

Evaluation of the Rabbit as an Animal Model for Determining  
the Bioavailability of Sustained-release Chlorpheniramine  
Dosage Forms

by

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THE BIOAVAILABILITY OF SUSTAINED-RELEASE CHLORPHENIRAMINE  
DOSAGE FORMS

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XUEYU CHEN

A thesis submitted to the Faculty of Graduate Studies of  
the University of Manitoba in partial fulfillment of the requirements  
of the degree of

MASTER OF SCIENCE

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## ABSTRACT

Five chlorpheniramine dosage forms: intravenous injection, oral solution, regular tablet and two repeat-action tablets, were given to five rabbits in a five-way crossover study. A HPLC procedure was used to determine the serum concentrations of chlorpheniramine.

The serum concentration versus time curves following the intravenous doses for all five rabbits were best described by biexponential equations with a mean terminal half-life of  $2.15 \pm 0.90$  hr. Absolute bioavailability for oral solution, conventional and two repeat-action tablets were  $15.98 \pm 1.19\%$ ,  $9.60 \pm 6.35\%$ ,  $7.68 \pm 3.02\%$  and  $8.69 \pm 3.50\%$  respectively. Mean absorption time for oral solution was  $0.70 \pm 0.98$  hr, and  $1.25 \pm 0.9$  hr,  $3.20 \pm 1.21$  hr and  $3.64 \pm 2.03$  hr for regular and two repeat-action tablets respectively. The areas under the serum chlorpheniramine concentration versus time curves from time zero to infinity were not significantly different for the oral solution, regular tablet and two repeat-action tablets. Two peaks in the serum concentration versus time plots were observed following administration of the two repeat-action tablets. These results indicate that rabbits can be used as an animal model in the bioavailability study of chlorpheniramine repeat-action dosage forms.

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Chapter I  
INTRODUCTION

1.1. Histamine

1.1.1. Properties of Histamine

Histamine ( $\alpha$ -aminoethylimidazole) is a physiologically active, endogeneous substance that exists in practically all mammalian tissues and body fluids in various concentrations(1,2,3,5). Its chemical structure is shown as follows:

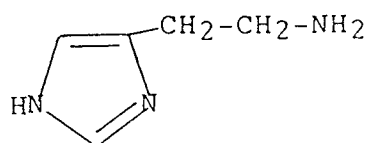


Fig.1 Histamine

Histamine is formed by decarboxylation of the amino acid histidine(2).

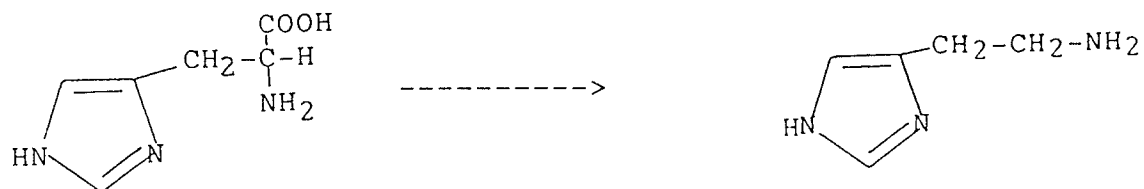


Fig.2 Formation of Histamine from Histidine

Histamine is stored chiefly in the lung, skin, and gastrointestinal mucosa. In these tissues, histamine is present in the mast cells as granules of an inactive histamine-anionic polymer complex. Histamine is released from tissues in free, active form by 1) destruction of cells, e.g. by bee stinging venom, bacterial toxins and injury; 2) dissolution of cytoplasmic granules which is caused by surfactants and radiation; and 3) histamine-liberators, such as drugs(morphine, d-tubocurarine), foreign proteins, etc.

The inactivation of histamine occurs in many tissues by N-methylation or oxidative deamination to form methylhistamine and ImAA(Imidazole Acetic Acid) by enzymes such as histamine-N-methyltransferase, diamine oxidase and histaminase. Methylhistamine and ImAA are further metabolized to form a number of other derivatives.

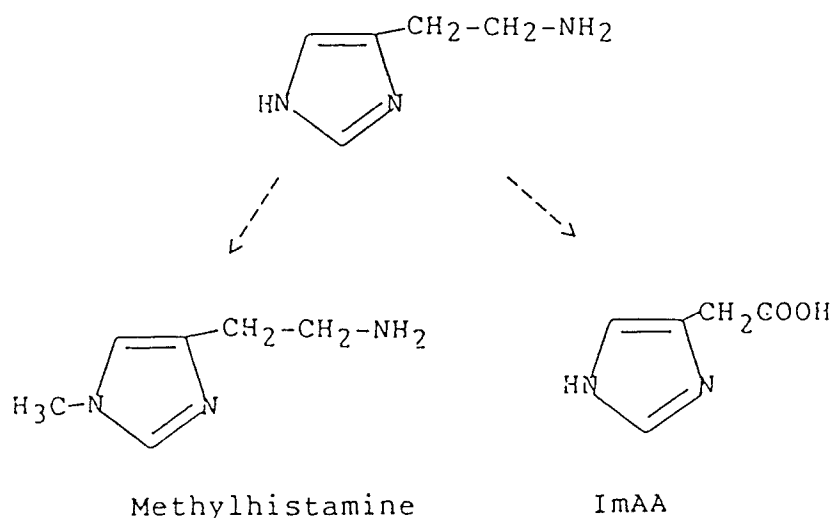


Fig.3 Biotransformation of Histamine

### 1.1.2. Mechanism of Histamine Action

The physiological effects of histamine involve the cardiovascular system, extravascular smooth muscle and exocrine glands(1,2). Histamine can also stimulate some nerve endings and this causes itching. Histamine effects are mediated through specific histamine receptors at various sites in the body: H1 and H2 types(1,2,4,5). Both histamine H1 and H2 receptors are located on the cell surface(2). H1-receptor stimulation will increase the concentration of cyclic AMP, while H2-receptor stimulation will increase the concentration of cyclic GMP in the responding cell(1,2,4,5).

In allergic reactions, IgE antibodies excreted by activated B lymphocytes enter the tissues and bind to receptor proteins on the mast cell and basophil surfaces that specifically recognize the Fc region of these antibodies. Thus, individual mast cells and basophils have cell-surface antibodies with a variety of different antigen binding sites. Antigen molecules cross link the Fab portion of the membrane-bound IgE antibodies, thereby activating the mast cell and basophil to release its histamine by exocytosis.

The interaction of histamine with both H1 and H2 receptors leads to capillary dilation and greatly increased permeability with leakage of proteins. In the skin, this gives rise to the classical "triple response" to local injury, reddening, wheal formation and flare(4).

The stimulation of H<sub>1</sub>-receptors results in constriction of smooth muscle in the tracheobronchial "tree" and gastrointestinal tract, lowering of the systemic blood pressure by the reduction of peripheral resistance, stimulation of exocrine gland secretion, mucus production and itching. Stimulation of H<sub>2</sub>-receptor results in increased gastric hydrochloric acid secretion.

## 1.2. Antihistamines

### 1.2.1. Classification of Antihistamines

Antihistamine is a term classically used to describe drugs that act as H<sub>1</sub>-receptor antagonists, while drugs which antagonize H<sub>2</sub>-receptors, such as cimetidine and ranitidine, are referred to as H<sub>2</sub>-receptor antagonists.

All classic H<sub>1</sub>-receptor antagonist have the same basic structure as shown below. (1,2,3).

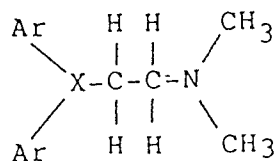


Fig.4 Basic Structure of Antihistamines

where X can be one of N, O, C or S and Ar can be phenyl, pyridyl, thiophenyl or other rings, with or without -Cl, -Br, -OCH<sub>3</sub> or other side groups. In addition,  $\begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{N} \\ \diagdown \\ \text{CH}_3 \end{array}$  may be incorporated into a ring structure such as  $\begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{N} \\ \diagdown \\ \text{CH}_3 \end{array}$ .



A large number of combinations can be made from these possible constituents. Thousands of such compounds have been synthesized and tested, and several dozens are in clinical use.

Antihistamines can be classified on the basis of X substitution into 6 groups(3).

#### 1. Ethylenediamine Derivatives

This group of antihistamines includes antazoline, pyrillamine, tripeleminamine and methapyrilene and has nitrogen in the X position.

#### 2. Ethanolamine Derivatives

This group of antihistamines includes bromodiphenhydramine, clemastine, carbinoxamine, dimenhydrinate, doxylamine, phenyltoluxamine, diphenhydramine, and diphenylpyraline and has oxygen in the X position.

#### 3. Propylamine Derivatives

In this group of antihistamines, a carbon atom is in the X position. Chlorpheniramine, brompheniramine, pheniramine, triprolidine, dexbrompheniramine, dexchlorpheniramine, dimethindene, pyrrobutamine, are included in this group.

#### 4. Phenothiazine Derivatives

This group of antihistamines includes methdilazine, promethazine and trimeprazine, and has nitrogen which is a part of a phenothiazine nucleus in the X position.

#### 5. Piperazine Derivatives

In this group, nitrogen, as part of a piperazine nucleus, is in the X position. Bucline, hydroxyzine, cetirizine, chlorcyclizine, cyclizine, and meclizine are in this group.

#### 6. Others

Azatadine, terfenadine, phenindamine, loratidine and cyproheptadine are included in this group.

#### 1.2.2. Mechanism of Antihistamine Action

The mechanism and site of action are virtually identical for all antihistamines (H<sub>1</sub>-receptor antagonists) (1,2,3,4,5). The basic structure of conventional antihistamines is very similar to the essential structure of histamine itself. This similarity is sufficient to permit the antihistamines to compete for the histamine H<sub>1</sub>-receptor sites on target cells, while the differences are such as to render them inactive as histamine substitutes. Antihistamines block most of the smooth muscle stimulating actions of histamine through competitively occupying H<sub>1</sub>-receptors of the GI tract, uterus, large blood vessels and bronchial muscle. They do not chemically or physiologically inactivate histamine nor do they prevent the release of histamine.

Antihistamines also effectively antagonize the action of histamine, which results in increased capillary permeability and the formation of edema. The H1-receptor antagonists also suppress flare and itching which accompany the endogeneous release of histamine. However, antihistamines(H1-receptor antagonists) do not block the stimulating effect of histamine on gastric acid secretion which is controlled by H2-receptors.

### 1.3. Chlorpheniramine

#### 1.3.1. Chemical and Physical Properties

Chlorpheniramine, ( $\pm$ )-3-(4-chlorophenyl)-NN-dimethyl-3-(2-pyridyl)-propylamine hydrogen, is an antihistamine which is a propylamine derivative(2,3). Its chemical structure is shown as follows:

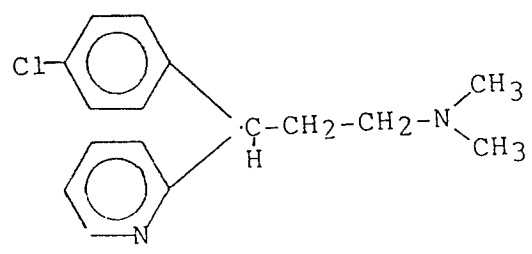


Fig.5 Chlorpheniramine

Chlorpheniramine is available only as the maleate salt which occurs as a white, crystalline powder. It is freely

soluble in water and soluble in alcohol. The pKa of chlorpheniramine is approximately 9.2.

### 1.3.2. Therapeutic use

Chlorpheniramine is generally considered to be a short acting antihistamine with weak sedative properties and a high therapeutic index(1,2,3,6). It has been widely used for symptomatic relief of the common cold and allergic conditions since 1951. It has been stated that its therapeutic effects start within 15 to 30 minutes, are fully developed in one hour, and will last for only four to six hours(1,3,49,54).

Chlorpheniramine is available in various dosage forms, including intravenous injection, syrups, conventional and repeat-action tablets and capsules(49,54). It is also available in combination with other drugs, such as pseudoephedrine sulfate, phenylpropanolamine HCl, phenylephrine HCl, acetylsalicylic acid, caffeine and codeine in various dosage forms(49,54).

There is minimal information about the pharmacodynamics and pharmacokinetics of chlorpheniramine. The literature describing the pharmacokinetics of chlorpheniramine is confusing, for example, the reported half-life in the human ranged from 2 to 30.4hr(35). The existing dosage regimens for chlorpheniramine are based on clinical effectiveness criteria. The recommended oral dose for adults is 4mg q4-6h,

or 8-12mg in the form of repeat-action tablets two or three times daily. Intravenous, intramuscular injection doses of 5-40mg (the maximum dose in 24h is 40mg) may be administered for anaphylaxis or other allergic conditions when oral therapy is impossible or contraindicated. Chlorpheniramine can also be used in treating rhinitis, or as an additional medication in the treatment of asthma(49).

### 1.3.3. Assays

The quality of the analytical methods used in pharmacokinetic studies is very important in order to permit correct interpretation of the pharmacokinetic data. Inconsistencies in reported biological half-lives of chlorpheniramine have been attributed to differences in analytical methods used(17,35).

