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IN MAN

UNIVERSITY ALBERTA

DEGREE Ph.D. YEAR GRANTED April 1970

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THE UNIVERSITY OF ALBERTA

"BIOPHARMACEUTICAL STUDIES OF CHLORPHENTERMINE IN MAN"

by

© HUNG WON JUN

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled:

"BIOPHARMACEUTICAL STUDIES OF CHLORPHENTERMINE IN MAN"

Submitted by

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in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

A procedure has been developed for the qualitative and quantitative determination of chlorphentermine in urine by gas-liquid chromatography. The influence of urinary pH and urine volume on the excretion profiles of the drug in humans has been investigated. Both urinary pH and urine volume were found to influence the excretion rate of chlorphentermine. The metabolic transformation of the drug has been found to be negligible.

The kinetics of absorption, distribution, and excretion of chlorphentermine have been examined using an electronic analog computer on the basis of urinary excretion data, after oral administration of 'free' dosage forms to man. This device also has been used to determine the *in vivo* rate of release of the drug from a prolonged-release preparation. *In vivo* drug release from the prolonged-release preparation was correlated with *in vitro* drug release data.

Blood level studies of chlorphentermine in man following oral administration of the drug in solution, as a prolonged-release preparation, and as an intravenous injection have been carried out by a modified analytical technique. The drug was found to have a long plasma half-life in the body, and the therapeutic need for a drug preparation of this type may therefore be doubtful. The significance of blood level studies in evaluating biopharmaceutical parameters is considered with respect to dosage form evaluation, even though such studies may not represent the therapeutic blood levels in cases where extensive drug localization occurs in tissues. The long plasma half-life and minor biotransformation of the drug is probably not due to extensive plasma protein binding.

Plasma levels and urinary excretion data following administration of a multiple dosage regimen of the prolonged-release preparation showed that a significant amount of the administered chlorphentermine was accumulated in the body. The pharmacological and therapeutic implications of drug accumulation are discussed.

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CHAPTER I.

AIMS AND OBJECTS OF THE PRESENT INVESTIGATION.

The primary aim of this study was to investigate the biopharmaceutical characteristics of chlorphentermine in man following its administration in various dosage forms. Additional factors such as metabolism, plasma protein binding, and tissue distribution which influence the drug's pharmacokinetics would also be studied. The information obtained would thus provide a proper evaluation of the influence of the dosage form on the therapeutic activity of chlorphentermine.

The results of the preliminary study showed that the drug had a long biological half-life which suggested that consideration be given to an investigation of the potential accumulation of the drug in the body following a multiple dosage regimen.

The proposed studies necessitated the development of a sensitive and specific analytical procedure for the determination of chlorphentermine in biological fluids which would allow accurate construction of blood level- and urinary excretion rate versus time curves.

CHAPTER II. LITERATURE SURVEY

A. CHLORPHENTERMINE AS AN ANOREXIGENIC DRUG

One of the more subtle and serious health hazards of our time is obesity. It develops so gradually and under such pleasurable circumstances that treatment is often difficult. The victim feeling hopelessly trapped in his own fat, tends to rationalize his condition. Not only can obesity become a physical impediment in itself, but it may predispose its victims to heart disease, diabetes, liver disease, and other complications (1). In particular, obese men have a higher death rate from degenerative cardiovascular and renal disease, diabetes mellitus and cirrhosis of the liver; whereas obese women have a greater excess mortality from diabetes mellitus and diseases of the gall bladder, especially gall stones.

Obesity has been shown to arise primarily from a marked increase in food intake and, therefore, the basic problem in obesity is the cause of the increased appetite. From studies with animals, Anand and Brobeck (2) and Brobeck, et al, (3) have shown that the appetite is mainly regulated by the hypothalamus which contains an appetite and a satiety center. In every case of obesity it has been proved that a sufficient reduction of calorie intake will produce loss of weight, but there is not necessarily an ability or willingness on the part of the patient to control the appetite.

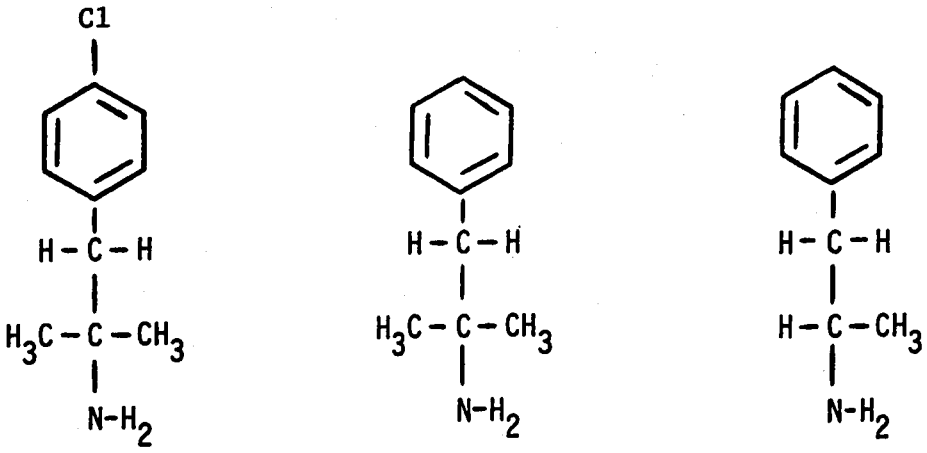
All presently available and accepted therapeutic methods of obesity treatment are basically dependent upon diminishing caloric intake by dietary restriction; the use of drugs to reduce appetite; increasing caloric output by exercise; the use of drugs to increase total metabolic rate. It is, however, easier to achieve a decrease in

food intake by the aid of drugs than to stimulate an increased caloric expenditure in the patient. Exercise can not be started too quickly in an obese individual; even if it is accomplished, it results either in extreme exhaustion or in increased appetite, which further increases the food intake, thus creating a vicious circle.

Chlorphentermine has been introduced as a marketable drug in an attempt to provide a compound with similar anorexigenic properties to amphetamine, but with less stimulation of the central nervous system. Restlessness, sleeplessness, and irritability are the major subjective complaints of amphetamine (4,5) which has been the most widely used drug for the treatment of obesity.

It is generally agreed that the anorexigenic amines exert their action largely via the central nervous system rather than a peripheral increase of metabolic rate (6). The first pharmacological evaluation of chlorphentermine was reported by Holm, et al, (7) in 1960, and subsequent studies by various authors (8,9) have shown that chlorphentermine has no significant CNS stimulating effects when used in therapeutic doses.

Chlorphentermine was synthesized by Bachman, et al, (10) in 1954. The chemical structure of chlorphentermine (p-chloro-α,α-dimethylphenethyl amine) is very similar to those of other sympathomimetic amines (see Figure 1.).



Chlorphentermine Phentermine Amphetamine

Figure 1. The Chemical Structures of Chlorphentermine and Related Compounds.

Chlorphentermine hydrochloride is soluble in water (>20%) and has a melting point ranging from 232° to 235°C.

B. BIOLOGICAL FATE OF CHLORPHENTERMINE

Little information has been reported concerning the biological fate of chlorphentermine in either man or animal.

Dubnick, et al, (11) reported a study of brain and whole body levels of chlorphentermine in mice compared to compounds related structually but lacking the benzene ring substituent, for example, phentermine, mephentermine, and d-amphetamine. The results obtained from this study indicated that chlorphentermine whole body levels disappeared more slowly than did the three related compounds. The concentration of chlorphentermine fell linearly to about 10 mcg/g (whole mouse) at 5 hours while there was a rapid fall to the same level with the three related compounds at one hour. Chlorphentermine was also shown to reach higher and longer lasting brain levels than the other three analogs. These authors compared the partition coefficients of the drugs under study between aqueous buffer (pH 7.4) and benzene. The results showed that chlorphentermine had a lipid/water partition coefficient seven times greater than mephentermine, ten times that of phentermine, and fifteen times that of d-amphetamine.

Jackson and Whyte (12) stated that chlorphentermine appeared to be metabolized differently to d-amphetamine, probably due to the para chloro substitution on the benzene ring, since it has been reported (13, 14) that the major route of metabolism of d-amphetamine is by p-hydroxylation.

Opitz and Weischer (15), using thin-layer chromatography, examined the urinary excretion of chlorphentermine and phentermine following administration of the drugs to rats. They found that chlorphentermine was excreted mainly unchanged in the urine over the course

of six to nine days, but that phentermine was mainly excreted as metabolic products. The excretion of chlorphentermine was stated to be dependent upon changes in urinary pH, but independent of urine volume. The cumulative urinary excretion of chlorphentermine in the rat was shown to be the same when the drug was administered orally or intraduodenally, with 13 to 20 mcg/ml chlorphentermine being found in the bile. Following administration of chlorphentermine and phentermine hydrochlorides to man, the urinary drug excretion patterns were similar to those seen in the rat. No urine pH control was maintained during any of these studies.

Portnoy, et al, (16) studied the biological fate of chlorphentermine in rhesus monkeys. They administered radio-isotopically labelled chlorphentermine-^{14}C by oral and intramuscular routes and observed that peak blood levels occurred 1 hour after intramuscular injection and 2-4 hours following oral doses. The blood levels were low at all times and became insignificant after 24 hours. Urinary excretion accounted for 68 to 83% of the drug administered and an additional 0.5 to 8.5% appeared in the feces. Parallel studies in human subjects gave similar results.

Brookes (17) examined the urinary excretion profiles of chlorphentermine in man under various urinary pH conditions and also with controlled urine flow rate. His finding that the rate of urinary excretion of chlorphentermine is influenced by the urine flow rate is not in agreement with the results of Opitz and Weischer (15). However, his observation of independence of urinary excretion rate with controlled urine flow rate was made with a drug dosage of only 6 mg of the drug administered as the hydrochloride salt.

Dubnick, et al, (18) also using radio-isotopically labelled

chlorphentermine made extensive studies of the distribution and metabolism of chlorphentermine in rats and mice. In these animal studies, they found a long duration of the pharmacological effects of chlorphentermine as well as its prolonged presence in various body tissues, notably brain, liver, and lung. They proposed that the long duration of action of the drug was probably due to its negligible metabolism since p-hydroxylation is blocked by the p-chloro substitution.

Many authors have related differences in biological disposition and pharmacological activity of phentermine and/or amphetamine and their halogenated derivatives, chlorphentermine and chloroamphetamine, to the presence of the halogen radicals which may hinder the metabolic route, p-hydroxylation (13, 14).

Pletscher, et al, (19) compared brain levels of p-chloro-N-methylamphetamine with those of amphetamine in the rat. The results of this study showed that the halogenated derivative, p-chloro-N-methylamphetamine, reached much higher brain levels (4 times) and persisted longer in the brain than the non-halogenated compound. They stated that the chlorine atom might be responsible for the relatively high affinity of the drug for the brain. Duhault and Fenard (20) also observed similar results following administration of fenfluramine (containing p-CF_3 substituent) and amphetamine in dogs and men. Fenfluramine was found to have higher tissue levels and a longer biological half-life than amphetamine.

Fuller and Hines (21,22) compared tissue levels of amphetamine and some of its chlorinated derivatives following intraperitoneal injection of equimolar doses in mice and rats. They found that in both species

p-chloroamphetamine disappeared more slowly from the body than amphetamine - the elimination half-lives for p-chloroamphetamine in rats and mice were six and five hours respectively, but the elimination half-life for amphetamine (one hour) was the same for both species.

