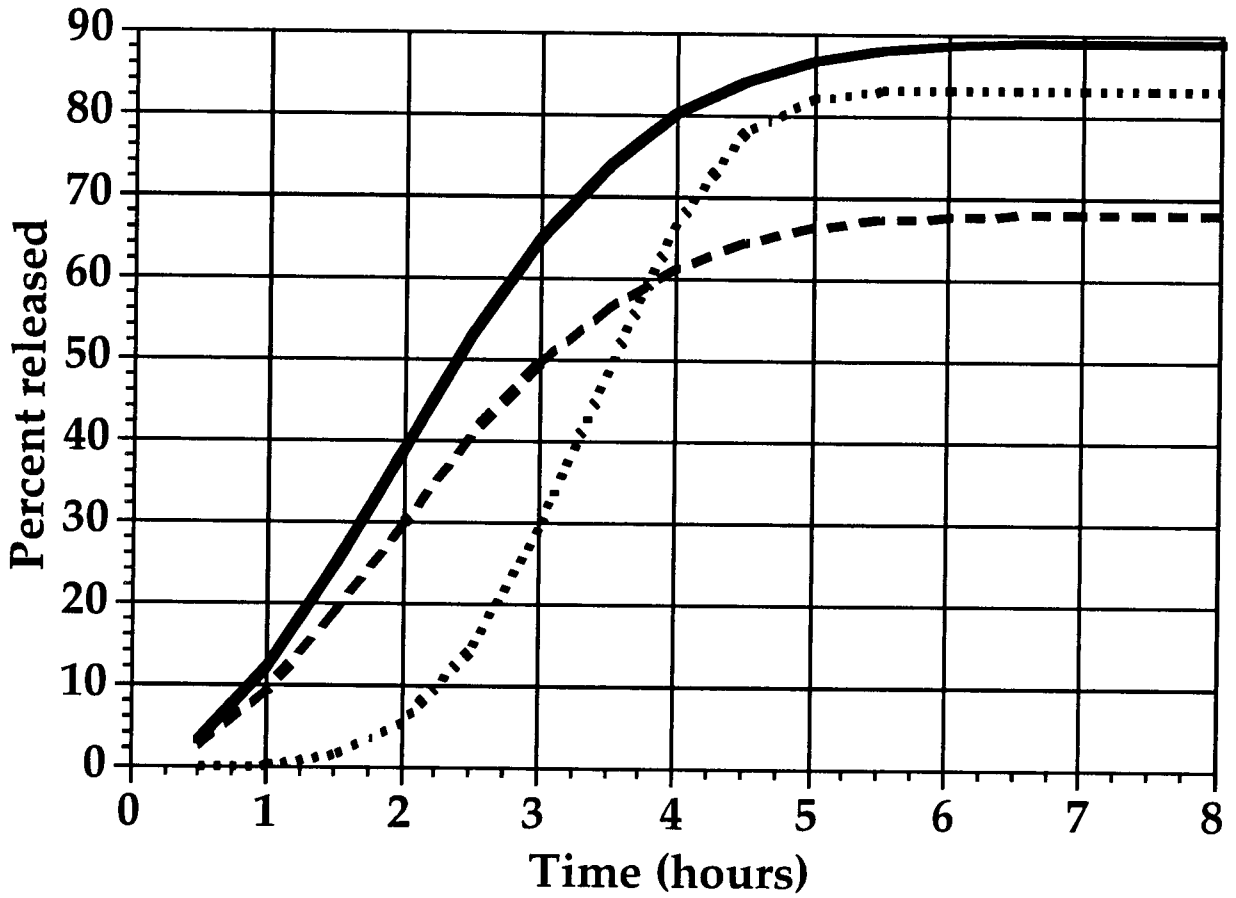

Figure III.6 Weibull ranges for *in vitro* dissolution profiles associated with bioequivalence. Dashed line, lower Weibull model limit (4 and 8 hours) β=2.0, τ=2.6, F_∞ =68; dotted line lower limit (1 and 2 hours) β=4.6, τ=3.6, F_∞ =83; solid line, upper limit (1, 2, 4, and 8 hours), β=2.0, F_∞ =89.

Table III.6 Dissolution ranges associated with bioequivalence, assuming that 20% variation in measured bioavailability parameters is acceptable.

Time (hours)	Weibull models	
	lower	upper
1	0.2	12.0
2	5.4	39.0
4	61.2	80.2
8	68.0	89.0

* Parameters for lower Weibull model limit (4 and 8 hours) β=2.0, τ=2.6, F_∞ =68; lower limit (1 and 2 hours) β=4.6, τ=3.6, F_∞ =83; upper limit (1, 2, 4, and 8 hours), β=2.0, F_∞ =89.