Many analytical methods including radioanalysis(8,9), GLC (10,11,12,13,14,15,21), thin-layer chromatography(16), HPLC(17,18) and mass fragmentography(19,20) have been published for quantitation of chlorpheniramine. Radioanalysis(8,9) and thin-layer chromatography(16) methods are mostly used for metabolic studies. GLC methods used either lack the required sensitivity, such as the method of Hanna and Tang(12), or are too elaborate and lengthy to be employed when a large number of samples have to be analyzed, such as the procedure of Towley et al. (21). Mass fragmentography(19,20) is reported to be the best assay available with

regard to sensitivity and analysis time, but instrumentation is too expensive to be employed routinely.

In some earlier studies, spectrophotometric methods were used to assay chlorpheniramine in urine for urinary excretion studies(56,57). More specific gas-liquid chromatographic (GLC) methods for the analysis of chlorpheniramine in biological fluids began to appear in the literature in later 1960's. Beckett and Wilkinson(10), using a GLC method for the assay of chlorpheniramine samples, showed that the renal excretion of chlorpheniramine in man was dependent on the urinary pH and flow-rate. Using the same method, Kabaskalian et al. (11) found N-desmethylated metabolites of chlorpheniramine: N-desmonomethylchlorpheniramine and N-desdimethylchlorpheniramine in urine. Another GLC method was used by Kamm et al. (8) to study the metabolism of chlorpheniramine in rats and dogs. They also showed that N-desdimethyl- and N-desmonomethylchlorpheniramine were major metabolites of chlorpheniramine. These GLC procedures, as well as the methods of some others, were developed only for the quantitation of chlorpheniramine and metabolites in urine.

Towley et al. (21) reported a GLC method that could measure nanograms of chlorpheniramine in plasma. However, their procedure is quite complicated involving six replicates of extractions between plasma and solvent and between solvent and aqueous acid or base, and requires up to 5ml of plasma for each assay. In a GLC method for the assay of chlorpheni-

ramine reported by Hanna and Tan(12), 1ml of plasma was required to detect 100ng of chlorpheniramine. Such sensitivity is not suitable for measuring chlorpheniramine in plasma after normal oral doses which produce plasma levels far below the detection limits of these GLC assays. Thompson et al. (19) used GLC-Mass spectrometry to measure chlorpheniramine in plasma. The sensitivity was reported to be 1-2ng/ml.

Lange et al. (16) used thin-layer chromatography(TLC) to separate chlorpheniramine from plasma, extracted the drug from the adsorbant on the developed TLC plates, and measured the fluorescence generated after reaction with rose bengal. This method was also elaborate and lengthy, and the sensitivity was less than that required for in vivo quantitation.

In 1979, Athanikar et al. (17) described a simple, highly sensitive method that detects chlorpheniramine and its two metabolites, N-desdimethyl- and N-desmonomethylchlorpheniramine, in plasma, saliva and urine by high performance liquid chromatography. In this method, a diethyl ether extract of the alkalinized biological samples was extracted with dilute acid which was chromatographed on a reversed-phase column using mixtures of acetonitrile and ammonium phosphate buffer as the mobile phase. Ultraviolet absorption at 254nm was monitored for the detection and brompheniramine was employed as the internal standard for quantitation. The sensitivity was reported to be 1ng/ml. This method and modi-

fied versions have become the most commonly used analytical procedure in pharmacokinetic studies of chlorpheniramine(17,22,23,24,25,26,27,37). Interassay reproducibility of this method is usually within 15 percent. No interference by pseudoephedrine, atropine, scopolamine, hyoscycamine, phenylpropanolamine and other drugs commonly used with chlorpheniramine has been observed with this assay(17).

#### 1.4. Pharmacokinetics of Chlorpheniramine

##### 1.4.1. Absorption

Chlorpheniramine maleate appears to be well absorbed following oral administration, and the absorption site is thought to be the small intestine because of its large surface area and the basicity of chlorpheniramine(25). Chlorpheniramine absorption is sensitive to local gastrointestinal conditions, such as water volume(11), presence of food(25), the effect of multiple dosing(11), and the formulation employed(25,29,30).

Chlorpheniramine undergoes substantial metabolism in the gastrointestinal mucosa during absorption and on the first pass through the liver(24,25). Limited data indicate that the oral bioavailability of chlorpheniramine is incomplete, ranging from 25-60% after a single dose of chlorpheniramine as conventional tablets, syrup, or solution(12,26).



Following oral administration of conventional tablets or solution in the human, chlorpheniramine appears in plasma within 30-60 minutes and peak plasma concentration generally occur at 2-6 hours. A mean absorption lag time of 0.7 hours has been reported after administration of syrups(29). In one study, the range of oral absorption half-lives was 0.35-1.04hr(26). The stomach emptying time appears to affect the rate of absorption of chlorpheniramine but not to affect the bioavailability, for example, in rabbits, the area under the serum concentration versus time curve(AUC) up to 12 hours, and the cumulative fractions absorbed up to 12 hours approached the same value under fasting and nonfasting conditions(25).

Several bioavailability studies have compared immediate-release and sustained-release products. Hanna and Tang(12) observed that 60-70% of the administered dose was absorbed from two chlorpheniramine syrups administered orally to human subjects. This result was higher than what was reported later by others(24,25,28,29), the difference may be attributed to the analytical methods used because the sensitivity of the GLC method used in their study was 100ng/ml.

Yacobi et al. (28) compared the bioavailability of a chlorpheniramine-pseudoephedrine sustained-release capsule administered q12h with conventional release chlorpheniramine and pseudoephedrine tablets administered q6h in 24 normal human volunteers. They showed that the AUC and the unchanged

drug excreted in urine, after a single dose and at steady state were identical and concluded that these two formulations were bioequivalent.

Barrett et al. (30) conducted a randomized, controlled, crossover, multiple dose bioavailability study of sustained-release tablets containing chlorpheniramine and phenylpropanolamine. They noted that at the steady state, the bioavailability parameters, namely areas under the plasma concentration curves, peak concentrations and time to reach peak concentrations produced by the sustained-release tablets, did not exhibit a significant difference from the same parameters achieved by the commercially available immediate release tablets of phenylpropanolamine or chlorpheniramine. Therefore, they concluded that the sustained-release tablets were biologically equivalent to the commercially available immediate-release phenylpropanolamine and chlorpheniramine tablets.

Vallner et al. (31) compared the relative bioavailability of chlorpheniramine conventional release tablets, 4mg q6h, repeated action tablets and barrier-coated, bead capsules, 8mg q12h, in 15 nonfasting healthy subjects. The AUC data for the different products did not differ significantly but high inter-subject variations were observed.

Kotzan et al. (29) also studied the bioavailability of chlorpheniramine regular release versus sustained-release

products in 15 subjects. Dosage forms used in this study were an 8mg barrier-coated, bead capsule, an 8mg repeat action tablet, two 4mg regular release tablets and 4- and 8mg doses, administered as oral syrups. The controlled release products extended the time necessary to attain peak drug levels compared to syrups and conventional tablets. However, the AUC data for the controlled release products was not equivalent to equal amounts of the regular release products. This result indicated that while controlled release chlorpheniramine products were successful in prolonging the time course of absorption, this was only achieved at the expense of incomplete bioavailability.

Athaniker and Chiou(24)observed that the absolute bioavailability of chlorpheniramine following oral administration in solutions was dose dependent. At 100mg dose, 36% of the orally administered dose was found to reach the systemic circulation in 6 dogs. At 50mg dose, the average bioavailability was only 9.4% in 4 dogs; while at 200mg dose, the average bioavailability in 4 dogs was 39.4%. They postulated that there might be a saturable first pass gut and hepatic elimination.

The above suggestions were confirmed by Huang et al. (25) on rabbits. They observed that the oral absolute bioavailabilities of chlorpheniramine in 4 rabbits, averaged 6%, 9% and 9% following a 3, 10.5 and 21 mg/kg dose respectively. The individual bioavailability data obtained following

different doses again suggested the existence of saturable presystemic elimination.

#### 1.4.2. Distribution

The results of pharmacokinetic tissue and organ distribution studies indicate a rapid and extensive distribution of chlorpheniramine in various parts of the body, which can be described by a two or three compartment open model depending on the sampling schedule in the distribution phase(23,24,26,29,32). The reported volumes of distribution are 5.9(33), 5.6(19), 3.36(26)and 2.5(9)l/kg after intravenous dosing and 7.51(31)and 7.0(27)l/kg after oral dosing. The steady state volumes of distribution of 3.81(32) and 3.17(26)l/kg have been reported after intravenous administration.

Tissue distribution studies on rabbits showed rapid and extensive uptake of chlorpheniramine by various organs. Highest concentrations were found in lung, kidney and brain after intravenous injection, as their concentrations were 160-,180- and 31-fold higher than the plasma level. Lower concentrations were found in the large intestine, muscle, stomach, etc(23). This result has been confirmed by an isolated overdose case(34). The accumulation in the brain might be related to chlorpheniramine's sedative effects. Chlorpheniramine is also distributed into saliva, and in small amounts into bile(35).

In most studies, the plasma level decaying profile can be described by bi- and triexponential characteristics(23,24,26,32). The rapid initial distribution phase probably reflects the entry of chlorpheniramine into tissue and the secondary slower disappearance rate, its re-entry into the blood from these compartments and its elimination from the body.

Chlorpheniramine is 70 percent bound to plasma proteins(9,23). Huang et al. (23) found that in vitro binding of chlorpheniramine to serum protein in human plasma was linear over a wide concentration range: 73% at 49-90 ng/ml, 72% percent at 280ng/ml and 69% at 1240ng/ml. The average protein binding of chlorpheniramine at 30 to 70 ng/ml was found to be 44% in rabbit sera and 70% for dog sera(25). They concluded that the lower percentage of protein binding in rabbits, as compared to those of dogs and humans(44% versus 70% and 73%) might contribute in part to the large volume of distribution in rabbits(15.5l/kg) compared to 5.25 and 3.36l/kg in dogs and humans respectively.

#### 1.4.3 Elimination

Chlorpheniramine is rapidly and extensively metabolized after absorption and undergoes metabolism in the GI mucosa during absorption and on the first pass through the liver following oral administration(23,24,26). However, the mechanism of the biotransformation of chlorpheniramine is not

completely understood. It is generally accepted that chlorpheniramine undergoes N-dealkylation to form monodesmethylchlorpheniramine and didesmethylchlorpheniramine (8,9), but it is principally metabolized to other unidentified metabolites(24,36). Recent studies(9,36) showed that chlorpheniramine can also be metabolized by an oxidative deamination mechanism to form polar metabolites: an alcohol and an acid in dogs and humans.

The gut and hepatic first-pass metabolism have been observed to be a saturable process(24,25)and enterohepatic cycling of chlorpheniramine has been observed in rats(8).

Chlorpheniramine and its metabolites are apparently excreted completely in urine. Beckett and Wilkinson(10)studied the influence of urine pH and flow rate on the renal excretion of chlorpheniramine. They found that renal excretion rates of chlorpheniramine varied with both urinary pH and the rate of urine flow.

Lai et al. (18)studied the urinary excretion of chlorpheniramine and pseudoephedrine in normal subjects who received a sustained-release dosage form containing 8mg chlorpheniramine maleate and 120mg pseudoephedrine hydrochloride every 12hr for 7 days with or without ammonium chloride administration. Excretion of chlorpheniramine and its two metabolites(mono-and didesmethylchlorpheniramine) was enhanced after ammonium chloride administration. At

steady state, a change in urine pH from 5.69 to 6.46 resulted in more than a 25% decrease in chlorpheniramine excretion.

Following a single oral or I.V. dose of chlorpheniramine maleate in healthy subjects with normal renal and hepatic function, Peets et al. (9) observed that about 20% of the dose was excreted in urine within 24 hours and 35% within 48 hours. Less than 1% was excreted in feces within 48 hours. About 3-7% of the dose was excreted in urine as unchanged drug within 48 hours, 2-4% as monodesmethylchlorpheniramine, 1-2% as didesmethylchlorpheniramine, and the remainder as unidentified metabolites.

In adults with normal renal and hepatic function, the reported elimination half-lives of chlorpheniramine range from 12-36.3 hours(22,26,28,33). Early studies suggested a half-life of only 2-4 hours(12,16). However, these values may have resulted from short sampling times and lack of sensitivity in the assays employed(23,35). The half-life from about 100 adults studied in published studies following different routes, doses and assays for chlorpheniramine ranges from 2 to 30.4 hours(35).

In children with normal renal and hepatic function, the half-life of chlorpheniramine reported averages 9.6-13.1 hours(range:5.2-23.1hr) (19,27,32,37), which is shorter than in adults. The mean overall half-life for 19 children stud-

ied so far was approximately half that reported for adults(11.94 versus 20.36). Since the Vd of chlorpheniramine in children is similar to that reported for adults, it is assumed that the shorter half-life in children is primarily due to a faster clearance rate. The reduction in metabolic enzyme activity with age may play a role since chlorpheniramine is metabolized extensively.

In patients with chronic renal failure undergoing hemodialysis(38), the elimination half-life of chlorpheniramine reported ranged from 280-330 hours.