Nielsen, et al, (23) carried out a similar study with amphetamine and its p-chloro-derivative in the serum of dog and rat and also in the brain of the rat. Rat brain levels of p-chloroamphetamine reached concentrations greater than twice those observed with amphetamine and declined slowly for 24 hours. This result is in agreement with the findings of Pletscher, et al, (19) and Fuller and Hines (21) for p-chloramphetamine. In this study, Nielsen, et al, qualitatively compared the urinary excretion of p-chloroamphetamine with that of amphetamine under similar conditions, following subcutaneous or intramuscular injections of 3 mg base/kg, using a paper chromatographic technique. P-chloroamphetamine was excreted unchanged, and was easily detected for as long as 48 hr after injection, but a fraction of the amphetamine dose was excreted as its p-hydroxy derivatives and only a trace of the unchanged drug was found in the urine at 48 hr.

C. KINETICS OF DRUG ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Pharmacokinetic studies are often performed to determine basic biopharmaceutical drug parameters such as rates of absorption, distribution, metabolism, and excretion of a drug or its metabolites under various experimental conditions. From a first glance at the complicated biological processes involved, the determination of such parameters appears to be difficult. However, most of these apparently diverse processes can be conveniently described by the application of a few basic concepts. One of these concepts is to use a mathematical model to simulate and simplify a real system (24, 25). These mathematical models assume that the body can be described as one or more connected compartments or pools in which an amount of drug or metabolite is homogeneously distributed throughout an apparent volume of distribution. The transfer of amounts of drug between compartments is usually found to be adequately described by first-order kinetics, This finding greatly facilitates the mathematical description of biological systems. Sometimes it is necessary to make further simplifications in order to mathematically describe the time course of the concentrations or amounts of drug, for the whole or part of the time, in the body. These mathematical expressions furnish a relatively easy and concise means of assessing a qualitative biological system in terms of its quantitative interpretation.

Mathematical models involving drug absorption, distribution, metabolism, and excretion primarily require measurement of the drug in serum, plasma, blood or urine with time. Implicit in such treatments are assumptions concerning the relationships expected to exist between different compartments. After fitting of the experimental data to the

theoretical equations of the mathematical model, the suitability of the model is judged on the basis of the agreement between the experimentally obtained and the theoretical values. Therefore, all useful models are, necessarily, based on specific and/or general assumptions which may not be an exact replica of the real system (26). Thus, the usefulness of any model depends upon the validity of these assumptions which must be rationalized and tested by the use of all the experimental data obtained.

The first extensive studies on the kinetics of urinary excretion of physiological substances were carried out by Widmark (27,28) in the 1920's, but it is within the last two decades that the kinetics of drug administration have been investigated. However, in the majority of cases the same principles apply for both classes of compounds. Using acetone as a test substance, Widmark studied the appearance and disappearance of acetone from the blood after its administration by several routes. He recognized that after absorption of the dose was complete the concentration in the blood decreased in an exponential manner, and that rate of elimination was directly proportional to blood concentration.

Later, Dominguez (29, 30) studied in considerable detail the relationship between plasma concentration and excretion rate, and other parameters involved in the elimination and distribution of creatinine and urea. Dominguez described these processes in terms of first-order kinetic equations. Another interesting study by Dominguez and Pomerene (31) was their development of a method to calculate drug absorption rate following oral administration. A term, volume of distribution, which Dominguez defined earlier was applied in this calculation.

Following the earlier kinetic studies of Widmark, Dominguez,

and others (32,33), Teorell conducted a general theoretical treatment of the kinetics of drug transfer across various body membranes and drug biotransformation (34,35). In the reports, Teorell introduced the concept of a two-compartmental model to describe the tissue distribution of a drug and derived differential equations for calculating the amount-time course of a drug at the absorption site, in the blood, in the tissues, the amount eliminated, and the amount biotransformed. Teorell (35) also described an example of constant-rate intravenous drug administration as well as instantaneous intravenous injection, and derived the appropriate expressions to indicate the amount-time courses of drug in blood and tissues, together with biotransformed and excreted drug amounts under these conditions of administration. His mathematical expressions were primarily based on the assumptions of drug transfer across body membranes by simple diffusion. Teorell's studies have greatly contributed to the mathematical basis of present day biopharmaceutics and pharmacokinetics.

Though several authors (36,37,38) have discussed in various degrees the application of the two-compartmental model to describe distribution of drug in the body, the majority of kinetic studies have been based on the single compartmental model. Recently, Riegelman, et al, (39,40,41) discussed the suitability of the two-compartmental model to depict the distribution of a drug in the body on the basis of physiological phenomena and on a mathematical viewpoint. They proposed several equations in order to calculate the kinetic parameters of a two-compartmental model. However, there is still controversy over the necessity of applying this more complicated model.

Since concentrations of a drug and its metabolites are usually much greater in urine than in blood, and as urine is relatively easy to

obtain, urinary excretion studies have been the most practical method of examining *in vivo* biopharmaceutical parameters. Such techniques assume that the time course of drug absorption is reflected in drug blood levels which, in turn, are considered to be directly proportional to the urinary excretion rate of the drug. However, care must be taken in interpreting results from determinations of drug or its metabolite concentrations in urine if a drug or its metabolites are only partially dissociated over the normal urinary pH range. This is because drug reabsorption from the kidney tubules back into the blood stream is considered to be a passive process selective only for the lipid-soluble undissociated forms present in the tubular luminal fluid (42,43). Excretion of drug will thus depend upon the concentration gradient of undissociated forms between the luminal and peritubular fluids. If the pH of the latter is considered to be constant at about pH 7.4, then the reabsorption of drug, and hence its excretion will be largely controlled by the urinary pH. An acidic urinary pH will result in a high excretion rate of a weakly basic drug whereas a more alkaline urine will result in a lower excretion rate. The converse will apply for weakly acidic drugs. Thus, drug excretion rates will reflect plasma drug levels only when the renal reabsorption of these drugs is negligible.

The degree of plasma protein binding and that of tissue localization also influences the rate of urinary excretion of drugs. Weiner, *et al* (44), reported that the slow biotransformation and negligible kidney elimination of dicoumarol results from the high degree of protein binding of the drug. Prolonged blood levels of chlortetracycline were explained by Sirota and Saltzman (45) on the basis of protein binding of the drug since the renal excretion was found to be solely the result of the pro-

cess of glomerular filtration. Kunin (46) has similarly related prolonged plasma levels, slow kidney clearance, and low apparent volume of distribution of methacycline to its degree of binding. Bertazzoli, et al (47), and DiCarlo, et al (48), have suggested that an interdependence does exist between the rates of elimination of sulfonamides and their degree of plasma binding.

Martin (49) presented the theoretical aspects of the kinetics of elimination of drugs possessing high affinities for plasma proteins. He discussed, using as an example, a model in which drug was distributed between residual water and plasma. Binding occurred to form a 1:1 drug-protein complex and drug disappearance proceeded at a rate which was directly proportional to the concentration of the free drug. Martin (50) also emphasized another characteristic attributable to the plasma binding of drugs having a high affinity for proteins; that there is a drug dosage range within which small increases in dose result in relatively large increases in the amount of non-protein bound drug in the body. He noted that this behavior could show interesting manifestations on both dose response characteristics and pharmacokinetic properties of some drugs.

The rate and extent of absorption are very important factors in determining the magnitude of blood and tissue levels of drug with respect to time after administration. Quantitative methods for estimating rate of absorption have been developed by various authors. The method of Dominguez and Pomerene (31) is applicable to blood or plasma drug levels versus time data, and the method of Nelson to drug urinary excretion rate versus time data (51).

Modifications of the methods of Dominguez and Pomerene (31), and Nelson (51), have been reported by Wagner and Nelson (52). The method of

Wagner and Nelson (52) is applicable to both blood concentration data and to urinary excretion measurements of unchanged drug. The method involves plotting the per cent absorbed drug versus time in order to calculate drug absorption rate. Nelson (53) later extended the method to allow the estimation of absorption rate from measurements of metabolite concentrations in blood; this method, however, is restricted to cases where little or no unchanged drug is eliminated via the urine. Scholer (54) also reported a method for the determination of absorption rates by integration of the rates of appearance and disappearance of the drug.

Levy and Miller (55) showed that the time of onset of a suitable pharmacological response, under conditions where a constant drug concentration gradient was maintained across the absorbing membranes, reflected drug absorption rate. Garrett, et al (56), estimated the rate constant for absorption by using an analog computer to make a best fit to a line passing through serum concentrations observed following oral drug administration. Other drug rate constant parameters of the system were previously estimated from intravenous drug administration studies in both normal and nephrectomized dogs. Levy and Hollister (57) applied Wagner and Nelson's equation (52) in their study of inter- and intra-subject variations in drug absorption kinetics.

The study of drug absorption from the gastrointestinal tract must first be considered in terms of the structure of the absorbing membranes. The classical study of Overton (58) on the permeability of animal cells to organic compounds led to the view that the cell boundary is lipoidal in nature. This suggests that a drug in its undissociated form would more readily penetrate across body membranes than the dissociated form due to the greater lipid solubility of the former.

Later, Schanker, et al (59), and Hogben, et al (60), studied the absorption of drugs in the rat and in man, which provided the concept that the absorption barrier between gastrointestinal lumen and blood had the characteristics of a lipoidal membrane permeable to lipid-soluble substances in their undissociated form. Accordingly, the lipid-soluble, undissociated forms of weak organic acids and bases are readily absorbed, whereas ionized drugs such as the quaternary ammonium compounds, are very poorly absorbed. A highly lipid-soluble substance, for example, thiopental is absorbed readily, and a poorly lipid-soluble drug, sulfaguanidine, is only slowly absorbed.

In addition to its lipid solubility, the property of a drug to complex with proteins or other body components may be an equally important factor affecting drug absorption. When a molecule contacts the cell membrane the molecule must adhere to the membrane before it can be absorbed into the cell. Schedl and Clifton (61) studied the binding of a series of steroids to albumin and found a direct relationship between steroid absorption rates and protein binding, as determined by perfusion studies with the small intestine of the intact rat. Interactions of drugs with substances present in, or secreted by, the gastrointestinal mucosa can be of considerable consequence in the absorption of some drugs. Quaternary ammonium compounds are absorbed poorly and irregularly from the gastrointestinal tract (62). While this probably is related to some extent to their poor lipid-solubility, Levine, et al (63), reported quaternary compounds can form nonabsorbable complexes with intestinal mucin.

Levy, et al, reported a series of investigations on the effect of complex formation on drug absorption in rats and goldfish. Levy and Reuning (64,65) suggested that the absorption rate of drugs can be

modified by formation of complexes which differ from the free drug mainly in size, or in lipid-water partition coefficient. Their studies were carried out with salicylic acid and its complexes with absorbable and nonabsorbable compounds. Levy and Anello (66,67) observed that polysorbate 80 enhanced the absorption of secobarbital by increasing the permeability of the biologic membrane to the drug, rather than by forming a more rapidly absorbed nonmicellar polysorbate-secobarbital complex.

Dissolution rate, stomach emptying time, enzymatic activity, and many other factors may also influence the rate of drug absorption in the gastrointestinal tract in various degrees (68,69,70).