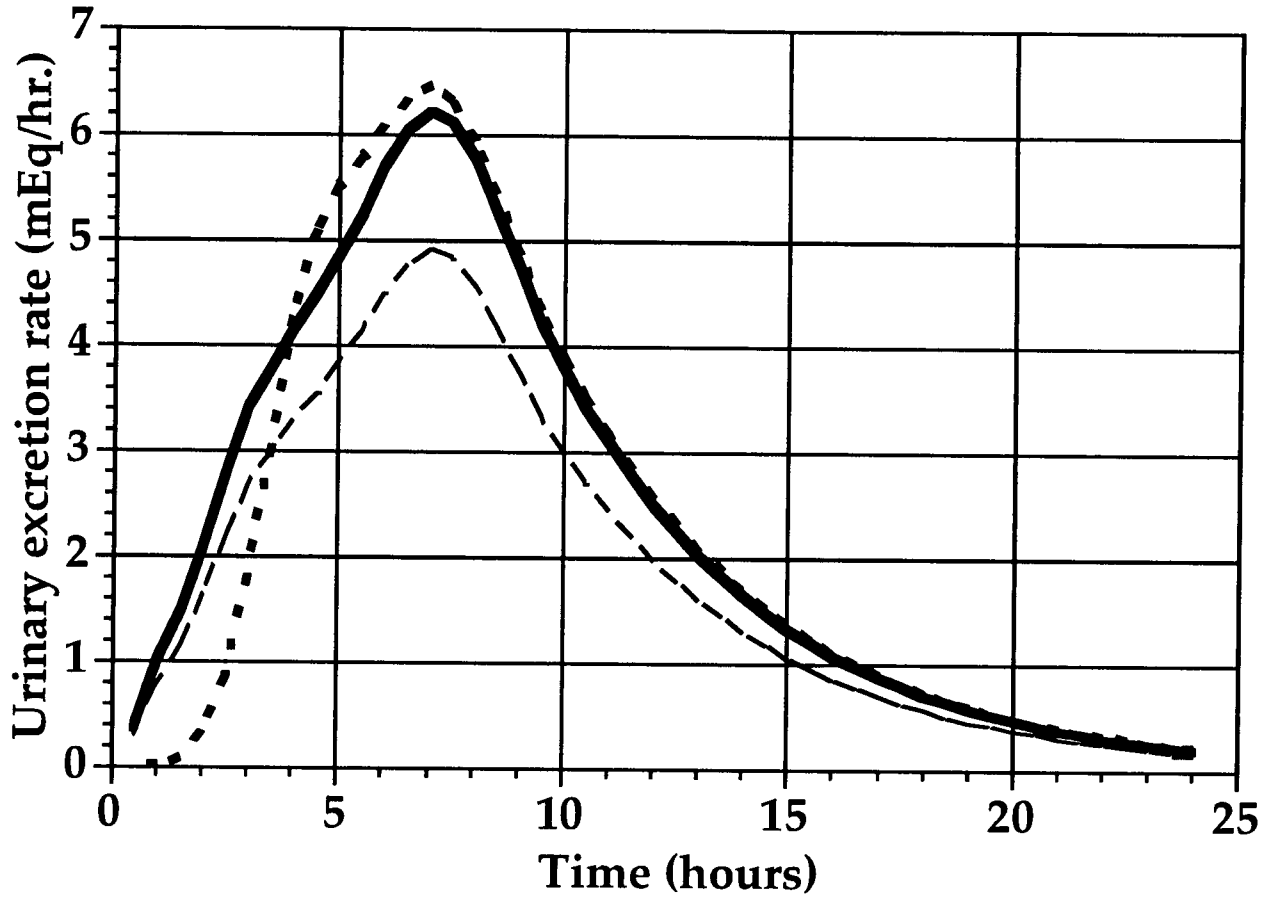

Figure III.7 Estimated urinary excretion rate based on Weibull dissolution models from figure 6. Dashed line, lower Weibull model limit (4 and 8 hours) β=2.0, τ=2.6, F_∞ =68; dotted line lower limit (1 and 2 hours) β=4.6, τ=3.6, F_∞ =83; solid line, upper limit (1, 2, 4, and 8 hours), β=2.0, F_∞ =89.

excretion rate simulations that are associated with bioequivalence to Micro-K. This shows that formulation of a product that produces a zero order release *in vitro* and is also bioequivalent is a difficult task. This is not surprising since the release rate of Micro-K is not zero order. The effect of variation of whole body elimination rate constant was insignificant through the narrow range investigated (.19-.22 hr^{-1}).