#### 1.5. Rationale of the Present Study

##### 1.5.1. Use of Rabbits in Bioavailability Studies

In bioavailability studies for various dosage forms, use of human subjects provides the most appropriate results, but due to difficulties in obtaining volunteers, relatively high cost and possible side effects, it would be desirable to have an animal model for the bioavailability studies. In addition, as in vitro dissolution tests and other pharmaceutical and chemical analyses are only designed for quality control and reproducibility, an animal model for comparative bioavailability studies in the quality control of dosage forms will be advantageous.

Various animals including dogs, rats, rabbits and monkeys have been used to clarify the correlation of GI drug absorp-



tion of orally administered drugs between humans and animals. The need for human trials in developing new drugs and dosage forms of increased bioavailability could be minimized if a good animal model is formed. Although rabbits are readily available and are not expensive to purchase and maintain, they are not considered useful for bioavailability and drug absorption studies, because rabbits are nocturnal animals and their GI anatomy is different from that of humans(39,40).

Chiou et al. (40) noted that the stomach of rabbits fasted for 24hr was as full as that of unfasted rabbits, although the rabbits were maintained on a wire mesh support which allowed feces to drop to the bottom of the cage, thus reducing coprophagy. Even after one week of fasting, the stomach still contained a significant degree of debris and solid materials. They concluded that the rabbit was not a suitable animal for the evaluation of GI absorption of drugs when stomach emptying is a limiting factor.

In research conducted by Maeda et al. (41), rabbits were given a special solid diet, which was prepared by removing alfalfa from the commercial solid diet, for 1 week, and the gastric content were washed out with saline. The rabbits were muzzled to prevent coprophagy during the night. The investigators noted that a special soft diet, which was prepared by adding 60 parts of water to 40 parts of the special solid diet, given to the rabbit was transferred exponential-

ly from the stomach into the small intestine and almost disappeared from the stomach within 5 hours in these so-called "stomach emptying-controlled rabbits". They also found that the absorption of griseofulvin, indomethacin or nalidixic acid was much faster and more complete in these rabbits than in rabbits fasted for 24 hours after being maintained on a normal diet. Good correlations were observed between the plasma level-time curves of these drugs in the stomach-emptying controlled rabbits and in human subjects.

In a later study, the same authors(42) studied the correlation between the dissolution rate and bioavailability of 3 different griseofulvin tablets in stomach-emptying-controlled rabbits and in humans. A good correlation was observed for the rank order of Cmax and AUC of these tablets between rabbits and humans. Their results suggested that the stomach-emptying-controlled rabbit is a useful animal model for examining comparative bioavailability of oral dosage forms.

Using a plastic catheter-rubber balloon device, Venlo and Eriksson(43) administered 3 different carbamazepine tablets to the rear pharynx of rabbits after 22 hours fasting. The bioavailability was measured from the carbamazepine and carbamazepine-10,11-epoxide serum levels for up to 24 hours. They found that carbamazepine was fairly constantly absorbed with no great variations from rabbit to rabbit, and the results agreed with previous observations for the bioavailability of these carbamazepine tablets in humans. Therefore,

at least with carbamazepine tablets, rabbits were useful in studying bioavailability.

There are no previous reports using rabbits as an animal model in investigating the time course and bioavailability of sustained-release dosage forms.

#### 1.5.2. Purposes of the Present Study

Chlorpheniramine repeat-action tablets contain either 8mg or 12mg chlorpheniramine maleate. The drug is divided equally between an outer layer for rapid absorption and an inner core protected by special timed barrier for releasing drug 3 to 6 hours after ingestion(44). A previous study compared the bioavailability of these repeat-action tablets with conventional tablets and syrups in humans(29).

The present study is designed to investigate whether rabbits can be used as animal model for studying the bioavailability of sustained-release dosage forms. Chlorpheniramine was chosen as the drug used here for two reasons. First, considerable research concerning the pharmacokinetics and efficacy of chlorpheniramine in man has been done in this laboratory and a sensitive, specific analytical method using HPLC for determining serum chlorpheniramine levels is available(27,37). Second, chlorpheniramine is marketed in a wide range of dosage forms as described above, which makes it easier to select dosage forms suitable for evaluation in animals.

Chapter II  
EXPERIMENTAL

2.1 Chemicals and Equipment

2.1.1. Chemicals

1. Chlorpheniramine maleate: Lot CM-0-G-16, Schering Canada Inc., Pointe Claire, Quebec, Canada.
2. Brompheniramine maleate: Lot M 656 A.H. Robins Montreal, Quebec, Canada.
3. Phosphoric Acid: Fisher Scientific Co. Fair Lawn, New Jersey, USA.
4. Ammonium Phosphate Monobasic: Fisher Scientific Co., Fair Lawn, New Jersey, USA.
5. Potassium Hydroxide: Fisher Scientific Co. Fair Lawn, New Jersey, USA.

2.1.2. Solvents

1. Diethyl Ether: Fisher Scientific Co. Fair Lawn, New Jersey, USA.
2. Acetone: Fisher Scientific Co. Fair Lawn, New Jersey, USA.
3. Acetonitrile (HPLC grade): Fisher Scientific Co. Fair Lawn, New Jersey, USA.

### 2.1.3. Supplies

1. Filter unit(0. 45 micron Millex-SR): Millipore Corporation, Bedford, MA, USA.
2. Sure-Sep II Serum Plasma Separator: General Diagnostics, New Jersey, USA.
3. Butterfly Infusion Sets(23G and 25G): Abbott Ireland Ltd. Sligo, Rep. of Ireland.
4. Syringes(5cc): Becton Dickinson and Co., Canada.
5. Disposable Test Tubes(16\*100mm): Fisher Scientific Co. Fair Lawn, New Jersey, USA.
6. 0.9% Sodium Chloride Injection(USP): Baxter Laboratories of Canada Limited. Malton, Ontario.

### 2.1.4. Equipment

1. Centrifuge(International Centrifuge): International Equipment Co. Boston, Mass., USA.
2. pH meter(Fisher pH meter, model 600): Fisher Scientific Co., Fair Lawn, New Jersey, USA.
3. Balance(Mettler AE 160): Mettler Instrument Corporation, New Jersey, USA.
4. Vortex mixer(Vortex Genie, model K-550-G): Scientific Industries Inc., Bohemia, USA.
5. Evaporator(The Meyer N-Evap Analytical evaporator: Organomatic Associates Inc., Northborough, MA, USA.
6. High Performance Liquid Chromatography: The HPLC was comprised of a 6000A high pressure pump, a U6K injector. The 480 U-V LC spectrophotometric detector is set at a wavelength of 225nm. The detector

was connected to a 720 data module. A 0.39mm\*30cm stainless steel column(uBondpak C18) was used for separation of chlorpheniramine. All of the above instruments are from Waters.(Waters Associates Inc., Millford, Mass, USA)

#### 2.1.5. Dosage Forms

1. Dosage Form 1: Chlor-tripolon Injectable, Lot 4CBZZ1  
Schering Canada Inc. Pointe Claire, Quebec.
2. Dosage Form 2: oral solution diluted from the injectable preparation with distilled water.
3. Dosage Form 3: Chlor-Tripolon tablets 4mg, Lot 4TWZG8  
Schering Canada Inc. Pointe Claire, Quebec.
4. Dosage Form 4: Chlor-Tripolon 12mg Repetabs, Lot 4AAEE2  
Schering Canada Inc. Pointe Claire, Quebec.
5. Dosage Form 5: Chlor-Tripolon 8mg Repetabs, Lot 5CCZE2  
Schering Canada Inc. Pointe Claire, Quebec.

The solution for injection and all solid dosage forms were commercially available products. The commercial available chlorpheniramine syrup, however, could not be used, because in the preliminary study, the syrup was given to rabbits in a dose of 12mg and no drug could be detected from serum samples taken at different times after administration.

## 2.2. Methodology

### 2.2.1. HPLC procedure for chlorpheniramine

The method used was that of Simons et al. (27) with some minor modifications.

One hundred  $\mu$ l brompheniramine solution (1 $\mu$ g/ml) which acts as the internal standard was added to 1ml of serum along with 250 $\mu$ l 10% KOH solution and 5ml of newly distilled ether. Extraction was achieved by mixing on a vortex mixer for 30 seconds followed by centrifuging for 5min at 4000 r.p.m. The aqueous portion was frozen in a dry ice/acetone bath and the ether layer was transferred to a clean 16\*100mm dry test tube. One hundred  $\mu$ l of 0.05% H<sub>3</sub>PO<sub>4</sub> solution was added, followed by mixing on a vortex mixer for 30 seconds and centrifuging for 5min at 4000 r.p.m. The aqueous portion was again frozen in a dry ice/acetone bath and the ether layer was discarded. The aqueous portion was exposed to a stream of dry nitrogen to remove remaining traces of ether. The remaining aqueous solution was then taken up in a syringe and injected onto the column.

### 2.2.2. Selection of Chromatographic Conditions

Aqueous solutions of chlorpheniramine maleate and the internal standard brompheniramine were initially chromatographed to select suitable chromatographic conditions, including flow rate, composition of the mobile phase and

sensitivity of the detector. Various portions of acetonitrile-phosphate buffer (20-25% acetonitrile) were tested as mobile phases. The phosphate buffer was prepared by acidifying ammonium phosphate solution (monobasic, 0.075M) with phosphoric acid to a pH about 2.5. All aqueous components of the mixture were passed through a 0.45um HA filter prior to mixing with acetonitrile. At the same time, various flow rates were tried in order to select the optimum value. From the chromatograms obtained, the chromatographic parameters of capacity ( $K'$ ), selectivity ( $\alpha$ ), the theoretical number of plates ( $N$ ) and resolution ( $R$ ) were calculated. The chromatographic conditions that gave the most desirable retention and resolution were then employed.

All chromatographic parameters were calculated according to standard equations (45). The resolution factor  $R$  was calculated using Equ. (1):

$$R = \frac{2 (tR^2 - tR^1)}{1.669 (W_{1/2}^2 + W_{1/2}^1)} \quad (1)$$

where  $tR^2$ ,  $tR^1$ ,  $W_{1/2}^2$ ,  $W_{1/2}^1$  are the retention times and the width of the peak at half-height of chlorpheniramine and brompheniramine respectively.

The capacity factor  $K'$  was calculated using

$$K' = \frac{(tR - t_m)}{t_m} \quad (2)$$



where  $t_R$  is the retention of chlorpheniramine or brompheniramine and  $t_m$  is the retention time of the front of solvent.

The separation factor  $\alpha$  is defined as :

$$\alpha = \frac{K_2'}{K_1'} \quad (3)$$

where  $K_1'$  and  $K_2'$  are the capacity factors of chlorpheniramine and brompheniramine respectively.

The number of theoretical plates of chlorpheniramine is calculated as following

$$n = 5.54 \frac{(t_R^2)^{1/2}}{(W_{1/2}^2)^2} \quad (4)$$

where  $t_R^2$  is the retention time of chlorpheniramine and  $W_{1/2}^2$  is the width of the chlorpheniramine peak at half-height.

### 2.2.3. Chromatographic separation and quantitation

The mobile phase used was acetonitrile-phosphate buffer (0.075M  $\text{NH}_4\text{PO}_3$ , pH 2.7 with  $\text{H}_3\text{PO}_4$ ) (22.5:77.5 v/v). The flow rate was set at 1.5ml/min. The effluent from the column was monitored by UV absorption at 225nm with 0.005 a.u.f.s. sensitivity setting. The chart speed of the data module was 0.5cm/min. Peak height ratios of chlorpheniramine and brompheniramine were used for the quantitation based on the calibration curves established during the study period.

The calibration curves were prepared from the results of assays on blank serum supplemented with known quantities of chlorpheniramine and the internal standard, brompheniramine. All the chromatographic separations were carried out at ambient temperatures. The serum concentrations of chlorpheniramine were calculated on the basis of chlorpheniramine maleate.

#### 2.2.4. Bioavailability Studies

Five New Zealand white rabbits (three males and two females) obtained from the Central Animal Care Services, University of Manitoba were used in this study. Their average weight was  $4.63 \pm 0.49$  kg. They were kept individually in metal cages which have a wire floor support to reduce coprophagy. Water and food were supplied ad libitum.

Dosage forms as listed in the table below were administered according to a five-way crossover design with all subjects receiving each dose.

Table 1. Dosage Forms

	Strength	Dose
Dosage Form 1	100mg/ml	12mg(0.12ml I.V. solution)
Dosage Form 2	8mg/ml	24mg(3.0ml oral solution)
Dosage Form 3	4mg	24mg(6 tablets)
Dosage Form 4	12mg	24mg(2 tablets)
Dosage Form 5	8mg	24mg(3 tablets)

The following scheme was used with a 1-week washing out period between each study.

Table 2. Study Schedule

---

Dosage Form	Study Period				
	1	2	3	4	5
	Rabbit No.				
Dosage Form 1	B	D	A	C	E
Dosage Form 2	C	A	E	B	D
Dosage Form 3	A	C	D	E	B
Dosage Form 4	D	E	B	A	C
Dosage Form 5	E	B	C	D	A

---

#### Intravenous Studies

All five non-fasted rabbits received an i.v. bolus dose of 12mg of chlorpheniramine maleate. The dose was injected through the ear vein. After injection, the infusion set was flushed with 5ml of normal saline to ensure complete delivery of chlorpheniramine. Then, 2.0-3.0ml of blood was withdrawn from the ear vein using a venipuncture set prior to injection and at 5,10,15,30,60,90 minutes and 2,3,4,5,6,7,8 hours after injection.