Multiple Dosage Regimens

Although blood level and urinary excretion measurements are usually obtained following single doses of drugs, a number of investigators (71,72,73) have made such measurements after multiple drug administration. Wagner, et al (74) pointed out the advantages of defining blood levels during a dosage interval at the equilibrium state, rather than to attempt to measure the peaks and nadirs after various single doses. This can be applied especially where drug therapy involves the administration of repeat or multiple doses. Sugarman and Rosen (75) pointed out that in order to make a valid comparison between dosage forms containing the same drug, but which release the drug at different rates, it is necessary to establish a steady state between drug input and output before measuring blood levels and urinary excretion as the basis of comparison. The urinary excretion study of Sugarman and Rosen (75), and the serum concentration study of Swintosky, et al (76) are illustrative of the use of multiple dosage experiments for dosage form comparisons. Green (77) studied the variability of equilibrium state serum concentra-

tions of salicylate following administration of aspirin in two different dosage forms. The variability observed was found to be less than that obtained after a single dose administration.

Koppanyi and Avery (78) pointed out that all drugs accumulate in the body providing rate of administration exceeds total rate of elimination (input > output). When a drug with a long biological half-life is given at regular time intervals the blood levels will build up appreciably since the rate of input far exceeds the rate of loss from the body.

After commenting on Krüger-thiemer and Bünger's concept (79,80) implying that drug accumulation is associated not with the final state but only with the transition between initial and final states, Wagner (81) proposed two indices of drug accumulation. The ratio of the average amount of drug circulating in the body and that lost from the body during a dosage interval at the equilibrium state, to the amount of drug absorbed and lost from the body in infinite time after a single dose of the drug, is the basis of one index. The greater the magnitude of this index, the greater is the chance of drug accumulation in the body following a multiple dosage regimen. Unfortunately, Wagner did not include a quantitative measure for the index to indicate the extent of accumulation and mention was not made of the possibility of drug accumulation in a particular organ of the body due to tissue localization.

Tissue localization and drug accumulation have been discussed by Boxer, et al (82). They (82) observed that repeated administration of a drug did not produce unlimited building up of the blood concentrations, in spite of the fact that a high, residual drug amount was present in the body at the times of readministration. Boxer, et al (82), further stated that even if an equilibrium state of blood level was reached

rapidly, drug accumulation in a deep tissue compartment or organ would probably continue as long as the amount of drug absorbed exceeded the amount excreted. Since the rate of excretion of drug is proportional to its blood concentration an equilibrium blood level is always reached after a certain dosage interval. However, drug may accumulate in various body organs. A good example would be the antimalarial drug atabrine.

D. ANALOG COMPUTER SIMULATIONS

Several methods are available for determining rate constants for drug transfer processes across various body membranes. These methods include classical mathematical analysis, graphical methods, and application of electronic digital and analog computer techniques. The use of automatic high speed digital and electronic analog computers results from relatively recent mechanical innovations. Digital computers have been successfully programmed to obtain solutions of mathematical equations established for the appropriate pharmacokinetic models (83,84,85). They have the the advantage of a high degree of accuracy and speed, but are expensive and relatively difficult to program.

Although analog computers are less accurate they are simpler and more flexible, particularly with respect to the solution of differential equations, and it is possible to visually compare computer generated curves with experimental data. Therefore, the analog computer has often been

applied to the elucidation of the mechanism and rates of transfer processes of drugs in *in vivo* and *in vitro* systems. Its use also permits the determination of compartmental models for absorption of drug from the site of administration into the blood, distribution into tissues, metabolic pathways, and excretion processes. Such determinations are carried out by programming the computer for any sequential, or parallel, changes in drug amount in the various body depots. The 'read-out' can be obtained as plotted curves of drug amount versus time for any selected compartment in the model chosen.

One of the earliest pharmacokinetic applications of analog computers was reported by Garrett, *et al* (86). Using psicofuranine as a test drug, they extensively examined the mechanism and rate of gastrointestinal absorption into the blood, diffusion into tissues, and elimination into the urine. This technique was also applied for the examination of multiple dose kinetics (87), steroid effect on calcium metabolism (88,89), pharmacokinetics of nalidixic and hydroxynalidixic acids (90), behavior of prolonged-action preparations (91,92, 93), simulation of drug distribution models (94,95). Silverman and Burgen (97) and Stelmach, *et al* (98), programmed an analog computer to give drug *in vivo* release and absorption kinetics from blood level versus time data.

Garrett and Lambert (99) described methods utilizing the analog computer as a laboratory aid in the preparation of drug formulations with improved therapeutic efficacy. In this study five basic dosage forms were used to show the versatility of the computer and the variety of programs that are possible. The dosage forms were as follows - single dosage, multiple dosage, amd three different types of prolonged-action formulations. The computer program used for the prolonged-action formulations included

a'loading' dose D_I representing immediately available drug and a 'maintenance' dose D_M representing that amount of drug required to maintain the therapeutic blood level for the desired period of time.

Beckett, et al, reported a series of kinetic studies for a number of sympathomimetic amines using an analog computer. These studies included the examination of the quantitative relationship between urinary pH and kidney reabsorption of drugs (100), pharmacokinetic and biopharmaceutical studies with amphetamine-type compounds (101), kinetics of buccal absorption of amphetamines (102), and prediction of the distribution and excretion of drugs under conditions of fluctuating urinary pH (103).

Determination of the quantitative relationship between measured urinary pH and kidney tubular reabsorption of drugs (100) was based on the use of drug excretion rate versus time profiles determined under normal conditions of controlled urinary pH, ie. constant acidic pH (5.0 ± 0.5) in the case of weakly basic drugs. The applications of the method were discussed with respect to the evaluation of drug formulations under normal urinary pH conditions.

Beckett, et al (103), established by this means a mathematical relationship between the percentage excretion of amphetamine and urinary pH. The mathematical relationship could be applied to predict the quantitative excretion of the drug under normal urinary pH conditions. Comparisons were made of computer predicted body levels of amphetamine after administration of the drug in a single solution dose, three divided solution doses, and in various prolonged-action formulations. The advantages of prolonged-action preparations were indicated and specifications for a theoretically ideal prolonged formulation were proposed.

Beckett, et al (102), have also applied an analog computer to determine the kinetic constants of buccal absorption of amphetamines. An analog computer program for this biological buccal absorption system was described as a simple three compartmental model. It was found that buccal absorption involved passive diffusion of the unionized form of the drug from an aqueous to a lipid phase. This is in agreement with the lipid-water partition theory for drug transfer across body membranes.

E. DOSAGE FORM EFFECTS ON DRUG ACTION

A prolonged-release preparation is designed to give drug body levels equivalent to those levels obtained after administration of a number of doses taken at intervals. The primary advantages, limitations, and difficulties of maintaining prolonged and uniform drug release to provide suitable therapeutic blood levels have been well documented (104,105). The primary advantage of prolonged-action formulations of any kind is that the necessity of dosing several times a day may be eliminated, with in some instances, correspondingly greater therapeutic benefits.

A number of reported studies (106,107) have dealt with the consideration of many of the important theoretical aspects of prolonged-action formulation. Some of these studies have shown how knowledge of

drug blood level, urinary excretion, and biological half-life data, when related to therapeutic response, may be used to design and evaluate prolonged-action dosage forms. Ideally drugs which are incorporated into such dosage forms must be those whose pharmaceutical properties, biological fates, therapeutic indications, and hazards have been well evaluated.

Prolonged-action dosage forms may be prepared by pharmaceutical, chemical, biological or medical methods. However, the majority of such formulations are prepared by pharmaceutical modifications. For example, encapsulated slow-release beads, tablets with slow-release cores, tablets with mixed-release granules, multiple-layer tablets, application of ion-exchange resins and porous inert carriers, and other miscellaneous examples. The advantage of such techniques is based on the fact that the technical manipulation of physical forms is more easily achieved than by chemical or biological methods. The primary aim of dosage form manipulations is to extend disintegration and/or dissolution rates with time.

However, the advantages of prolonged-action formulations are not always achieved <u>in vivo</u> as many other complicated factors are involved (108,109). Variations in gastrointestinal physiology between individuals, for example, stomach emptying rate and intestinal transit time, have been pointed out as complicating factors in achieving the objectives of prolonged-action preparations (109,110). Oral administration of a drug preparation brings many more variables into account compared to local application or parenteral administration. The stomach may be full or empty, may contain little or much gastric juice, and may retain the drug preparation for a few minutes or for as long as several

hours. The intestine may also vary considerably in its contents of digestive fluids, pH, and motility. These factors account, in part, for the known variation in drug absorption between individuals. Gruber, et al (111), have shown that even the physical posture of the patient has an influence on the rate of passage of drug out of the stomach.

Chapman, et al (112), and Campbell, et al (113), have discussed the importance of physiological availability of drugs in the body. They examined the physiological availability of drugs in various dosage forms as a basis for the establishment of standards for disintegration tests. Using riboflavin and sodium p-amino-salicylate tablets, they showed that when the in vitro disintegration timeswere more than 60 minutes, the substances were not completely available. In their conclusions, emphasis was made on the necessity of determining physiological availability of prolonged-action formulations. In further studies, Chapman, et al (114), examined prolonged-action formulations of creatinine, acetylsalicylic acid and amphetamine sulfate by comparing the rate of urinary excretion of the drugs after single doses of prolonged-action forms with the rate of excretion following doses of the non-formulated drugs. One amphetamine product exhibited prolonged-action properties in vivo, but the other products tested showed no evidence of prolonged-action.

Shenoy, et al (115), found that the physiological availability of amphetamine varied from about 25 to 100 % in human subjects in an examination of eight different prolonged-action preparations in pelleted form. Only two of the products showed prolonged drug urinary excretion for about twenty-four hours, and were quantitatively available. Several of the products were poorly absorbed and relatively unavailable to the subjects. Two others showed no evidence of prolonged effect.

Furthermore, there are instances in which use of prolonged-action preparations would seem to be unnecessary. According to Dragstedt (110), drugs for which precision of dosage is important, such as the digitalis glycosides and glyceryl trinitrate, or those whose absorption is normally erratic, should not be given as prolonged-action preparations. This could be of particular importance in the case of drugs having a low therapeutic index. Drugs which have intrinsically long biological half-lives may also not be good candidates for prolonged-action formulation.

The need for quantitative evaluation of prolonged-action formulation has been recognized and such tests must be supported by careful *in vitro* and *in vivo* examination. Although no single universal standard test method has been developed a wide variety of methods have been used to determine the effectiveness of prolonged-action products.

During the development of the dosage form, *in vitro* testing serves as a guide in estimating the drug amount released per unit time. Once an *in vitro*/*in vivo* correlation has been shown by animal and clinical studies, the *in vitro* test serves a very useful quality control purpose (116). Reproducibility of the release data in the *in vitro* test is indicative of the manufactured batches meeting the dosage form specifications and of similar therapeutic activity from batch to batch. *In vitro* tests, in which attempts are made to simulate conditions within the intestinal tract, are probably the most widely used procedures for examining the release of drugs from prolonged-action dosage forms.

Although these *in vitro* test methods may differ depending upon the dosage form tested most of these procedures make use of simulated gastric and intestinal fluids maintained at 37° C, and with some mechanical device used to move the product tested through the fluid

at a constant rate (117,118). The time required for the preparation to disintegrate may be determined, or analyses of the drug may be carried out at intervals on portions of the eluate or on the residual undissolved material. Some workers have assumed that, since simulated gastric juice and intestinal juice are used in these tests, the time required for a preparation to disintegrate *in vitro* is directly comparable to that required *in vivo*. However, since the varying conditions within the human gastrointestinal tract are not yet completely understood and because of the inability to directly simulate *in vivo* conditions, such an assumption appears rather unwarranted. Furthermore, different *in vitro* tests can give different results. Blythe (119) and Campbell, *et al* (113), have stressed the fact that while *in vitro* procedures are valuable tools for regulatory and control purposes, they have no real value by themselves unless the results obtained are correlated with a quantitative *in vivo* procedure.