The modeling approach used herein is not limited to analysis of K^+ sustained release products, but is applicable to any drug that satisfies model assumptions. For K^+, the assertion that necessary model assumptions are fulfilled is supported by the pharmacokinetics of K^+ as reported in the literature. The assumption that absorption of K^+ is dosage form limited is supported by the observation that administration of oral solutions produces a nearly instantaneous increase in plasma concentration of K^+ (6). Plasma peak concentrations following administration of an oral syrup occur within 30 minutes (8). Sustained release formulations produce rates of absorption so slow that no discernible change in plasma concentration may occur (5,6). Therefore, the assumption that rate of absorption from the gastrointestinal tract is associated with a concomitant change in plasma concentration is apparent with formulations that deliver K^+ rapidly. However, for sustained release formulations of K^+, plasma appears to be a poor indicator of rate or extent of K^+ absorption since the change is small enough not to be statistically different from baseline . Thus, reliable determination of absorption rate from the gastrointestinal tract and bioavailability of K^+ from sustained release dosage forms cannot be made from plasma vs time data. An appropriate alternative to plasma concentration vs time data is urinary excretion rate data.

Use of urinary excretion rate changes to estimate absorption rate requires that a mathematical relationship between changes in absorption rate from the

gastrointestinal tract and urinary excretion rate is known. In our analysis we have assumed that change in rate of absorption of K^+ from the gastrointestinal tract produces a parallel increase in urinary excretion rate (eq. 1). Rabinowitz, et al., (9) reported that during the intravenous infusion of K^+ to ewes, changes in urinary excretion rate paralleled the infusion rate. Three different infusion rates were examined and in each case urinary excretion rate was the same fraction (~0.3) of infusion rate. When high doses of K^+ are administered as oral solutions in man, increase in rate of urinary excretion parallels the increase of K^+ in plasma (10). Unfortunately the rate of IV infusion reported by Rabinowitz et al. is an order of magnitude greater then what has been observed for absorption rate in our research. Thus the assumption of a simple linear relationship between urinary excretion rate during periods of slow absorption of K^+ from a sustained release dosage form must be made with caution.

The relationship between *in vivo* % absorbed and *in vitro* % released is quite different (Figure 4) for the generic sustained release and Micro-K products evaluated in the bioavailability study. Since both formulations were evaluated in the same *in vitro* dissolution media, differences must exist in how each dosage form interacts with luminal contents or surface of the gastrointestinal tract. Specific interactions may include, but are not limited too, response of polymers to change in pH, bile salts or other surface active agents. Shape and size of the dosage form may also effect transit time through the gastrointestinal tract and may contribute to the differences observed in the *in vitro-in vivo* relationship of the two formulations. Because of the difference in the *in vitro-in vivo* relationship of the two formulations, the model described here is best applied to formulations that have similar physical-chemical properties, basic formulations, and processing variables. The use of *in vitro* dissolution media that contains surfactants, or enzymes may identify the source of difference *in vitro-in vivo*

relationship between the two formulations. Other limitations have also been presented in the application of the model.

The model accurately predicts mean cumulative urinary excretion in 24 hours, time to peak urinary excretion rate, and peak urinary excretion rate in rate averaged data but significantly underestimates the average of peak urinary excretion rates of individuals. The model when applied to mean data does not detect this behavior, however the model can also be applied to individuals within the study population. Table 5 shows that peak urinary excretion rates of individuals are much higher then the mean rates given in table 2. Statistical methods may be applied to build a more complex model that would correctly predict the mean of peak excretion rates.

In order to identify sub-populations with peak characteristics attributable solely to absorption rate, the complexity of the result obtained by numerical deconvolution must be reduced so that statistical methods may be applied. Calculation of the first moment of cumulative excretion rate with respect to time (analogous to mean absorption time, MAT, that is applied to plasma vs time data), may reduce the complexity of individual absorption rate data obtained from individuals. This reduction in complexity may provide the means for identification of sub-populations and thereby enable the construction of a model which predicts the high mean peak excretion rate observed in individual data. The use of statistical moments to reduce the complexity of deconvolution estimates of rates of absorption of drugs from the gastrointestinal tract, and the correlation of these moments to bioavailability parameters has been reported (2,11).

Estimation of rate of absorption from the gastrointestinal tract is probably the single greatest source of error present in this analysis. The explicit deconvolution method used here was chosen for several reasons. We assumed

from the outset that absorption rate from the gastrointestinal tract was not likely to be described by a simple first or zero order process. This assumption is consistent with the problems commonly associated with mathematical modeling of the pharmacokinetics of sustained release dosage forms. In fact, modeling mean urinary excretion rate with a single 0 order or first order input was examined prior to use of deconvolution. While a single 0 order input did produce a fair estimate (data not shown), it is our belief that deconvolution produces a more precise estimate of absorption rate and thus is more likely to be useful in generating meaningful simulations.

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