Blood samples were collected in 16\*100mm test tubes without anticoagulants. Sera were separated by placing Sure Sep-II separators on the top of test tubes and centrifuging at 4000 r.p.m for 15min. Sera were stored frozen(-20<sup>o</sup>c) until analyzed.

## Oral Studies

All oral studies were performed under nonfasting conditions and the rabbits were restrained only at the time of blood sampling. Chlorpheniramine tablets were applied to the rear pharynx of the rabbit with a plastic rod through a hole in a wooden stick holding the mouth open and the tongue sufficiently protruded out of the mouth. Each rabbit received 10ml of water at the time of oral dosing. Rabbit chow was supplied two hours after drug administration while water was supplied ad libitum.

Blood samples were obtained from the ear vein using a different venipuncture set at the time of each sampling. In the case of oral solution and regular release tablets, 2.0-3.0ml blood was withdrawn prior to drug administration and at 5,15,30,60,90 minutes and 2,3,4,5,6,7,8 hours after dosing. In the case of repeat-action tablets, 2.0-3.0ml blood was withdrawn prior to drug administration and at 15,30,60,90min and 2,3,4,5,6,7,8,9,10 and 12 hours after dosing.

Concentrations of chlorpheniramine were analyzed using the HPLC procedure described above.

### 2.3. Data Analysis

Data from the bioavailability studies were analyzed using PKCALC(46) on an IBM PC. PKCALC is a BASIC program which

performs standard statistical and pharmacokinetic analysis of multisubject data sets, including means, standard deviations, standard errors, of variation, half-lives of absorption and elimination, coefficients, areas under the concentration versus time curves, mean residence time, and total clearance.

PKCALC can be linked to an augmented copy of the program ESTRIP(47) which can strip serum concentrations-time data automatically using different polyexponential equations. Models giving the least sum of squared derivations and the best coefficient of determination were selected.

The elimination rate constant( $\beta$ ) was determined from the slope of the terminal linear portion in concentrations vs time plot by the least square regression method. The elimination half-life was calculated using Equation 1(48):

$$T_{1/2} = 0.693/\beta \quad (5)$$

Area under the concentration vs time curve(AUC) was calculated by the trapezoidal rule from the time 0 to time t of the last sample and extrapolated to infinity according to the formula:

$$AUC_{0 \rightarrow \infty} = AUC_{0 \rightarrow t} + C_t/\beta \quad (6)$$

where  $C_t$  is the concentrations of chlorpheniramine in the last sample at time t.

Results are expressed in terms of ng\*hr per ml.

The total plasma clearance (CL<sub>p</sub>) was calculated using Equation 7:

$$CL_p = \text{Dose} / AUC_{0 \rightarrow \infty} \quad (7)$$

Results are expressed as millilitre per minute.

The apparent volume of distribution (V<sub>d</sub>) was calculated using Equation 8:

$$V_d = \text{Dose} / (A+B) \quad (8)$$

where A and B are intercepts for the bioexponential decay of plasma levels of chlorpheniramine after intravenous injections and were calculated by the computer. Results are expressed in terms of litres per kg.

The bioavailability was calculated using Equation 9:

$$F = \frac{(\text{Dose})_{i.v.} (AUC_{0 \rightarrow \infty})_{oral}}{(\text{Dose})_{oral} (AUC_{0 \rightarrow \infty})_{i.v.}} \quad (9)$$

Mean Residence Time (MRT) after an oral dose was calculated using

$$MRT_{po} = AUMC_{0 \rightarrow \infty} / AUC_{0 \rightarrow \infty} \quad (10)$$

where AUMC<sub>0→∞</sub> is the area under the first moment of the serum concentration versus time curve. Mean Absorption Time (MAT) was then obtained by

$$MAT = MRT_{po} - MRT_{i.v.} \quad (11)$$

where  $MRT_{i.v.}$  and  $MRT_{po}$  are the mean residence times after intravenous and oral doses respectively.



## Chapter III

### RESULTS

#### 3.1. HPLC assay

##### 3.1.1. Selection of Mobile Phases

Chromatograms with different degrees of resolution were found from the mobile phases with different acetonitrile compositions ranging from 18% to 25% and different pH values of the phosphate buffer of pH 2.5 and 2.7. Chromatographic parameters  $k'$ ,  $\alpha$ , R and N calculated from these chromatograms are summarized in Table 3.

Table 3. Effects of Composition and pH of the Mobile Phase on Chromatographic Parameters of Chlorpheniramine and Brompheniramine

Mobile Phase	k1'	k2'	$\alpha$	R	N
acetonitrile-					
phosphate buffer(pH2.5)					
25:75	1.06	1.41	1.33	2.33	3366
23:77	1.70	2.28	1.34	2.51	3420
20:80	2.10	2.85	1.35	2.95	2986
acetonitrile-					
phosphate buffer(pH2.7)					
22.5:77.5	2.01	2.67	1.33	2.92	4248
20:80	2.43	3.18	1.31	3.08	4139
18:82	2.87	3.82	1.33	3.32	3289

From this table, the mobile phase composed of 22.5% acetonitrile in pH 2.7 phosphate buffer gave the highest number of theoretical plates, suitable retention and separation, therefore, it was used as the mobile phase in later studies.

### 3.1.2. HPLC Chromatograms

Representative HPLC chromatograms for chlorpheniramine and the internal standard brompheniramine are shown in Fig.6.

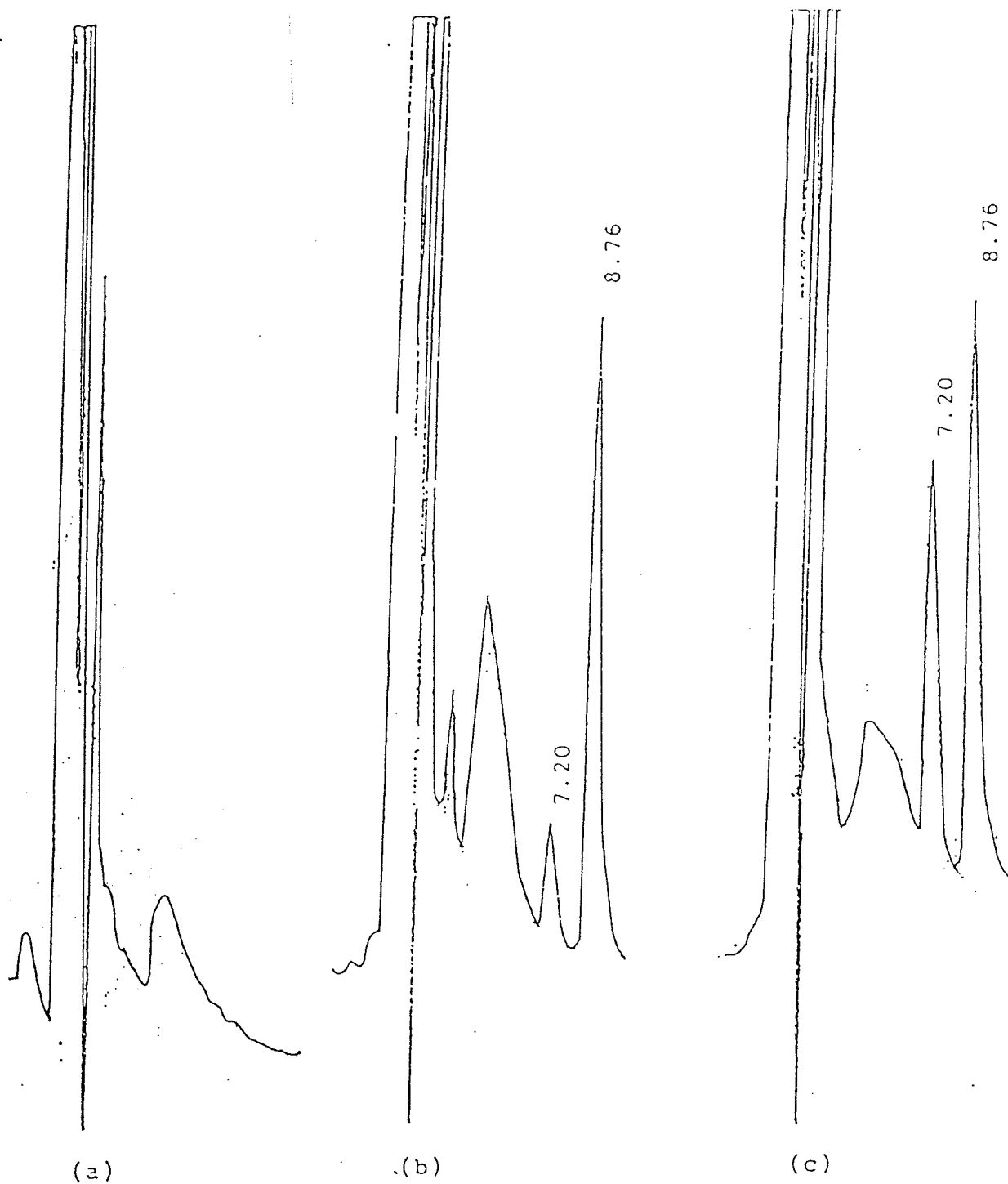


Fig.6 HPLC chromatograms for Chlorpheniramine  
and Brompheniramine

(a) Control serum extract (without brompheniramine)

(b) 15ng/ml chlorpheniramine spiked serum extract (with  
brompheniramine)

(c) 80ng/ml chlorpheniramine spiked serum extract (with  
brompheniramine)

Retention times of chlorpheniramine and brompheniramine were 7.20 and 8.76 minutes respectively. There were no interfering peaks.

### 3.1.3. Calibration Curves for Chlorpheniramine

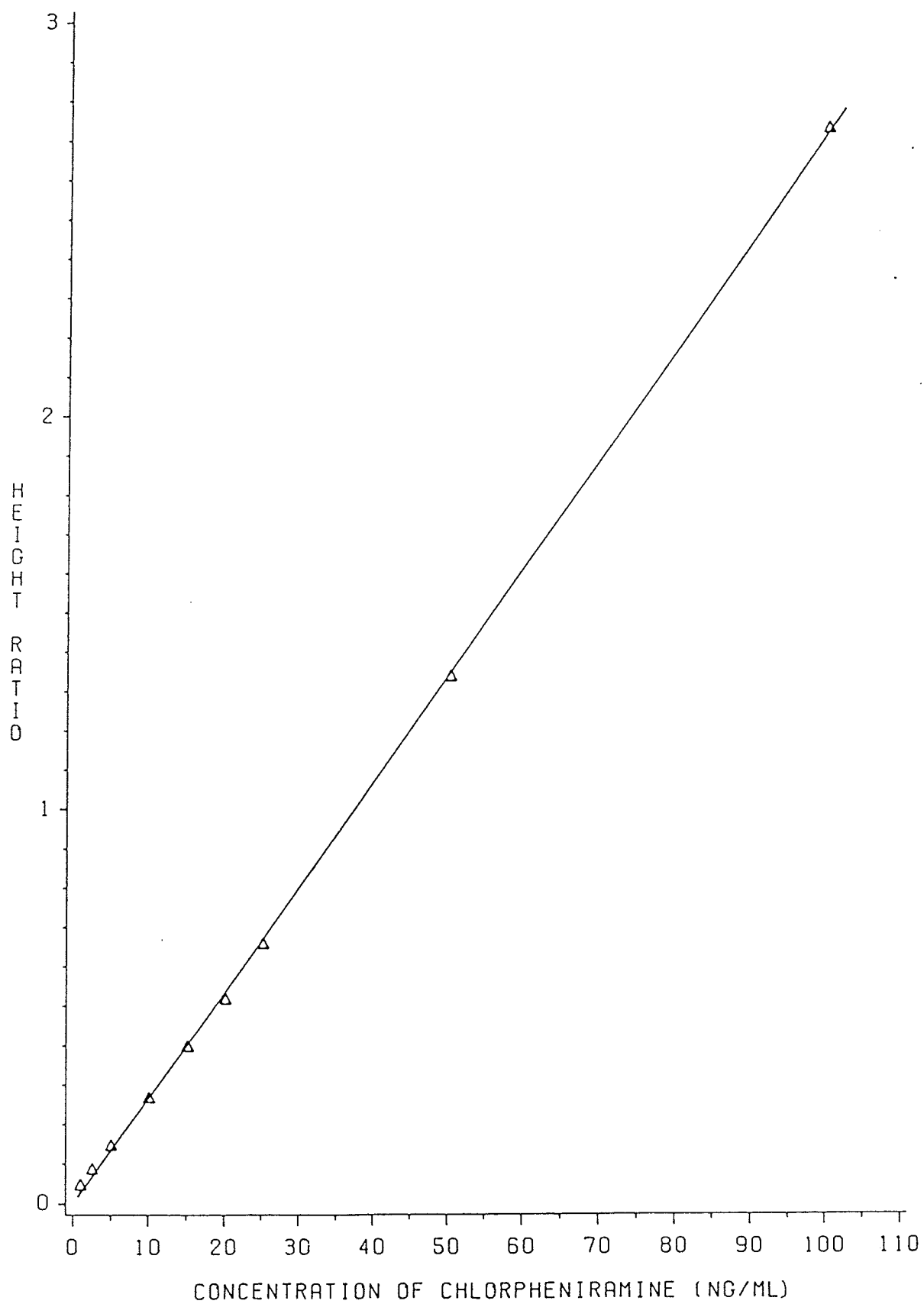
The calibration curves for chlorpheniramine were constructed by plotting peak height ratios of chlorpheniramine to brompheniramine versus concentrations of chlorpheniramine. Calibration curves were analyzed periodically during the study period using concentrations of chlorpheniramine from 1.02ng/ml to 101.51ng/ml. The calibration curves were linear over this range of concentration. The variability in the calibration curves over a period of 8 months, calculated as the coefficient of variation is shown in Table 4 and the calibration curve is shown in Fig.7.