The measurement of drug blood levels is usually considered to be the most unequivocal method of evaluating the *in vivo* performance of dosage forms. This approach is particularly valuable if drug blood levels parallels pharmacological activity (121). However, the feasibility of blood level studies as a general approach to the evaluation of dosage forms is severely limited by the bioanalytical problems arising from the low therapeutic dosage and extensive extravascular distribution of many drugs as well as the inconvenience to subjects of frequent blood sampling.

Using prednisolone as a tracer, Wagner, *et al* (120), measured drug plasma levels in man and dog following administration of prolonged-

action products and their rapidly available forms. The plasma levels for the prolonged-action products were sustained for twelve hours in man but only for seven hours with the conventional form. Abrahams and Linnell (122) showed blood-level curves obtained for creatinine after its administration in both prolonged-action and conventional forms. They found that the initial drug absorption rate decreased with prolonged-action formulation. Brudney (123) examined blood levels following ingestion of penicillin V complexed with an ion exchange resin and compared the obtained levels with previously published results for uncomplexed penicillin V and penicillin G. The results indicated that the complexed penicillin gave a sustained drug level for three hours which then steadily decreased. The uncombined penicillin compounds gave high initial blood levels and then decreased rapidly.

Urinary excretion studies have also been used as in vivo technique for evaluating prolonged-action dosage forms. For certain drugs a direct relationship has been shown to exist between drug excretion rate and the amount of the drug in the blood (124). Heimlich, et al (125), compared the urinary excretion of trimeprazine and its metabolites after administration of the drug as prolonged-action products to conventional dosage forms administered in three divided doses every four hours. Total drug and metabolite excretion following both administration schedules was similar indicating complete availability of the drug from the prolonged-action dosage forms.

The release of phenylpropanolamine hydrochloride contained in a prolonged-action product to that of the drug in the untreated form has also been compared by means of urinary excretion studies. The results

indicated that the treated drug was excreted in a manner similar to the unprocessed drug when an equal dose of the latter was given every four hours in three divided doses (126). Urinary excretion studies and the measurement of drug disappearance from blood can facilitate the design of prolonged-action dosage forms when blood level and urinary excretion data can be related to therapeutic response (127, 128).

F. ANALYTICAL TECHNIQUES FOR THE ANALYSIS OF CHLORPHENTERMINE AND RELATED COMPOUNDS IN BIOLOGICAL FLUIDS

A number of different analytical techniques have been described for the determination and identification of chlorphentermine and related compounds in biological fluids. The most commonly used techniques are based on specific color reactions, thin-layer chromatography(TLC) or gas-liquid chromatography(GLC).

One of the most common color reaction methods for determination of these compounds has been the methyl orange assay procedure (129, 130). The basis of this method is that methyl orange, an acidic dye, interacts with bases, such as chlorphentermine, to form colored complexes. These complexes are distinguished from uncomplexed methyl orange by their solubility in organic solvents, therefore the complex is extracted into an organic phase and determined colorimetrically. The analytical method used by Dubnick, et al (11), in their investigation of whole body levels

of chlorphentermine in mice was based on the methyl orange complexation procedure developed by Brodie, et al (131), as modified by Axelrod (132). Dubnick, et al, however, further modified the method by substituting perchloric acid as the protein precipitant. Keller and Ellenbogen (133), and Chapman, et al (114), also have applied the methyl orange reaction for the determination of amphetamine in blood and urine.

TLC has been used as a technique for the separation and identification of amphetamine and related compounds in biological fluids. This technique is capable of detecting 2-5 mcg of amphetamine, and many rapid screening procedures based on this method of analysis have been developed in recent years. Debackere and Massart-Leen (134) have applied TLC to identify and determine the metabolism of amphetamine in biological fluids of horses injected with clinical doses of amphetamine. Opitz and Weischer (15) quantitatively determined chlorphentermine and its analogs in urine by TLC. The amines were converted into their respective dinitrophenyl derivatives, extracted with cyclohexane, and separated by TLC. The dinitrophenyl-amine content of the appropriate chromatographic zone was determined spectrophotometrically using methanol as the extracting solvent. However, TLC could not be applied for the routine quantitative analysis of these compounds.

The lack of sufficiently sensitive and specific analytical methods has until recently prevented the quantitative analysis of these compounds in vivo. Since GLC has been found to be a highly sensitive, specific and versatile analytical method it has been used to determine these compounds in biological fluids. For chlorphentermine and related compounds the sensitivity limit is in the order of 0.1 mcg per ml.

The work of Fales and Pisano (135),and Brochmann-Hanssen and Svendsen (136) was the first published GLC analysis of the sympathomimetic amines in biological fluids. Fales and Pisano sucessfully applied GLC to estimate amphetamine in urine using a chromatographic column containing 4% SE-30 siloxane polymer as liquid phase. They obtained a well-defined peak for amphetamine extracted from urine following the oral administration of a 5 mg tablet of amphetamine sulfate. Parker, et al (137), reported the separation and identification of some sympathomimetic amines by GLC. Peaks were obtained from 0.1 to 3.0 mcg of the free base or salts with 5% Carbowax 20 M as the liquid phase. Kazyak and Knoblock (138) have described a similar procedure for the routine screening of toxicological extracts for a wide variety of drugs of this type.

Cartoni and de Stefano (139) have used GLC to determine amphetamine and methylamphetamine in biological fluids. These authors used a Carbowax 1500 alkali coated capillary column, assaying the compounds in the presence of an internal marker. Beckett and Rowland (140) have also used GLC to detect and estimate amphetamine in urine and further characterized the amphetamine peak by derivatizing it with acetone.

None of these techniques were however applied to the determination of these drugs in blood. However, Axelrod (132) used a colorimetric procedure to determine amphetamine in plasma and tissues of animals that had received relatively large doses of the drug.

With the development of the electron capture detector and formation of halogenated derivatives of chlorphentermine and related amines, (e.g. heptafluorobutyrates), the sensitivity of the GLC technique has been much increased (141,142) and drug concentrations as low as 1 ng

can be detected. This would be of great significance in determining blood levels following administration of these extravascularly concentrated drugs. Bruce and Maynard, Jr. (142) and Rowland (143) recently reported investigations of blood levels of these compounds by electron capture GLC. Both authors analyzed the amines by forming halogenated derivatives. Bruce and Maynard, Jr. (142) found that preconditioning of the column with heptafluorobutyric anhydride appeared to an essential part of the method.

Ultraviolet spectrophotometric methods (144) and radio-isotopic tracer techniques (145, 146, 147) have also been used for the determination of such compounds in biological fluids. Dring, et al (145), used radio-isotopically labelled amphetamine to examine its metabolic pathway in man, rat, rabbit and dog. ^{14}C-excretion (drug and metabolites) was estimated by scintillation counting, while the excretion of metabolites alone was determined by paper chromatographic separation following a reverse isotope dilution method. Alleva (146) examined the excretion profiles of amphetamine in the rat following administration of amphetamine-^{14}C.

Rosen, et al (147), studied the absorption and excretion rates of d-amphetamine sulfate following administration to man of a prolonged-action preparation. They determined blood and urine levels of drug following administration of amphetamine-^{14}C. Later, Rosen, et al (148), carried out an identical study in man and dog to compare the absorption and excretion of amphetamine-^{14}C sulfate in sustained-release and non-sustained-release dosage forms. A direct comparison of the results of these studies showed that the various dosage forms performed in a similar way in both man and dog.

Beckett, et al (149), have also determined blood and urine

levels of amphetamine after oral administration of amphetamine-^{14}C sulfate to humans under normal and controlled acidic urinary pH conditions. They observed that the decline in blood concentration of the drug was more rapid under the controlled acidic conditions than under conditions of fluctuating urinary pH.

CHAPTER III. EXPERIMENTAL METHODS

A. URINARY EXCRETION STUDIES

Introduction

Urinary excretion studies have been used for many drugs as the most practical method of examining their in vivo biopharmaceutical parameters (150,151). Furthermore, the importance of the finding that the urinary excretion rate of some drugs is pH dependent and in some instances, urine volume dependent, necessitated the examination of the urinary excretion characteristics of chlorphentermine under various urinary pH conditions.

Quantitative analysis of chlorphentermine in biological fluids has previously been performed by thin-layer chromatography, spectrophotometric methods, radioisotopic techniques, and gas-liquid chromatography. In these experiments, a gas-liquid chromatographic method was selected as the quantitative analytical method of choice for the measurement of chlorphentermine in urine.

Extraction Procedure

The extraction procedure was similar to that previously reported for the analysis of amphetamine (139). A 5 ml urine sample was pipetted into a 15 ml glass centrifuge tube, and 0.5 ml of 20% w/v sodium hydroxide solution was added. To the tube was then added 2.5 ml of redistilled 'Reagent' grade diethyl ether. The tube was tightly stoppered and shaken for 5 min on a shaker[a] (18 rev/min), followed by centrifugation at 2,000 r.p.m. for 10 min. The upper ethereal layer was transferred to a 12 ml glass sedimentation tube containing 1 ml of a 10 mcg/ml solution of

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a Lab-Tek Aliquot Mixer, Ames Lab-Tek Inc., Westmont, Ill.

azobenzene in diethyl ether (internal marker). Further 2.5 ml portions (2 times) of diethyl ether were added to the centrifuge tube, and the procedures of shaking, centrifugation, and transference of the ethereal layer repeated. Prior to chromatography the combined ethereal extracts were reduced to a small volume (about 50 µl). Concentration was best achieved by evaporation of the ether extracts in sedimentation tube placed on a water bath at 40°C; boiling was promoted by addition of a small boiling stone. Then, 2-5 µl of the concentrate was injected onto gas-liquid chromatographic column.

Gas-liquid Chromatographic Procedure

A F and M Model 700 gas chromatograph[b] equipped with a dual flame ionization detector and a 0-10 mV Speedomax W recorder[c] with a chart speed of 0.5 inch/min and a 1-sec full-scale response, was used throughout the experimental work. All samples were injected with a 10-µl syringe[d].

The chromatographic column was a 1/8 inch o.d. stainless steel column, 6 feet in length, and packed with 10% W98[e] on Diatoport S[f] (80-100 mesh). The column was silanized *in situ* with 2 x 6 µl injections of hexamethyldisilizane, and equilibrated for 24 hr at the operating conditions.

The oven temperature used was 150°C. The sample injection port temperature was maintained at 295°C and the detector block at 310°C. Helium was used as the carrier gas, at a rate of 60 ml/min with an inlet pressure of 40 lb/sq in. Air and hydrogen inlet pressures were 25 and

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b Hewlett-Packard Co., Ltd., Avondale, Pa.
c Leeds & Northrup Co., Inc., Philadelphia, Pa.
d Hamilton No. 701 Syringe, Whittier, Calif.
e Silicone Gum Rubber
f Diatomaceous Earth

15 lb/sq in , respectively. All determinations were made with attenuator settings between 1 x 10 and 1 x 50.

The optimum air pressure (25lb/sq in) and hydrogen pressure (15lb/sq in) were selected for the maximum response of chlorphentermine and internal marker, azobenzene, by measuring peak heights at different gas pressures. Peak heights of chlorphentermine and azobenzene were plotted against gas pressure.