Table 4. Variability in HPLC Calibration Curves for  
Chlorpheniramine

concentrations(ng/ml)	Peak Height Ratio	C.V.
1.02	0.05	0
2.54	0.09±0.01	13.26
5.08	0.15±0.00	3.28
10.15	0.27±0.01	4.69
15.23	0.40±0.02	4.13
20.3	0.52±0.01	1.83
25.38	0.66±0.01	1.44
50.76	1.34±0.16	11.97
101.51	2.73±0.18	6.68

The coefficients of variation of chlorpheniramine were found to be less than 15%.

Fig.7 The Calibration Curve for Chlorpheniramine



### 3.2. Bioavailability Studies

#### 3.2.1. Serum Concentrations of Chlorpheniramine after Administration of Various Dosage Forms

To determine whether rabbits should be fasted before oral administration, 24mg oral solution were administered to rabbit B under fasting and nonfasting conditions respectively. Table 5 lists serum concentrations of chlorpheniramine obtained from these two conditions and these concentrations were plotted against time in Fig.8.

Table 5. Serum Concentrations of Chlorpheniramine after Administration of 24mg Oral Solution under Fasting and Nonfasting Conditions in Rabbit B \*\*

Time(hr)	Fasting	Nonfasting
0.088	4.65	29.45
0.16	6.63	****
0.25	****	39.20
0.50	6.08	****
1.0	16.25	30.28
1.5	****	19.13
2.0	8.29	15.56
3.0	4.32	10.87
4.0	4.98	6.79
5.0	3.98	4.73
6.0	2.70	3.90
7.0	4.32	2.43
8.0	3.56	++++

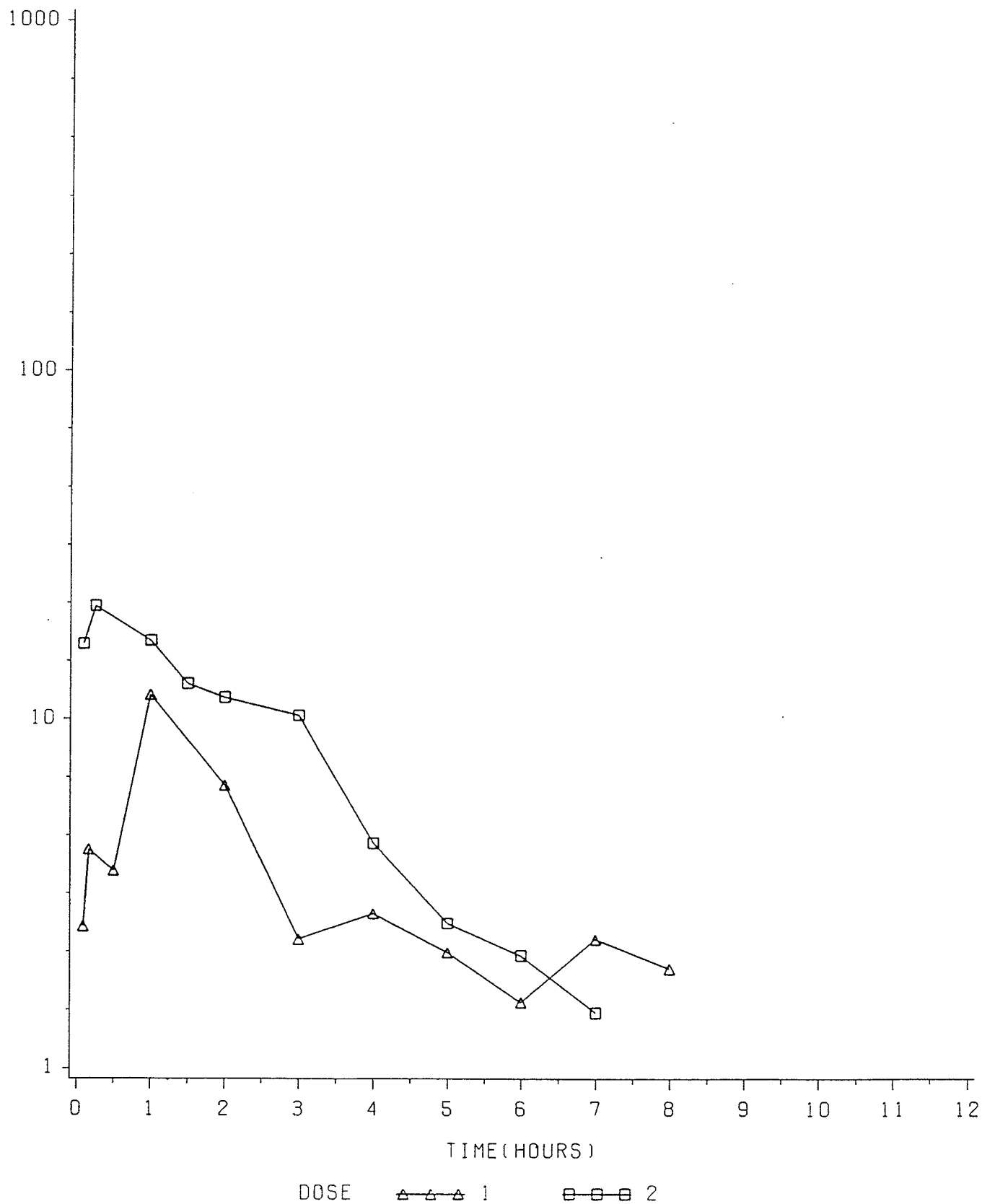
\*\*\*\* means no sample obtained.

++++ means that the concentration is below the limit of detection.

\*\* The unit of the above concentration is nanogram per millilitre.



Fig.8 Serum Concentration of Chlorpheniramine After Administration of 24mg Oral Solution under Fasting and Nonfasting Conditions in Rabbit B



The serum concentrations of chlorpheniramine in serum from five non-fasted rabbits after administration of five dosage forms were shown in Table 6 to Table 10 and plotted against time in Fig.9 to Fig.13.

Table 6. Serum Concentrations of Chlorpheniramine after Administration of Five Dosage Forms in Rabbit A \*\*

Time (hr)	Dosage Forms				
	1	2	3	4	5
0.088	220.06	14.95	39.5	****	****
0.16	174.9	****	****	****	****
0.25	186.38	19.13	50.9	++++	3.95
0.50	128.85	27.49	50.7	2.32	5.96
1.0	97.33	11.62	25.46	7.26	5.99
1.5	60.84	9.10	19.30	6.89	****
2.0	21.18	8.07	11.51	3.03	2.83
3.0	20.5	4.97	8.33	3.16	3.49
4.0	13.89	2.66	7.23	****	2.67
5.0	8.80	2.36	4.45	1.84	3.14
6.0	****	1.60	4.86	2.53	3.07
7.0	4.84	++++	4.45	****	3.29
8.0	3.29	++++	3.25	4.40	2.85
9.0	****	****	****	****	****
10.0	****	****	****	3.99	2.75

\*\*\*\* means no sample obtained.

++++ means the concentration is below the limit of detection.

\*\* The unit of the above concentrations is nanogram per millilitre.

Fig.9 Serum Concentrations of Chlorpheniramine after Administration of Five Dosage Forms in Rabbit A

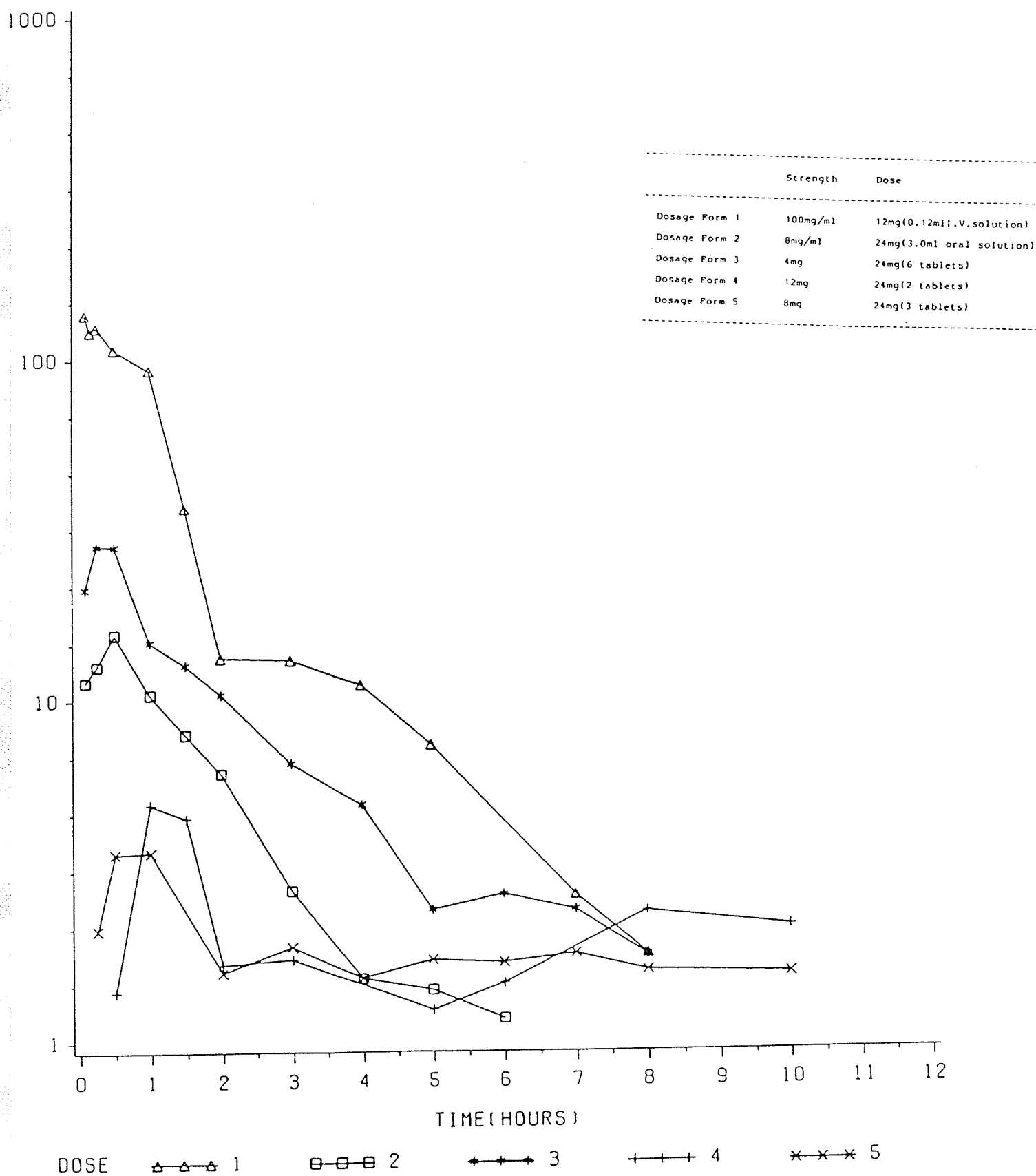


Table 7. Serum Concentrations of Chlorpheniramine after  
Administration of Five Dosage Forms in Rabbit B \*\*

Time (hr)	Dosage Forms				
	1	2	3	4	5
0.088	688.77	29.45	14.24	****	****
0.25	385.18	39.20	29.86	6.74	6.18
0.50	310.60	****	22.00	19.95	9.71
1.0	175.70	30.28	8.48	****	15.69
1.5	159.87	19.13	7.31	8.37	****
2.0	121.51	15.56	7.21	4.14	5.21
3.0	73.80	10.87	6.05	5.68	3.90
4.0	67.20	6.79	****	3.82	2.62
5.0	40.02	4.73	7.72	8.84	4.04
6.0	37.65	3.90	3.55	5.36	4.37
7.0	17.70	2.45	2.41	2.57	6.18
8.0	10.04	++++	++++	2.52	2.70
10.0	****	****	****	4.04	++++

\*\*\*\* means no sample obtained.

++++ means that the concentration is below the limit of detection.

\*\* The unit of the above concentrations is nanogram per millilitre.