Analysis of 'blank' samples of urine collected from each subject were performed for every excretion trial.

The absolute recovery of chlorphentermine hydrochloride extracted from urine was determined by comparing peak ratios of a known amount of the drug dissolved in ether to that obtained for an equal amount of drug dissolved in blank urine estimated by the gas-liquid chromatographic procedure.

The reproducibility of the extraction procedure was measured by extracting twelve urine samples each containing 10 mcg/ml chlorphentermine hydrochloride.

In order to examine any possible deterioration of chlorphentermine in urine, a urine sample containing the drug was refrigerated at $4^{o}C$ for 4 weeks and analyzed at selected time intervals.

A calibration curve was prepared from stock urine solutions containing known amounts of chlorphentermine hydrochloride. Urine containing 1.0 - 60.0 mcg/ml chlorphentermine hydrochloride was assayed by the described analytical technique. Peak heights of chlorphentermine and azobenzene were measured with calipers. A calibration curve was then constructed by plotting the ratio of the peak heights of chlorphentermine

to azobenzene against known concentrations of chlorphentermine hydrochloride in urine. The calibration curve procedure was repeated at two-month intervals.

The necessity of determining the exact volume of the ethereal concentrate to be injected onto the column was avoided by using the internal marker technique. Chlorphentermine response was not measured directly but always relative to a known amount of internal marker.

Urinary Excretion Trials

General Method.

Three healthy male volunteers (aged 24 - 30 years) participated in these experiments. Each subject received an oral dose of 78 mg of chlorphentermine hydrochloride (≡65 mg base) in 10 ml aqueous solution thirty minutes after a light breakfast of coffee and toast. At least a one month interval was allowed between each experiment.
None of the subjects were allowed any other medication for the day before or during the period of the experiments.

Urine samples were collected every hour for the first 6 hr, at 2 hr intervals for the period 6 - 12 hr, and again at 22, 26, 30, 34, 38, and 48 hr after drug administration. If urine sampling was not at the above times, the exact time was noted. Blank samples were obtained just prior to taking the drug. Urine volumes were measured for each sample, and urinary pH determined with a pH meter[g] as soon as possible after collection. No urinary pH control was required.

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g Beckman Expandomatic pH meter

Acid Urine Conditions.

The general method was followed but an acid urine was induced and maintained by the oral administration of ammonium chloride (0.5 g enteric coated tablets). A typical dosage regimen was 1.0 g ammonium chloride every four hours taken on the day before the trial and 1.0 g at three hourly intervals on the day of the trial. When more than one day's urine collection was required the ammonium chloride regimen for the subsequent day(s) of the trial was the same as for the first day.

Urinary acidification induced by administration of ammonium chloride has been found useful (152,153) for the maximum exploitation of urinary excretion studies of weakly basic drugs.

Alkaline Urine Conditions.

The general method was followed but an alkaline urine was induced and maintained by the oral administration of sodium bicarbonate solution (10% w/v). The normal dosage regimen was 4.0 g sodium bicarbonate in solution before retiring on the day preceding the trial, then 4.0 g at four hourly intervals on the day of trial. When more than one day's urine collection was required the regimen for the subsequent day(s) of the trial was the same as for the first day.

Urinary alkalinization induced by administration of sodium bicarbonate has been shown to minimize the excretion of weakly basic drugs (154).

B. ANALOG COMPUTER SIMULATIONS

Introduction

The kinetics of absorption, distribution, and excretion of chlorphentermine were examined using an electronic analog computer on the basis of urinary excretion data, after oral administration of 'free' dosage forms to man under controlled acidic urinary pH conditions. This device was also used to determine the *in vivo* rate of drug release from a prolonged-release preparation. The objectives of these experiments were to determine whether a simple compartmental model could mathematically describe the pharmacokinetics of chlorphentermine after its administration in various dosage forms, and to evaluate the *in vivo* release rate of the drug with the *in vitro* release rate from the prolonged-release dosage form.

The analog computer simulates the assumed physiological models by an electrical network. The computer uses electrical voltage to represent drug dosage, and can perform mathematical operations, for example, summation, multiplication, and integration by groups of electronic components (155). Systematic variation of the rate potentiometers of the computer was performed within the possible limits of the chosen compartmental model in an effort to fit the computer generated curves to the experimental data previously plotted on recorder paper. Thus, the compartmental model and the experimental data could be evaluated as to the best possible fit, and the mathematical equations, and rate constants evaluated.

Pharmacokinetic Models

The following assumptions were made for the use of the simple compartmental models to investigate the kinetics of absorption, distribution,

and elimination of chlorphentermine administered as various dosage forms, under acidic urine conditions:

(i) the rate of urinary excretion of the drug is proportional to its concentration in the plasma,

(ii) drug transfer from one compartment to the other, excluding the tissue compartment, is irreversible,

(iii) transfer rate of drug from one compartment to another is directly proportional to the amount of drug in that compartment, ie. drug release, absorption, distribution, and excretion are apparent first-order kinetic processes with rate constants having units of reciprocal time,

(iv) compartments are uniform and homogeneous throughout the transfer process,

(v) there is no decomposition of the drug at the absorption site nor diffusion of the drug from the blood into the stomach,

(vi) the rate constant for drug absorption is unchanged along the gastrointestinal tract,

(vii) excretion of unchanged drug by pathways other than via the kidney is negligible and little or no metabolism of the drug occurs,

(viii) absorption and elimination rate constants are independent of dosage form as are distribution constants also.

The validity of these assumptions will be discussed in relation to correlations observed from experimental results and computer simulations.

Model (I): applicable to 'free' forms of chlorphentermine(see Figure 2). Pharmacokinetic Model (I) was proposed to describe the kinetics of absorption, distribution, and excretion of chlorphentermine in man after oral administration of 'free' dosage forms of the drug under controlled acidic urine condition.

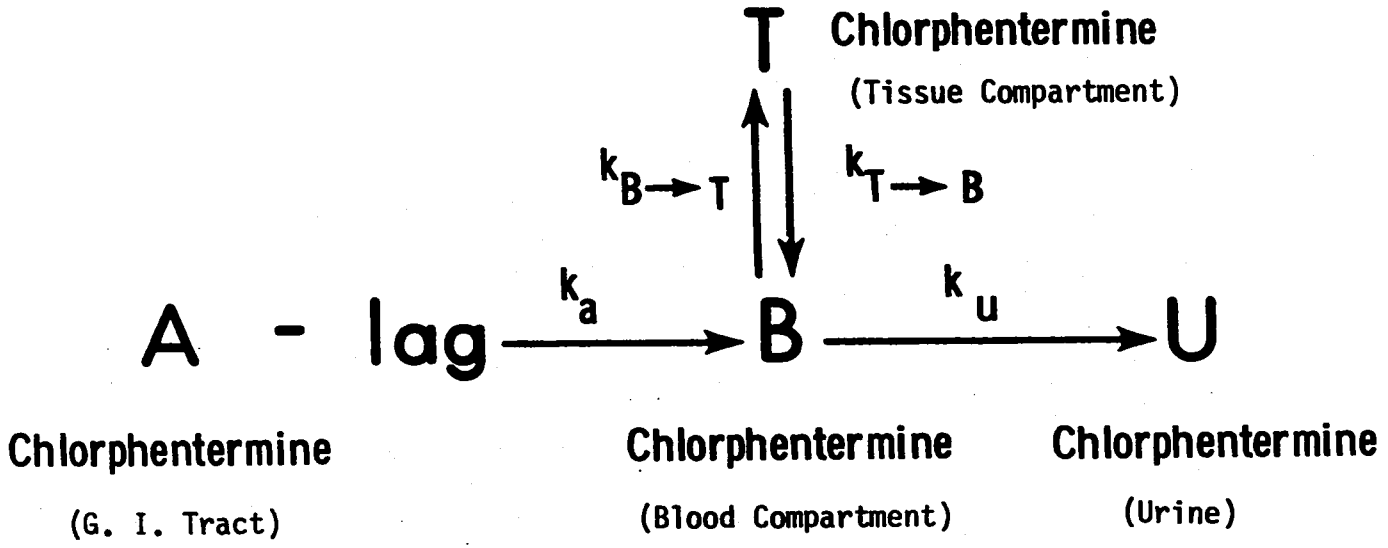

Figure 2. Compartmental Model (I) for 'Free' dosage forms.

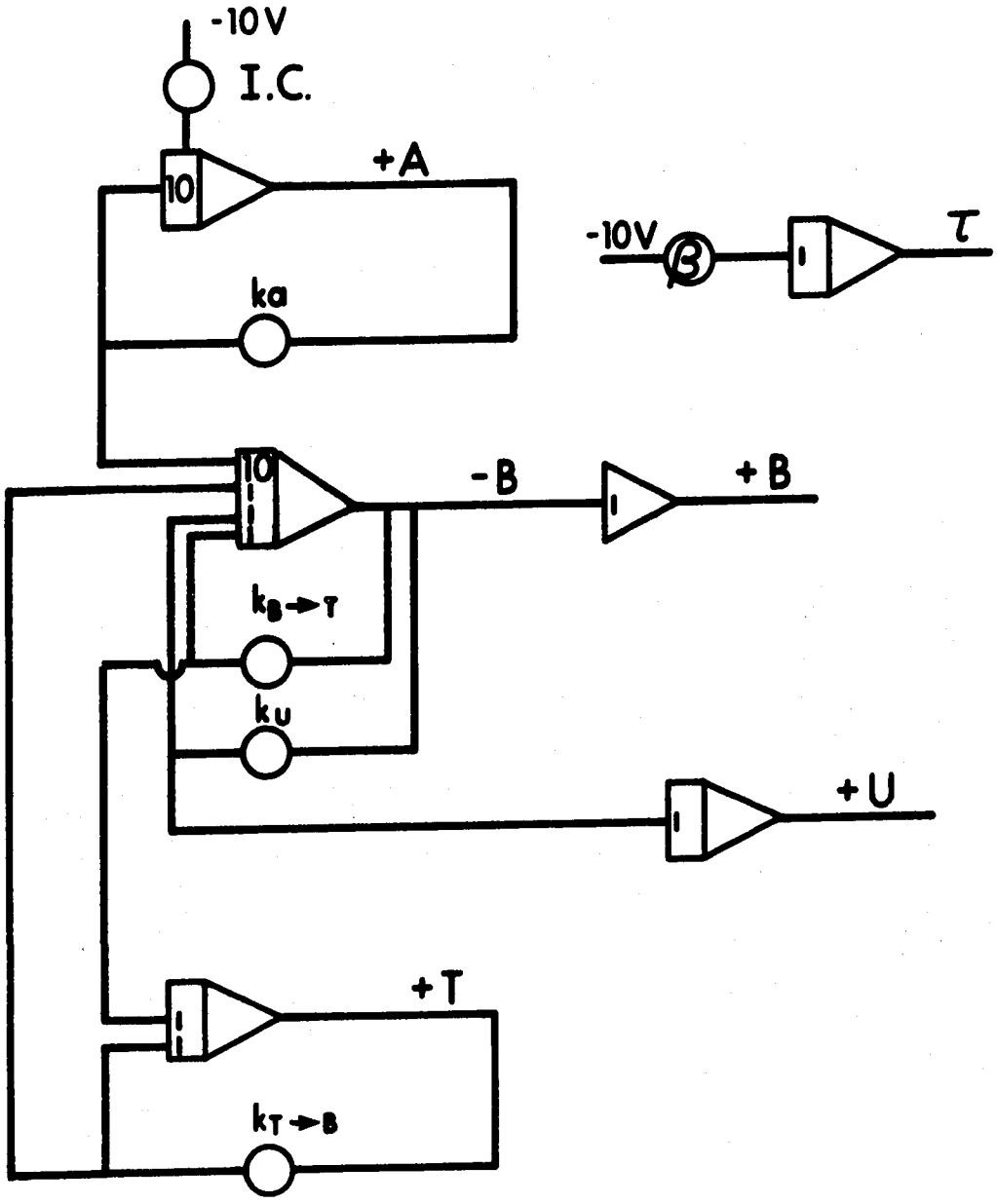

Figure 3. Analog Computer Program for Model (I).