Fig.10 Serum Concentrations of Chlorpheniramine after Administration of Five Dosage Forms in Rabbit B

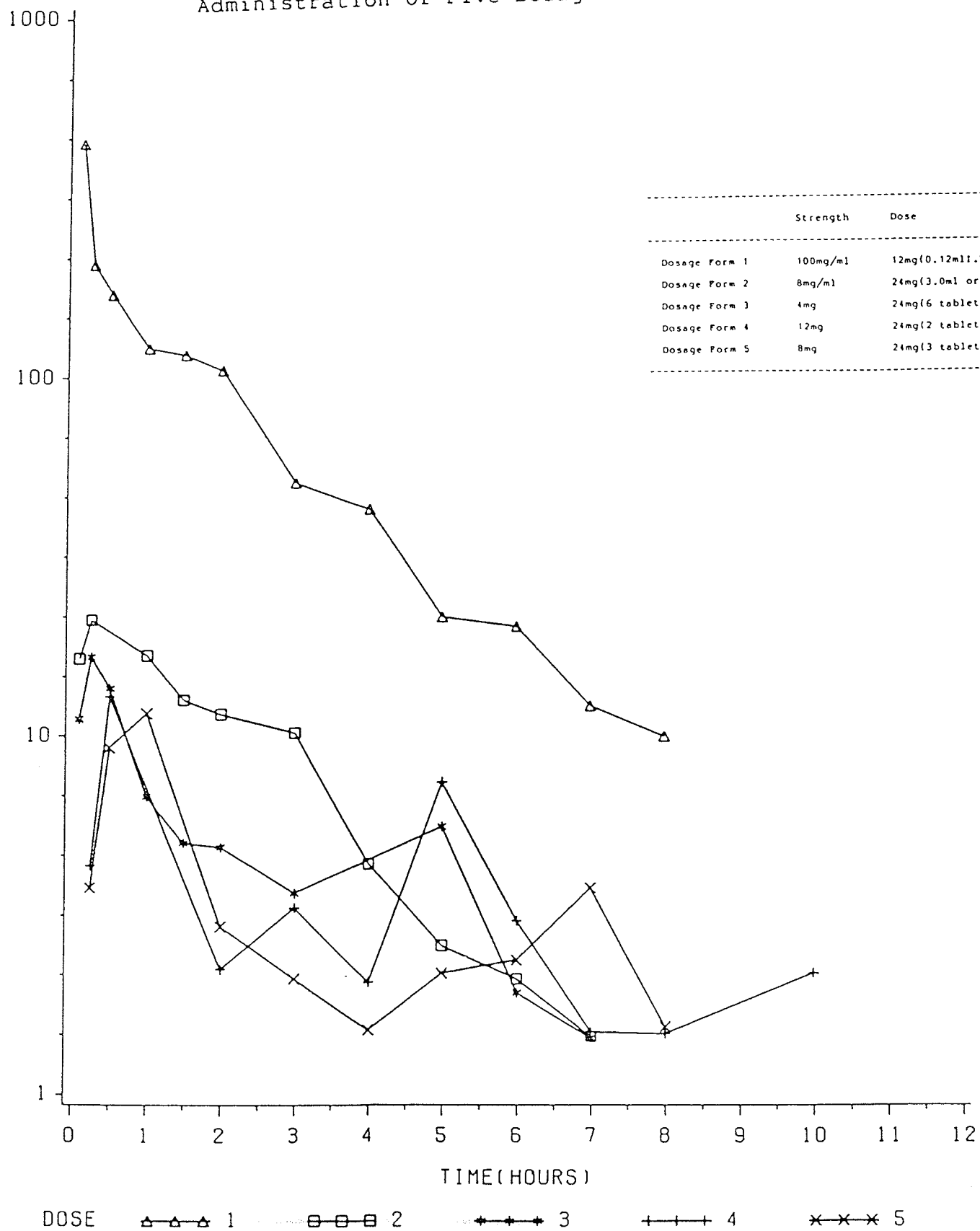


Table 8. Serum Concentrations of Chlorpheniramine after Administration of five dosage Forms in Rabbit C \*\*

Times (hr)	Dosage Forms				
	1	2	3	4	5
0.088	****	16.37	9.45	****	****
0.25	128.6	17.70	13.84	++++	2.50
0.50	125.5	11.75	12.61	1.92	5.71
1.0	114.51	11.39	****	2.62	4.78
1.5	78.26	****	****	3.10	3.90
2.0	48.67	10.00	****	4.73	4.73
3.0	40.09	7.15	8.40	3.43	****
4.0	21.00	6.60	5.40	3.16	2.51
5.0	14.76	5.84	4.24	3.43	3.86
6.0	****	6.13	4.99	2.53	3.74
7.0	****	5.46	4.45	4.37	4.63
8.0	9.45	****	****	2.43	8.80
9.0	****	****	****	1.50	****
10.0	****	****	****	++++	6.82

\*\*\*\* means no sample obtained.

++++ means that the concentration is below the limit of detection.

\*\* The unit of the above concentrations is nanogram per millilitre.

Fig.11 Serum Concentrations of Chlorpheniramine after Administration of Five Dosage Forms in Rabbit C

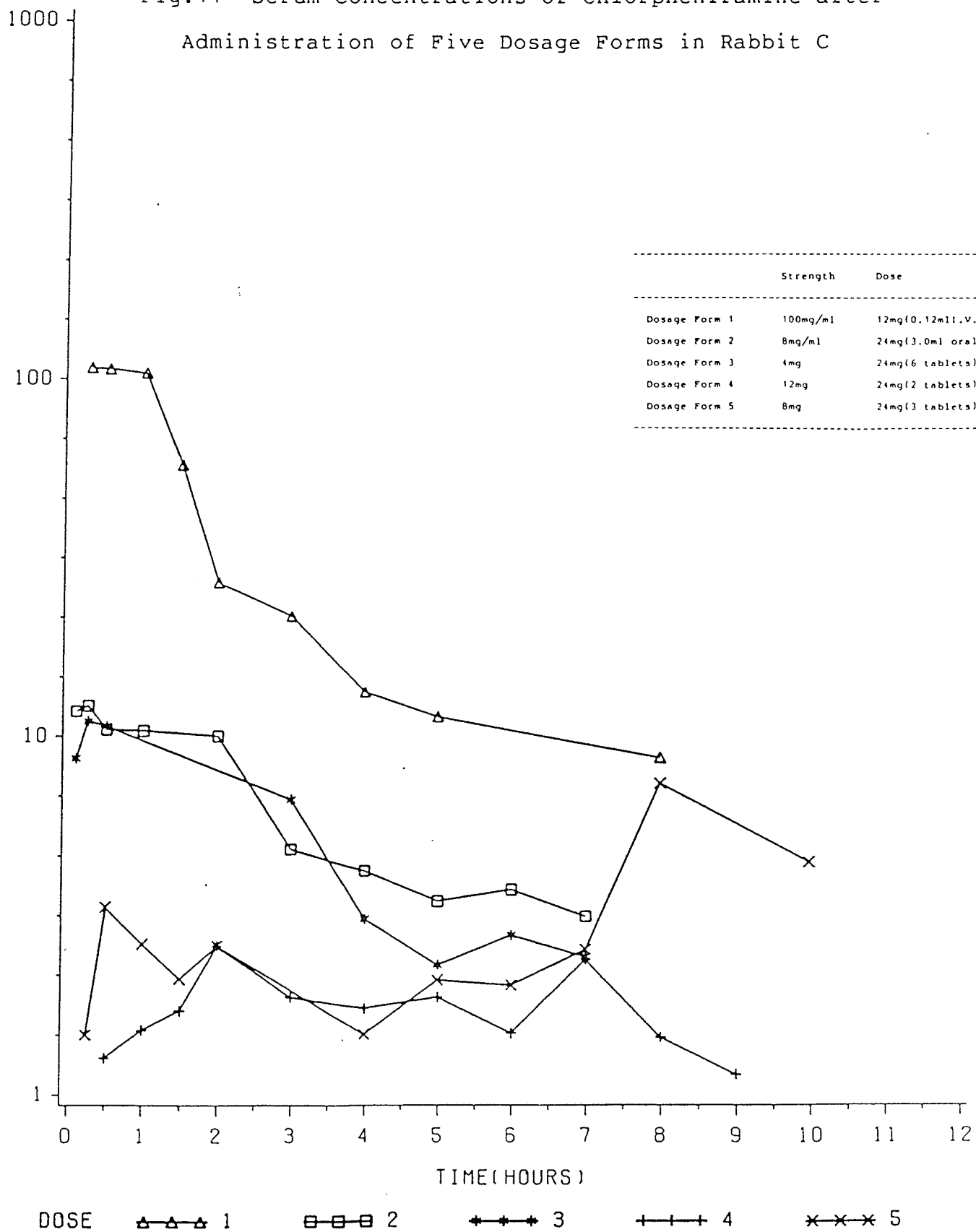




Table 9. Serum Concentrations of Chlorpheniramine after Administration of Five Dosage Forms in Rabbit D \*\*

Times (hr)	Dosage Forms				
	1	2	3	4	5
0.088	402.46	90.00	5.51	****	****
0.25	246.80	171.38	8.37	****	2.38
0.50	166.21	145.32	9.45	12.32	4.47
1.0	85.72	82.66	12.66	10.65	5.24
1.5	102.34	56.20	13.70	7.38	24.44
2.0	50.33	40.28	10.51	****	13.37
3.0	39.81	29.52	8.70	6.17	11.70
4.0	34.00	21.82	6.27	5.60	7.30
5.0	15.69	****	4.47	5.00	4.20
6.0	13.50	10.36	3.33	9.94	7.05
7.0	10.63	8.07	2.52	5.66	5.49
8.0	8.78	6.43	++++	****	2.89
9.0	****	****	****	3.95	++++

\*\*\*\* means no sample obtained.

++++ means that the concentration is below the limit of detection.

\*\* The unit of the above concentrations is nanogram per millilitre.

Fig.12 Serum Concentrations of Chlorpheniramine after Administration of Five Dosage Forms in Rabbit D

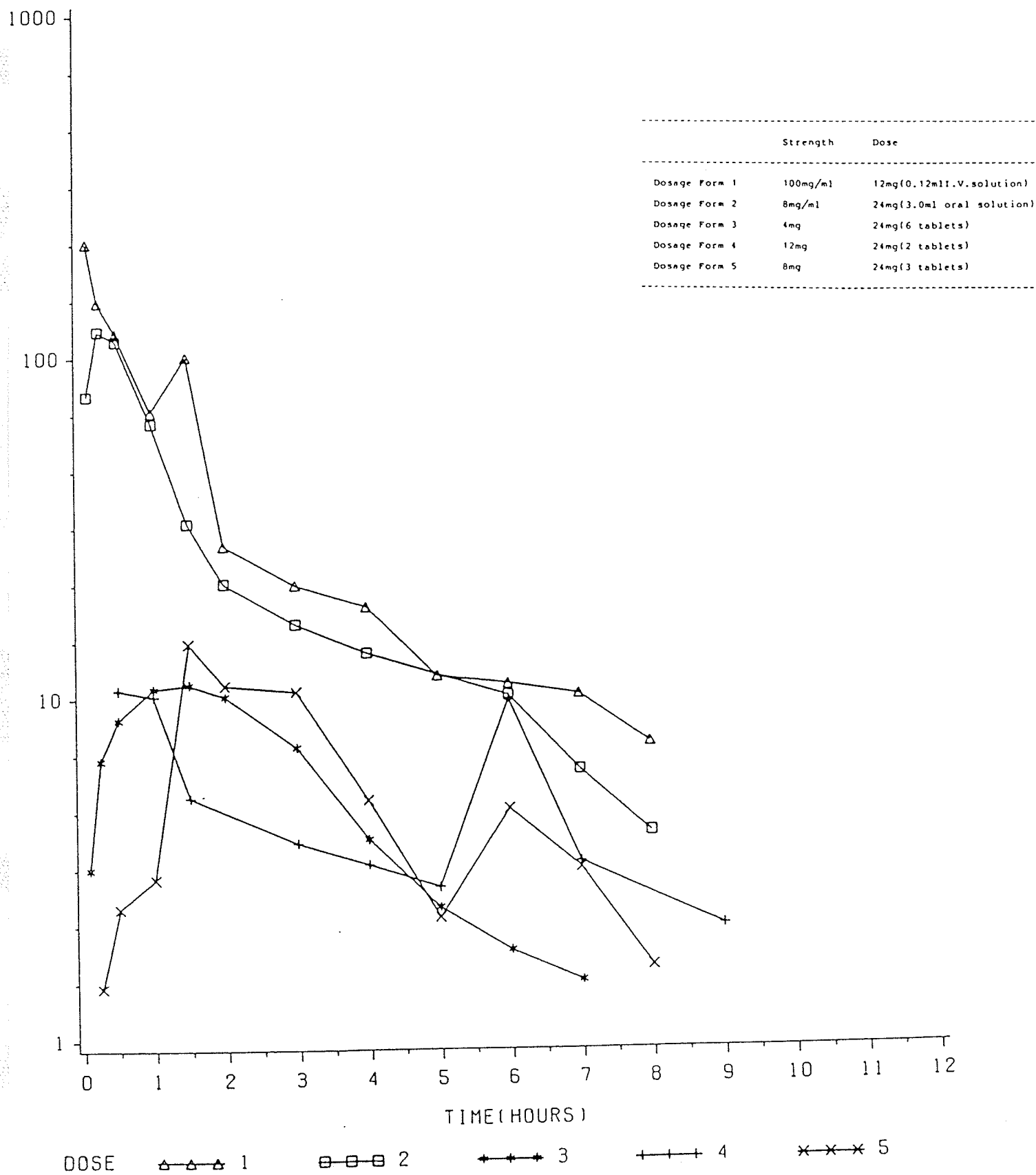


Table 10. Serum Concentrations of Chlorpheniramine after Administration of Five Dosage Forms in Rabbit E \*\*

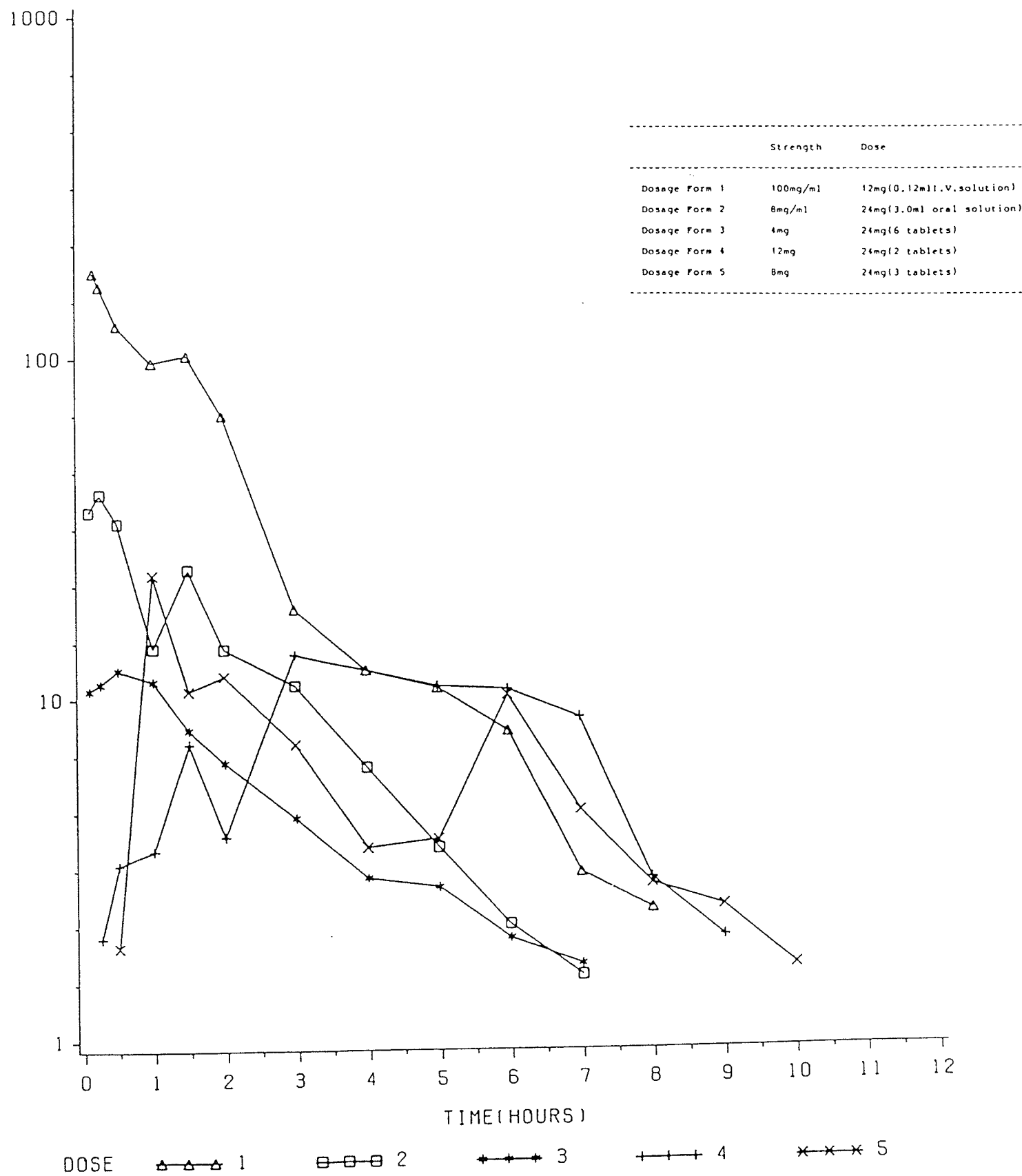
Times (hr)	Dosage Forms				
	1	2	3	4	5
0.088	****	59.50	12.38	****	****
0.16	326.89	****	****	****	****
0.25	290.98	64.16	14.14	3.70	****
0.50	187.51	56.50	17.70	5.62	3.46
1.0	98.90	23.38	14.70	6.00	42.74
1.5	106.81	44.15	9.18	8.81	12.00
2.0	84.74	23.07	8.31	6.36	15.90
3.0	33.53	13.33	6.84	21.49	8.78
4.0	17.27	8.17	5.25	****	6.05
5.0	12.58	6.05	5.00	13.00	6.27
6.0	9.11	4.02	3.66	12.05	10.54
7.0	5.38	2.67	2.96	9.45	7.01
8.0	4.41	++++	++++	5.22	5.06
9.0	****	****	****	3.67	4.47
10.0	****	****	****	++++	2.89
12.0	****	****	****	++++	++++

\*\*\*\* means no sample obtained.

++++ means that the concentration is below the limit of detection.

\*\* The unit of the above concentrations is nanogram per millilitre.

Fig.13 Serum Concentrations of Chlorpheniramine after Administration of Five Dosage Forms in Rabbit E



### 3.2.2. Curve Fitting

The serum concentration-time curves following the intravenous doses for all five rabbits were best described by biexponential equations. The coefficients and exponents are summarized in Table 14.

Table 11. Coefficients and Exponents of Bioexponential Equations for Chlorpheniramine Given by I.V. Doses to Rabbits

RABBIT	A	B	C	D	E
BW (kg)	4.48	4.63	4.73	5.33	3.97
Dose (mg/kg)	2.68	2.59	2.54	2.25	3.02
C1 (ng/ml)	175.22	810.11	122.45	418.36	323.41
C2 (ng/ml)	47.71	301.97	41.49	143.02	173.56
$\beta$ (hr <sup>-1</sup> )	0.3291	0.4030	0.1879	0.3794	0.4916
$\alpha$ (hr <sup>-1</sup> )	1.1202	7.9093	0.7083	5.1063	3.6380

The serum concentration-time curves were best described by triexponential equations following oral administration of solution and regular release tablets. The coefficients and exponents are summarized in Table 12. Data from the administration of the two repeat-action tablets can not be fitted into any pharmacokinetic models.

Table 12. Coefficients and Exponents of Triexponential Equations for Chlorpheniramine Given Orally to Rabbits

RABBIT	A	B	C	D	E
oral solution					
dose(mg/kg)	5.36	5.18	5.08	4.50	6.04
C1(ng/ml)	-33.38	-47.89	-69.82	-680.02	-70.51
C2(ng/ml)	20.42	22.87	58.06	605.43	28.61
C3(ng/ml)	12.96	25.02	11.76	74.5	41.90
Ka (hr <sup>-1</sup> )	6.2025	13.3371	13.2168	5.677	25.3887
$\alpha$ (hr <sup>-1</sup> )	1.4902	0.9887	8.8891	3.2568	0.8383
$\beta$ (hr <sup>-1</sup> )	0.3520	0.3251	0.1210	0.3145	0.3925
regular release tablets					
dose(mg/kg)	5.36	5.18	5.08	4.50	6.04
C1(ng/ml)	-82.69	-39.07	-15.36	-21.90	-25.38
C2(ng/ml)	70.46	28.69	4.91	4.17	12.86
C3(ng/ml)	12.23	10.38	10.48	17.73	12.59
Ka(hr <sup>-1</sup> )	10.0290	7.1848	12.0116	1.9360	10.4131
$\alpha$ (hr <sup>-1</sup> )	1.5178	1.6663	1.6952	0.7035	2.6117
$\beta$ (hr <sup>-1</sup> )	0.1599	0.1707	0.1350	0.2787	0.2036

### 3.2.3. Pharmacokinetic Parameters

The pharmacokinetic parameters calculated for chlorpheniramine in rabbits after five dosage forms are summarized in Table 11. Their means are presented in Table 14.

Table 13. Pharmacokinetic Parameters of Chlorpheniramine

Dosage Forms	Rabbits				
	A	B	C	D	E
i.v.					
T <sub>1/2</sub> (hours)	2.11	1.72	3.69	1.83	1.41
β (hour <sup>-1</sup> )	0.3291	0.4030	0.1879	0.3794	0.4916
AUC <sub>0--&gt;∞</sub> (ng/ml*hr)	261.31	775.76	351.10	418.06	442.84
CL <sub>p</sub> (ml/min)	759.48	257.81	569.64	478.40	451.63
V <sub>d</sub> (L/kg)	7.39	2.61	17.30	4.43	6.96
MRT(hr)	1.88	2.41	2.91	2.38	1.86
oral solution					
t <sub>max</sub> (hours)	0.50	0.25	0.25	0.25	0.25
C <sub>max</sub> (ng/ml)	27.49	39.20	17.70	171.38	64.16
AUC <sub>0--&gt;∞</sub> (ng/ml*hr)	47.52	94.00	98.05	303.24	128.63
K(hour <sup>-1</sup> )	0.3520	0.3251	0.1210	0.3145	0.3925
T <sub>1/2</sub> (hours)	1.97	2.13	5.73	2.20	1.77
MRT(hr)	2.46	2.49	5.33	2.49	2.17
MAT(hr)	0.58	0.08	2.42	0.11	0.31
F	9.09%	6.06%	13.96%	36.27%	14.54%



(continued)

## regular release tablets

tmax(hours)	0.25	0.25	0.25	1.5	0.5
Cmax(ng/ml)	50.9	29.86	13.84	13.7	17.7
AUC0--> $\infty$ (ng/ml*hr)	104.75	59.29	76.19	57.45	56.98
K(hour <sup>-1</sup> )	0.1600	0.1707	0.1365	0.2787	0.2036
T1/2(hours)	4.33	4.06	5.13	2.49	3.40
MRT(hr)	3.01	2.82	5.69	3.31	2.90
MAT(hr)	1.13	0.41	2.78	0.93	1.04
F	20.04%	3.2%	10.85%	6.87%	6.43%

---

## repeat action tablets(12mg)

tmax1(hours)	1.0	0.50	2.0	0.5	1.5
Cmax1(ng/ml)	7.26	19.95	4.73	12.32	8.81
tmax2(hours)	8.0	5.0	7.0	6.0	3.0
Cmax2(ng/ml)	4.4	8.84	4.37	9.94	21.49
AUC0--> $\infty$ (ng/ml*hr)	47.61	67.65	34.97	69.97	102.97
MRT(hr)	7.00	4.20	6.24	5.23	4.79
MAT(hr)	5.12	1.79	3.33	2.85	2.93
F	9.11%	4.36%	4.98%	8.34%	11.63%

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(continued)

repeat action tablets(8mg)

tmax1(hours)	1.0	1.0	0.5	1.5	1.0
Cmax1(ng/ml)	5.99	15.69	5.71	24.44	42.74
tmax2(hours)	7.0	7.0	8.0	6.0	6.0
Cmax2(ng/ml)	3.29	6.18	8.80	7.05	10.54
AUC0-->∞(ng*hr/ml)	41.59	51.32	85.33	71.94	101.53
MRT(hr)	6.26	4.23	9.81	5.18	5.16
MAT(hr)	4.38	1.82	6.90	2.80	2.30
F	7.96%	3.31%	12.15%	8.60%	11.46%

Table 14. Mean Pharmacokinetic Parameters of Chlorpheniramine

Parameters	Dosage Forms				
	1	2	3	4	5
T1/2(hours)	2.15±0.90	2.76±1.67	3.88±0.99	****	****
tmax1(hours)	****	0.3±0.11	0.55±0.54	1.1±0.65	1.0±0.35
Cmax1(ng/ml)	****	63.99±62.49	25.0±15.74	10.61±5.90	19.53±14.80
tmax2(hours)	****	****	****	5.80±1.92	6.80±0.84
Cmax2(ng/ml)	****	****	****	9.81±7.00	7.17±2.74
AUC0->∞	503.39±182.75	134.29±89.54	70.93±18.35	64.64±25.87	70.34±24.8
MAT(hr)		0.70±0.98	1.25±0.90	3.20±1.21	3.64±2.03
F		15.98%±1.19%	9.60%±6.35%	7.68%±3.02%	8.69%±3.50%

Table 15. Multiple Range Tests of Pharmacokinetic Parameters

Dosage Form	Mean	Grouping	F	PR>F
AUC0--> $\infty$				
Dosage Form 1	450.22	A	2.34	0.0004
Dosage Form 2	131.76	B		
Dosage Form 3	79.98	B		
Dosage Form 4	64.63	B		
Dosage Form 5	70.34	B		
Time to Peaks(tmax)				
Dosage Form 2	0.30	A	28.10	0.0001
Dosage Form 3	0.55	A		
Dosage Form 4(tmax1)	1.10	A		
Dosage Form 5(tmax1)	1.0	A		
Dosage Form 4(tmax2)	5.80	B		
Dosage Form 5(tmax2)	6.80	B		
Peak Concentrations(Cmax)				
Dosage Form 2	63.99	A	1.59	0.2284
Dosage Form 3	25.00	A		
Dosage Form 4(Cmax1)	10.61	A		
Dosage Form 5(Cmax1)	19.53	A		

(continued)

Half-life(hours)

Dosage Form 1	2.15	A	14.84	0.0006
Dosage Form 2	2.35	A		
Dosage Form 3	3.88	B		

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Mean Absorption Time(MAT)

Dosage Form 2	0.70	A	9.15	0.0005
Dosage Form 3	1.26	A		
Dosage Form 4	3.20	B		
Dosage Form 5	3.64	B		

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## Chapter IV

### DISCUSSION

#### 4.1. HPLC Assay of Chlorpheniramine

Chromatograms with differing degrees of resolution were found from the mobile phases with different acetonitrile composition (18%-25%) and different pH of the phosphate buffer (pH 2.5 and pH 2.7). Chromatographic parameters  $K_1'$ ,  $K_2'$ ,  $\alpha$ , R and N calculated from these chromatograms are shown in Table 3. The mobile phase used in the present study was acetonitrile-phosphate buffer ( $\text{NH}_4\text{H}_2\text{PO}_4$ , 0.075M) (22.5:77.5 v/v), which is slightly different from that of Simons *et al.* (27) and Athanikar *et al.* (17), giving a better separation of chlorpheniramine maleate and brompheniramine maleate and suitable retention times.