Based on this model, the following rate equations may be written (all symbols are defined in Appendix A).

Post lag time;

$$\frac{dA}{dt} = -k_a A \qquad \text{Equation 1}$$

$$\frac{dB}{dt} = +k_a A - k_{B\to T} B + k_{T\to B} T - k_u B \qquad \text{Equation 2}$$

$$\frac{dT}{dt} = +k_{B\to T} B - k_{T\to B} T \qquad \text{Equation 3}$$

$$\frac{dU}{dt} = +k_u B \qquad \text{Equation 4}$$

The analog computer program for the solution of Model (1) is shown in Figure 3.

Model (II): applicable to prolonged-release preparation of chlorphentermine (see Figure 4). Model (1) was modified to describe the kinetics of release, absorption, distribution, and excretion of the drug after administration to man of prolonged-release preparation A under controlled acidic urine conditions. The modification consisted of the addition of a formulation compartment D_M (see Appendix A) containing the total dose at zero time and from which the drug is released into the gastrointestinal tract by sequential first-order processes governed by the rate constants k_{r_1} and k_{r_2} .

In addition to the equations given for Model (1), Equation 5 also applies,

$$\frac{d D_M}{d t} = - k_r D_M \qquad \text{Equation 5}$$

where k_r is initially k_{r_1} and becomes k_{r_2} after the 'break-time' (see Appendix A).

Drug absorption using this model is given by Equation 6,

$$\frac{d A}{d t} = + k_r D_M - k_a A \qquad \text{Equation 6}$$

The analog computer program for the simulation of Model (II) is shown in Figure 5.

The lag time was estimated by manually setting the abscissa zero of the X-Y recorder, and the change over from k_{r_1} to k_{r_2} at the 'break-time' was accomplished by the use of a signal voltage comparator.

Dosage Forms

Solution: aqueous solution of chlorphentermine hydrochloride. Dose; 78 mg/10 ml

Tablet : compressed tablet of chlorphentermine hydrochloride in lactose base. The tablet was prepared in our laboratory such that it had rapid disintegration and dissolution, thus giving a dosage form readily available for drug absorption from the gut. Therefore, this was

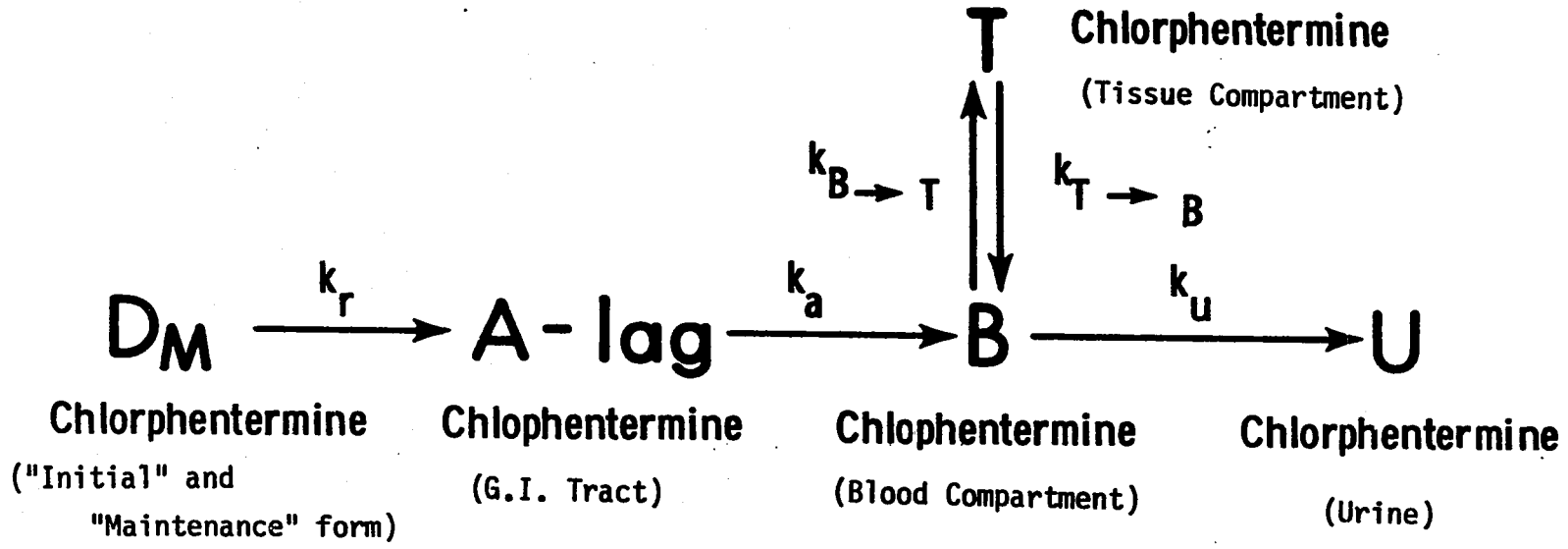

Figure 4. Compartmental Model (II) for 'Prolonged-release' preparation.

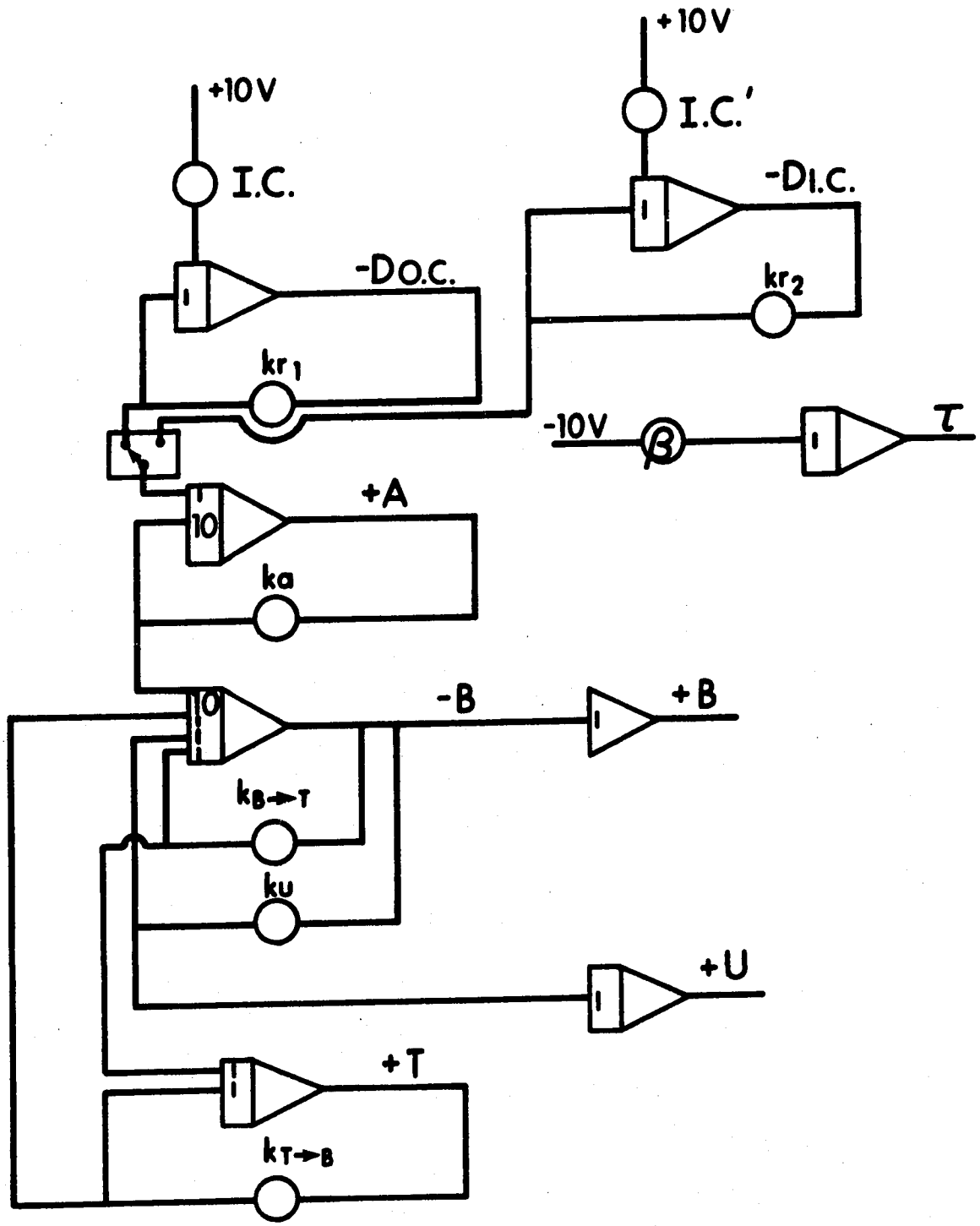

Figure 5. Analog Computer Program for Model (II).

considered to be a 'free' dosage form. Dose; 78 mg.

Prolonged-release preparation A : commercial prolonged-release inner core tablet containing chlorphentermine hydrochloride. Dose; 78 mg.

In Vitro Experiments

In vitro data for drug release was determined by the manufacturer of prolonged-release preparation A. The dissolution test used to evaluate drug release was the rotating bottle method (116).

The effect of ammonium chloride on *in vitro* drug release was determined as follows. Two tablets of prolonged-release preparation A were placed in the wire basket of a U.S.P. XVII disintegration apparatus; six grams of ammonium chloride (representing approximately that present in the gut in *in vivo* situations) were dissolved in the simulated intestinal juice, (600 ml) and the U.S.P. disintegration procedure followed. Samples of solution (3 ml) were taken at 15 min intervals for 1 hr, and then every 30 min for a further 2 1/2 hr. Chlorphentermine content in the samples was determined by the analytical method described (see p.36). The procedure was repeated in the absence of ammonium chloride for comparison. If extrapolation could be made from the results to the *in vivo* situation then the effect of ammonium chloride administration on *in vivo* drug release could be determined.

Urinary Excretion Trials

The dosage forms were given to healthy male subjects whose urinary pH was maintained acidic (5.0 ± 0.5). Each of three subjects received the dosage forms of chlorphentermine hydrochloride with at least one month time interval between each trial. The protocol regarding

time of drug administration, times of urine sampling, measurement of urine pH , and the dosage regimen for ammonium chloride has been described previously (see p.38,39). Chlorphentermine in urine was determined by the described method.

Computer Simulations

A PACE TR-20[h] analog computer was used together with an X-Y recorder[h]. The appropriate pharmacokinetic model to describe absorption, distribution, and excretion was programmed. The experimental urinary excretion data was plotted on the X-Y recorder as drug cumulative excretion. In some cases, the absorption points derived from the data using the equation of Wagner and Nelson (52) were also plotted (see Figure 13). The settings of the rate potentiometers were then systematically varied in an effort to fit the computer generated curves to the experimental points. When the best fit was obtained, the settings of these potentiometers were read from the computer voltmeter. The suitability of the model was judged on the basis of the fit obtained to the experimental data.

Fitting of prolonged-release preparation data from appropriate subjects was made, as far as possible, using values of the rate constants for absorption, distribution, excretion, and lag time, similar to those used in previous simulations of 'solution' and 'tablet' data.

In vivo drug release rate was plotted by taking the output from the integrator representing the formulation compartment D_M. Hence, direct comparisons of in vivo and in vitro drug release rates could be made.

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h Electronic Associates Ltd., Princeton, N.J.

C. BLOOD LEVEL STUDIES

Introduction

Urinary excretion studies of chlorphentermine analogs have been carried out by a number of workers as a method for evaluating the kinetic behavior of these drugs in the body (17, 101). This technique, however, is not completely satisfactory as a number of assumptions are implicit in the method (see p.41). To overcome some of these shortcomings blood level studies have been advocated (143).

A method to determine blood levels of chlorphentermine in man was developed using flame ionization gas-liquid chromatography, and blood levels of the drug examined following oral administration of the drug in solution, as a prolonged-release preparation, and as an intravenous injection.

Extraction Procedure

The extraction procedure for the drug from blood samples was based on that used by Reynolds and Beckett (156) for the determination of a series of local anesthetics. A 5 ml blood sample was diluted with an equal volume of water in a 15 ml glass centrifuge tube, and 1 ml of 20% w/v sodium hydroxide was added. Four ml of redistilled 'Reagent' grade diethyl ether was then added. The tube was stoppered tightly and shaken for 10 min, followed by centrifugation at 2,000 r.p.m. for 5 min. The upper ethereal layer was transferred to a second centrifuge tube, and the procedure repeated for two further ether extractions. The combined ethereal extracts were shaken twice for 10 min with 3 ml 0.1 N HCl. The aqueous layer containing the extracted drug was transferred to a third centrifuge tube and adjusted to approximately pH 11 with

20% w/v sodium hydroxide (about 1 ml). Three ml of redistilled diethyl ether was added. The tube was again stoppered tightly and shaken for 5 min, followed by centrifugation at 1,500 r.p.m. for 3 min. The upper ethereal layer was transferred to a 12 ml glass sedimentation tube containing 1 ml of a 2 mcg/ml solution of azobenzene in diethyl ether (internal marker). Further 3 ml portions (2 times) of diethyl ether were added to the centrifuge tube, and the procedures of shaking, centrifugation, and transference of the ethereal layer repeated.

The sedimentation tube was placed in a water bath at 40°C and the contents evaporated to near dryness. The tube was then stoppered and placed in an ice bath to produce a final volume of about 5 µl. A 2 - 3 µl sample of this concentrate was then injected onto the gas chromatographic column.

Gas-liquid Chromatographic Procedure

A Fand M Model 700 gas chromatograph equipped with a dual flame ionization detector and a 0 - 10 mV Leeds and Northrup Speedomax W recorder was used.

The chromatographic column was a 1/8 inch o.d. stainless steel column, 6 feet in length, and packed with 1% OV-17[i] on 80-100 mesh Chromosorb G (AW-DMCS[j]). The column was silanized *in situ* with 2 x 6 µl injections of hexamethyldisilizane, and equilibrated for 24 hr at the operating conditions.

The operating conditions for chromatography were the same as

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i Silicone Gum Rubber, Hewlett-Packard Co., Ltd.

j General Purpose Solid Support, Hewlett-Packard Co., Ltd.

described in the urinary excretion studies section (see p. 36) except for one modification; helium flow rate was 40 ml/min.

Analysis of 'blank' sample of blood was performed to determine whether any interferring peaks would be observed with the same retention times as chlorphentermine or azobenzene.

The absolute recovery of chlorphentermine hydrochloride extracted from blood was determined by comparing peak ratios of the ether extracts for a known amount of the drug dissolved in ether to that obtained for an equal amount of drug dissolved in 'blank' blood and estimated by the gas-liquid chromatographic procedure.

The reproducibility of the extraction procedure was determined by extracting ten blood samples each containing 0.5 mcg/5ml of chlorphentermine hydrochloride.

In order to examine any possible deterioration of chlorphentermine in blood, a blood sample containing the drug was refrigerated at $4^{o}C$ for 4 weeks and analyzed at selected time intervals.

A calibration curve was obtained from stock blood solutions prepared by adding known amounts of chlorphentermine hydrochloride. Blood samples containing 0.25 - 3.00 mcg/5 ml of chlorphentermine hydrochloride were assayed by the previously described extraction procedure. Peak heights of chlorphentermine and azobenzene were measured with calipers. A calibration curve was then prepared by plotting the ratio of the peak heights of chlorphentermine to azobenzene against known concentrations of chlorphentermine hydrochloride in blood.

Chlorphentermine responses were not measured directly but

always relative to a known amount of internal marker, azobenzene. Azobenzene solution was freshly prepared prior to each blood level study.

Blood Level Trials

Oral Administration.

Chlorphentermine hydrochloride was administered in aqueous solution as a 100 mg dose to four healthy male subjects (aged 25 - 30 years) approximately 30 min after a light breakfast of coffee and toast. Blood samples (7 ml) were taken intravenously using E.D.T.A. pretreated Vacutainer tubes[k] at 30 min time intervals for 3 hr, then at 7, 11, 13, 25, 35, and 48 hr after drug administration. Urine samples were also collected irregularly over the 48 hr time period, and in some cases for a further 8 days.

The drug was also administered as a 78 mg dose in the form of prolonged-release preparation to three of the four subjects who had received the drug in aqueous solution. Blood samples were taken as previously at 60 min intervals for 4 hr, 120 min intervals for a further 8 hr, and at 24, 28, 32, 36, and 48 hr after drug administration. Urine samples were also taken irregularly over the total experimental period.

Intravenous Administration.

Chlorphentermine hydrochloride injections were prepared in our laboratory. The injections were prepared by dissolving chlorphentermine hydrochloride in pyrogen free redistilled water to obtain a drug concentration of 25 mg/ml. The solutions were sterilized by passing

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k Becton, Dickinson & Co., Clarkson, Ont.

through a bacterial filter[1]. Ampoules were filled with the solution under aseptic conditions to give a final concentration of 100 mg/4 ml of the drug as the hydrochloride salt. The ampoules were autoclaved and tested for sterility[m]. Drug content was determined by the analytical procedure described.

The drug was infused intravenously over a 2 min period as a 50 mg dose to two subjects who had previously received the drug in solution. Blood samples were taken at 2, 5, 10, 20, 40, 60, and 120 min, and at 4, 7, 10, 24, 36 and 48 hr after drug administration. Urine samples were again taken irregularly.

Accumulation Study.

Chlorphentermine has been found to have a long apparent elimination half-life (see p.77) and since normal clinical therapy with this drug involves the administration of multiple drug doses, it was decided to examine blood levels of the drug following a multiple dosage regimen. In this study, three subjects who had participated in previous experiments were given one oral dose of prolonged-release preparation A daily for seven consecutive days. Subject's urinary pH was uncontrolled.

Blood samples (7 ml) were collected periodically throughout the experimental time period. The first sample was taken just prior to receiving the first tablet and further samples were taken at 3, 6, 9, and 12 hr following drug administration. Sampling times for subsequent days were as for the first day. Blood samples, collected in E.D.T.A. pretreated Vacutainer tubes, were kept at 4°C until analyzed.

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1 Standard Millipore Filter Type HA, B.D.H. (Canada) Ltd., Montreal, Que.
m U.S.P. XVII Sterility Test for Injections.

D. METABOLISM STUDIES

Introduction

It has been shown that parahydroxylation and deamination are major metabolic pathways for the biotransformation of amphetamine (14) and of mephentermine (157) in animals and man. Since these metabolic pathways are likely to be hindered or completely blocked in chlorphentermine as consideration of its chemical structure shows a p-chlorine atom in the benzene ring and an α,α-dimethyl substitution in the side chain (see Figure 1), chlorphentermine would appear not to undergo metabolic transformation. This has been substantiated in both man (16) and rat (18). It is possible that the long duration of the pharmacological effects of the drug and the prolonged presence of high drug concentrations in various tissues of rats and mice (18) is due to the negligible metabolism of chlorphentermine.

However, the metabolism of chlorphentermine has been shown to be both species and sex dependent. Dubnick, et al (18), using radioactively labelled chlorphentermine in male and female rats and mice, reported that while 70 - 90 per cent of the administered drug was excreted unchanged in rats, female mice excreted only about 25 per cent of the dose as unchanged drug with 60 per cent as an acidic conjugate that could be separated by countercurrent fractionation of the urine. Attempts at isolation and identification of the acidic metabolite have been so far unsuccessful.

In Vitro Metabolism Study

Three male Sprague-Dawley rats, weighing about 300 g were used in these experiments. Each rat was killed by decapitation, the liver quickly removed, and a weighed section (4 g) was homogenized with 5 volumesof ice-cold 1.15% potassium chloride solution in a stainless steel blender[n] . The homogenate was centrifuged for 30 min at 10,000 g. at 4°C to remove all debris and the supernatant fraction decanted.

To the supernatant (1.0 ml), in seventeen 10 ml Erlenmyer flasks was added 2 μM NADP, 20 μM glucose-6-phosphate, 25 μM magnesium chloride, 50 μM nicotinamide, 60 μM phosphate buffer (pH 7.4) and 40 mcg of chlorphentermine hydrochloride. Each of the additives plus the drug was added in solution in as small a volume as possible. A number of flasks (7 flasks) were used as controls containing all the additives except the drug.

The flasks were placed in a metabolic incubator aerated with oxygen, and maintained at 37°C for one hour. The flasks were removed and the supernatant protein precipitated with 2 ml of 20% zinc sulfate solution followed by addition of a saturated solution of barium hydroxide dropwise until no further precipitation occurred.

The supernatant was extracted with redistilled diethyl ether (3 times) under alkaline conditions (pH 11) followed by an acidic extraction (pH2), also with ether (3 times). The solution was finally neutralized with conc. NH_4OH and extracted with ethylacetate.

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n Waring Blender, Waring Products Co., Winsted, Conn.

A portion of the supernatant had been set aside for the estimation of conjugated products. This portion was hydrolyzed for 30 min in a boiling water bath with 6 N HCl, followed by extraction with ethylacetate.

The extracts were evaporated to about 100 µl and chromatographed on 20 x 20 cm silica gel plates. Extracts from the control flasks and chlorphentermine standards were chromatographed at the same time.

In Vivo Metabolism Study

Bulk 24 hr urine samples were collected from three subjects who had received a 78 mg oral dose of chlorphentermine hydrochloride solution. The samples were extracted for metabolites under acidic, alkaline and neutral pH conditions respectively as described for the *in vitro* metabolism study (see p. 56). The extracts were examined for the presence of metabolites by thin-layer chromatography (see p. 58). Blank urine samples were similarly treated and used as controls.

Aliquots of the 24 hr urine samples were hydrolyzed by the method of Dubnick, *et al* (18), which consisted of prolonged heating (3hr) of the urine sample acidified with 1 N H_2SO_4 . Since these workers found that the acidic conjugate was hydrolyzed to free chlorphentermine the hydrolyzed urine samples were extracted for chlorphentermine (see p.35) and analyzed for drug content by gas-liquid chromatography. Comparison of peak height of chlorphentermine to internal marker was performed for both non-hydrolyzed urine and hydrolyzed urine samples.