For serum samples, the retention times for chlorpheniramine and brompheniramine are 7.20 and 8.76 respectively. There are no interfering peaks from the serum.

Within the concentration range of 1.02 ng/ml to 101.51 ng/ml of chlorpheniramine maleate, the drug-internal standard peak height ratios were found to be linear. The coefficients of variation obtained over a period of 8 months were shown to be less than 15%.

## 4.2. Bioavailabilty Studies

### 4.2.1. Curve Fitting

The computer program PKCALC(46), together with an augmented copy of ESTRIP (47) strips serum concentrations versus time data according to the following generalized equation:

$$C = \sum_{i=1}^n A_i e^{-B_i t} \quad (12)$$

where  $c$  is the concentration of the drug at the time  $t$ ;  $n$  is the number of exponential terms; and  $A_i$  and  $B_i$  are parameters to be determined.

After stripping, the computer will print out parameters associated with each exponent, sum of squared derivations and coefficients of determination.

Attempts were made to fit the data obtained after intravenous injection into bi- and triexponential equations. Data obtained after administration of oral solution and regular tablets were fitted into tri- and tetraexponential equations. Data obtained after administration of Dosage Form 4 and 5 could not be fitted to any model since they are repeat-action dosage forms and no theory is available to fit them into any pharmacokinetic models.

The decline in serum concentrations of chlorpheniramine with time in all five rabbits studied following intravenous injections follows the biexponential equation:

$$C = A e^{-\alpha t} + B e^{-\beta t} \quad (13)$$

corresponding to a two-compartment open model. Coefficients of determination ranged from 0.969 to 0.992, indicating that the biexponential equation adequately describes the time course of serum chlorpheniramine concentrations after intravenous injections.

The biphasic serum level decline of chlorpheniramine has been reported previously both in humans and in animals(9,23,24,32). Peets et al. (9) in their report on the metabolism of chlorpheniramine in man, have shown a biphasic decline of plasma levels of chlorpheniramine, which was confirmed by Thompson et al. (32) in their study of the pharmacokinetics of chlorpheniramine in children. A biphasic decline has also been demonstrated both in dogs by Athanikar and Chiou(24)and in rabbits by Huang et al. (23). Huang et al. (23,26) also observed the triphasic decline of plasma levels of chlorpheniramine in human and rabbits. They noted that about 90% of the initial distribution phase would be gone in about 10min in rabbits with triexponential decay following intravenous injections. The lack of serum data for the first 10min due to difficulties in getting blood samples in a short period of time might explain why only biexponential decay profiles were observed in this study.

The serum concentration versus time data after administration of oral solution and regular release tablets were best fitted into an equation for a two-compartment open model with first order absorption:

$$C = Le^{-K_1t} + Me^{-\alpha t} + Ne^{-\beta t} \quad (14)$$

With the oral solution, coefficients of determination ranged from 0.88 to 0.99, while coefficients of determination ranged from 0.87 to 0.98 with the regular tablets. These results indicate that the triexponential equation adequately describes the time course of serum concentrations following administration of these dosage forms.

Kotzan et al. (29) fitted their data obtained after administration of syrups into a similar model. No other reports on animals are available.

#### 4.2.2. Bioavailability Studies

The pharmacokinetic parameters of five rabbits are shown in Table 13. Their means are shown in Table 14.

The mean  $t_{1/2}$  in five rabbits after intravenous injection of 12mg chlorpheniramine maleate was 2.16hr with a range of 1.41-3.69hr. These values were similar to those found in rabbits and dogs previously (8,23,24,25) (mean 2.52 and 1.73hr respectively). These values were much shorter than those were reported for humans, 15.6hr from Thompson and Leffert(32), 28hr from Peets et al. (8) and 22.5 from Huang et al. (26).

An early study(40)indicated that during the 24 hr fast-  
ing, the stomach emptying was significantly prolonged in rabbits. The slowed gastric emptying could therefore delay the transfer of chlorpheniramine to the small intestine,



which is thought to be the main absorption site because of its large surface area and the basic absorption characteristics of chlorpheniramine. Huang et al. (25) reported that the absorption rate constants were decreased after fasting for 24 hours.

During the present study, 24mg of chlorpheniramine solution were administered to one rabbit (Rabbit B) under fasting and nonfasting conditions. The serum concentration-time data were shown in Table 5 and plotted together in Fig.10. Following administration of oral solution under fasting conditions, peak concentration appears at 1hr, while it was 0.25hr under nonfasting conditions. The  $t_{1/2}$  calculated from the data obtained from the fasting animal (6.2hr) is much longer than that obtained in i.v. studies [1.9hr in the present study and in literature(22)], which is consistent with the result reported by Huang et al. (25) that fasting can prolong the stomach emptying time, thereby delaying the absorption of chlorpheniramine. However, the  $AUC_{0 \rightarrow \infty}$  data obtained under fasting (74.20 ng\*hr/ml) was smaller than that obtained under nonfasting conditions (94.0ng\*hr/ml). Therefore, all following oral studies were carried out under nonfasting conditions.

In preliminary studies, commercially available chlorpheniramine syrup was given to rabbits in a dose of 12mg. No drug could be detected from serum samples taken at different times after administration. This may be due to the fact that a

large volume of chlorpheniramine syrup (24ml) had to be administered because of the low concentration of drug (2.5mg/ml). The large volume of syrup in the stomach may prolong the stomach emptying and thus adversely affect the absorption of chlorpheniramine.

Chlorpheniramine is also available in a liquid sustained-release dosage form in combination with phenylpropanolamine HCl(55). It was not used in this study because the chlorpheniramine concentration in this dosage form is low (0.8mg/ml) and a large volume would have to be administered to make the dosage equal to that used in other dosage forms. There is concern that a large volume of liquid in the stomach may affect the absorption of chlorpheniramine as in the case of the oral syrup.

Statistical tests show that there are significant differences in the elimination half-lives obtained following administration of intravenous injections, oral solution and regular release tablets. The elimination  $t_{1/2}$  of regular release tablets was significantly higher than that of intravenous injections and oral solutions as measured both by Tukey and Bonferoni multiple range tests. It is believed that the difference can be attributed to the slower release of chlorpheniramine from the tablets when compared with oral solution from which the drug is immediately available.

The peak concentrations of chlorpheniramine varied widely both among dosage forms and among rabbits. However, multiple range tests failed to show that there are any significant difference among them. The lack of significance may be attributable to the large intra- and intersubject variability which has been previously reported both in humans and in animals(23,24,29).

There is no significant difference among AUC<sub>0-->∞</sub> of oral solution, regular release tablets and two repeat action tablets. The absolute bioavailability values calculated are 15.98%, 9.60%, 7.68% and 8.69% respectively, which is also consistent with the literature(23,24).

The method of statistical moments, which is independent of any specific pharmacokinetic model, has been used to interpret the data on oral administration(50,51,52). Jackson and Chen(53) used this method, together with other usually used parameters such as C<sub>max</sub> and t<sub>max</sub>, to evaluate bioequivalence of dosage forms.

Mean absorption times(MAT) after administration of various dosage forms on different subjects were calculated and an ANOVA was run to examine whether there was any significant difference among them. The MAT obtained from the two repeat action tablets(Dosage Form 4 and Dosage Form 5) were significantly longer than those obtained after administration of oral solution and regular release tablets, which is to be expected.

The mean residence time after the intravenous dose was 2.29hr, which is not significantly different from the mean absorption time when giving oral solution and regular release tablets. This result indicates that after oral administration, chlorpheniramine kinetics are not rate limited by absorption.

Chlorpheniramine repeat action tablets contain 8mg and 12mg of chlorpheniramine maleate and they are so designed that the "dosage is divided equally between an outer layer for rapid absorption and an inner core protected by special timed barrier for releasing 3-6hr after ingestion"(44).

In this study, two peaks were observed following administration of repeat action tablets. The first peak concentration occurred at a time ranging from 0.5 to 1.5hr, while the second one occurred at a time ranging from 3 to 8hr. Multiple range tests indicate that there are no significant differences for the time to peak concentrations among four oral dosage forms ( $t_{max1}$  for Dosage Form 4 and Dosage Form 5). However,  $t_{max2}$  of Dosage Form 4 and Dosage Form 5 are significantly longer than the  $t_{max}$  of Dosage Form 1 and Dosage Form 2 and  $t_{max1}$  of Dosage Form 4 and dosage Form 5. No lag time in absorption was required to fit the data from the present study in contrast to a lag time of 0.70hr which was needed to fit data in humans reported previously(29). This confirms the previous conclusion that chlorpheniramine kinetics are not rate-limited by absorption(24,25,26,29).

Although there is no significant difference in  $AUC_{0-\infty}$  among the four oral dosage forms, an examination of  $AUC_{0-\infty}$  data did show that the amount of chlorpheniramine released from the repeat action tablets is less than that of oral solution, but similar to that of regular release tablets.

There are three kinds of rabbit models available for bioavailability studies. The "stomach-emptying-controlled" rabbits were found to be better than the fasted rabbits for the bioavailability study of griseofulvin tablets(41,42). Fasted rabbits were found to be a good animal model for the bioavailability study of carbamazepine(43). Huang et al. (25) showed that the non-fasted rabbit was preferable to the fasted rabbit when studying the bioavailability of chlorpheniramine oral solution. The non-fasted rabbit model has not been compared to the "stomach-emptying-controlled" rabbit model at this time(41,42,43,25). In the present study, non-fasted rabbits were used as the animal model for the bioavailability study of chlorpheniramine repeat-action tablets, based on the results of Huang et al. (25) and some preliminary studies conducted in this laboratory(Table 5 and Fig.8).

In the present study, the absolute bioavailability of the two repeat-action chlorpheniramine tablets in the rabbit is in the same order as those observed in the human, and the serum concentration versus time plots for the two repeat-action tablets are similar to those found in the

human(26,29,31,44). Therefore, it is concluded that the rabbit is an useful animal model for studying bioavailability of chlorpheniramine repeat-action tablets.

## Chapter V

### SUMMARY AND CONCLUSION

The object of the present study was to evaluate the usefulness of the rabbit as an animal for measuring the bioavailability of drug dosage forms, especially sustained-release products. Five dosage forms of chlorpheniramine: intravenous injection, oral solution, conventional and repeat-action tablets, were given to five rabbits in a five-way crossover study. Blood samples were taken at different times. Modified extraction and HPLC procedures were used to determine the serum concentration of chlorpheniramine with a detection limit of 1.0ng/ml.

Data obtained after administration of different dosage forms were fitted to different compartmental models using the BASIC program: PKCALC, and various pharmacokinetical parameters including  $t_{1/2}$ ,  $AUC_{0 \rightarrow \infty}$ , MAT and absolute bioavailability were calculated. The serum concentration versus time curves following intravenous doses were best fitted to a two-compartment model from which a mean elimination half-life of  $2.15 \pm 0.90$  hr was obtained, while the serum concentration versus time curves following administration of oral solution and regular tablets were best fitted to a two-compartment model with a first-order absorption phase.

Absolute bioavailability for the oral solution, conventional and repeat-action tablets are 15.98%, 9.60%, 7.68% and 8.69% respectively. There are no statistically significant differences among them, however, there are significant differences of MAT among the four oral dosage forms. The MAT obtained after administration of repeat-action tablets are significantly longer than that obtained after administration of oral solution and regular tablets, which is expected.

Following administration of two repeat-action tablets, two peaks in serum concentrations were observed. The first one occurred at 0.5 to 1.5hr, while the second one occurred at a time ranging from 3.0 to 8.0hr. These results are consistent with the design of these repeat-action dosage forms.

Results from the present study indicate that rabbits can be used as an animal model for the bioavailability studies of repeat-action chlorpheniramine dosage forms. Further studies with other drugs and various sustained-release products are needed to determine if this animal model can be used for the determination of bioavailability of other sustained-release products.



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