Thin-layer Chromatography

All thin-layer chromatographic separations were performed on

20 x 20 cm glass plates coated with an adsorbent layer of 250 μ of Silica Gel G (Stahl). The plates were prepared by the following procedure: 34 g of Silica Gel G were dissolved in 80 ml of distilled water. The gel was spread on the plate to provide a thickness of 250 μ. The plates were left at room temperature for 10 to 15 min, then activated at 100°C for 1 hr. After cooling, the plates were placed in a desicator until used.

The composition of the solvent systems (v/v) were as follows -

a) Basic Extract - Benzene : Acetone : Alcohol : conc. Ammonium Hydroxide
(50 : 40 : 5 : 5)

b) Acidic Extract- Chloroform : Alcohol : glacial Acetic Acid
(90 : 10 : 5)

c) Neutral Extract & Hydrolyzed Sample - Benzene : Methanol : glacial Acetic Acid
(90 : 16 : 8)

The following spray reagents were used in an attempt to identify any possible metabolites -

a) Dragendorff's reagent (yellow-orange with secondary amines)
b) Diazotized p-nitro aniline (pink with primary amines)
c) Bromocresol purple or green (yellow with weak acid)
d) Diazotized sufanilic acid (yellow - pink with weak acid)

E. BINDING STUDY

Introduction

There is a general consensus of opinion that, because macromolecules and macromolecular complexes pass across membranes only with difficulty, protein binding *in vivo*, particularly with plasma proteins, can influence the distributional, pharmacological, and pharmacokinetic properties of certain drugs. The influence of protein binding on the kinetic behavior of drugs in the body has been investigated by a number of authors (44,49). Many qualitative and semiquantitative observations have indicated that binding can be an important parameter in the biopharmaceutical characterization of a drug. Brodie and Hogben (158) have suggested that the prolonged biological half-life and very slow rate of metabolic transformation of some drugs in man may result from the binding of the drugs by plasma protein.

Since little information was available with regard to the protein binding characteristics of chlorphentermine and related compounds in the blood, it was felt that a preliminary investigation would provide some useful information.

Reagents

Bovine serum albumin[o] (fraction V), chlorphentermine hydrochloride, reagent grade sodium hydroxide and monobasic potassium phosphate were used in these experiments. All solutions were prepared immediately prior to use. Reagent grade potassium chloride was also

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o Armour Pharmaceutical Co., Chicago, Ill.

used to adjust the ionic strength of the buffer solutions.

Equilibrium Dialysis Method

The general approach and technique employed in this study were essentially the same as those employed by Patel and Foss (159), in their study of the interaction of pharmaceuticals with macromolecules, with the exception that seamless cellulose membranes[p] were employed in this investigation.

The membrane was placed in water and heated to 90°C for one hour, then washed with distilled water several times in order to remove potentially contaminating substances.

The dialysis cells consisted of two plexiglass blocks, each block containing a cylindrical cavity. The cells were assembled by placing a small square of cellulose membrane over the cavity and then clamping the two blocks together. Into one cavity of the dialysis cell was pipetted ten mls of a solution of bovine serum albumin and into the other cavity, ten mls of chlorphentermine hydrochloride solution. The cells were then agitated in an Aminco water bath[q], equipped with a rotational shaker, at 36°C ($\pm$ 1°), until equilibrium was established.

Time sufficient to ensure equilibrium was ascertained by using a control cell containing a buffer solution in place of the protein solution. Identical concentrations of the drug on both sides of the membrane were indicative of equilibrium. Equlibrium was established at the end of 20 hours.

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p Fisher Scientific Co. Ltd., Pittsburg, PA

q Constant Temperature Bath, American Instrument Co.Inc.,Silver Spring, Md.

Because the pH of plasma is 7.4, the experiments were conducted at this pH using phosphate buffer, and the temperature was kept at 36°C (±1°). A protein concentration of 1.0×10^{-4} M was used with three different concentrations of chlorphentermine hydrochloride (0.002, 0.02, and 0.05 % w/v).

Analytical Procedure

At the end of the equlibrium time, 2 ml aliquots were removed from both sides of the membranes and diluted with 2 % NaOH solution (about 3 ml) in order to make the solutions alkaline. The extraction procedure and gas-liquid chromatographic technique for determining drug concentration were as previously described (se p. 35).

Five equilibrium dialysis cells and two control cells were employed for each different drug concentration studied.

F. DETERMINATION OF THE pKa VALUE OF CHLORPHENTERMINE

The pK_a value of chlorphentermine was determined in methanol-water by titration method. A standard concentration (1×10^{-2} M) of chlorphentermine base was prepared in various concentrations of methanol-water and titrated at 25°C with 0.1 N HCl. The titrant was added in successive 0.2 ml quantities from a semimicro buret, the pH recorded and the titration curves constructed. The pK_a values were estimated from the titration curves obtained for each methanol-water system and these values were plotted against the concentration of methanol-water. The

extrapolated value to zero per cent methanol would give the pK_a value of chlorphentermine in aqueous solution at 25°C.

All pH measurements were made using a Beckman Expandomatic pH meter.

CHAPTER IV. RESULTS

A. URINARY EXCRETION STUDIES

Analytical Procedure

The extraction procedure for chlorphentermine from urine samples was found to be very efficient giving an absolute recovery of 95 - 100 % (see Table I.).

Table I.

Recovery of Chlorphentermine added to Blank Urine

Chlorphentermine Hydrochloride added, mcg/ml	Recovered[a], mcg/ml	Recovery, %
2.0	1.9	95
4.0	3.8	96
8.0	7.6	95
10.0	9.8	98
20.0	19.6	98
40.0	40.0	100
60.0	57.0	95

a Average of 5 replicates

The standard deviation of twelve analyses of urine samples each containing 10 mcg/ml of chlorphentermine hydrochloride was found to be $\pm$ 0.14 mcg/ml.

No deterioration of chlorphentermine in urine occurred when refrigerated at 4^{o}C for 4 weeks.

Analysis of blank samples of urine from the subjects used in the studies showed no interfering peaks with the same retention times as chlorphentermine or azobenzene.

Figure 6 shows a typical chromatogram of a urine extract containing chlorphentermine and internal marker, azobenzene. Good symmetrical peaks were obtained for both chlorphentermine and azobenzene. The retention times for both peaks were within seven minutes.

Figure 7 illustrates a calibration curve for the drug in urine. The curve is linear over the range 2.0 - 60.0 mcg of drug per ml of urine and passes through the origin. Calibration curves prepared at later times did not show significant deviation from the original constructed curve.

The optimum air and hydrogen pressures for maximum response of drug and internal marker were found to be 25 lb/sq in and 15 lb/sq in respectively.

Urine Excretion Trials

Figure 8 represents the urinary excretion profile of chlorphentermine following oral administration of a 78 mg solution dose to one subject (H.J.) whose urinary pH was uncontrolled. Fluctuations in the excretion rate of the drug occurred which were paralled to a large extent by changes in urinary pH and urine volume. Urinary pH appeared to be the major factor influencing the excretion rate of the drug, but under acidic urinary pH control fluctuations in excretion rate still occurred, which could be mainly attributed to changes in urine volume.

Figure 9 shows the large differences noted in the cumulative excretion of chlorphentermine in the urine of one subject (H.J.) under varying urinary pH conditions.

When the urinary pH of the three subjects was maintained alkaline by oral administration of sodium bicarbonate, the percentage

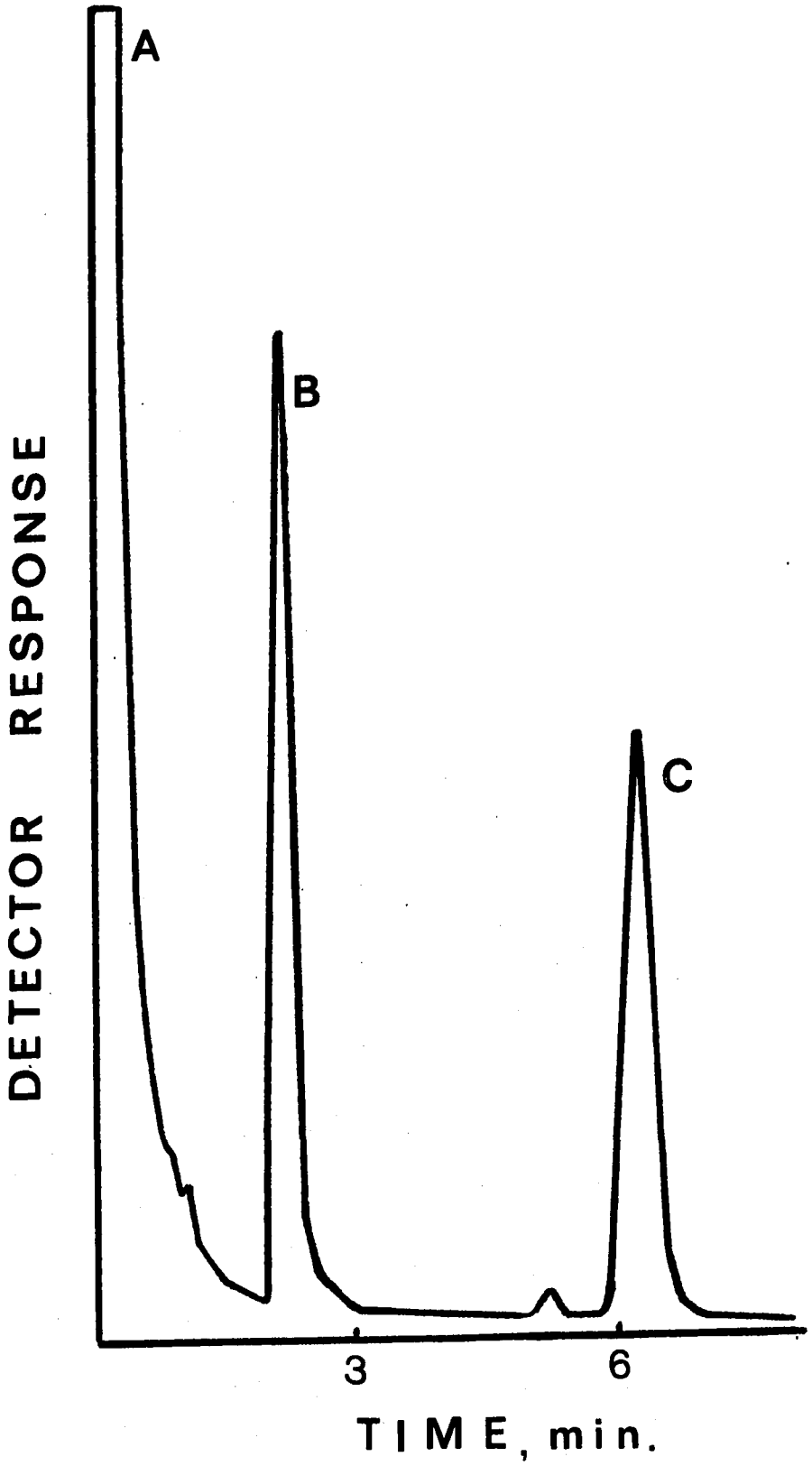

Figure 6. Typical Chromatogram of Urine Extract Containing Chlorphentermine; A = Solvent (ether), B = Chlorphentermine, C = Azobenzene (internal marker).

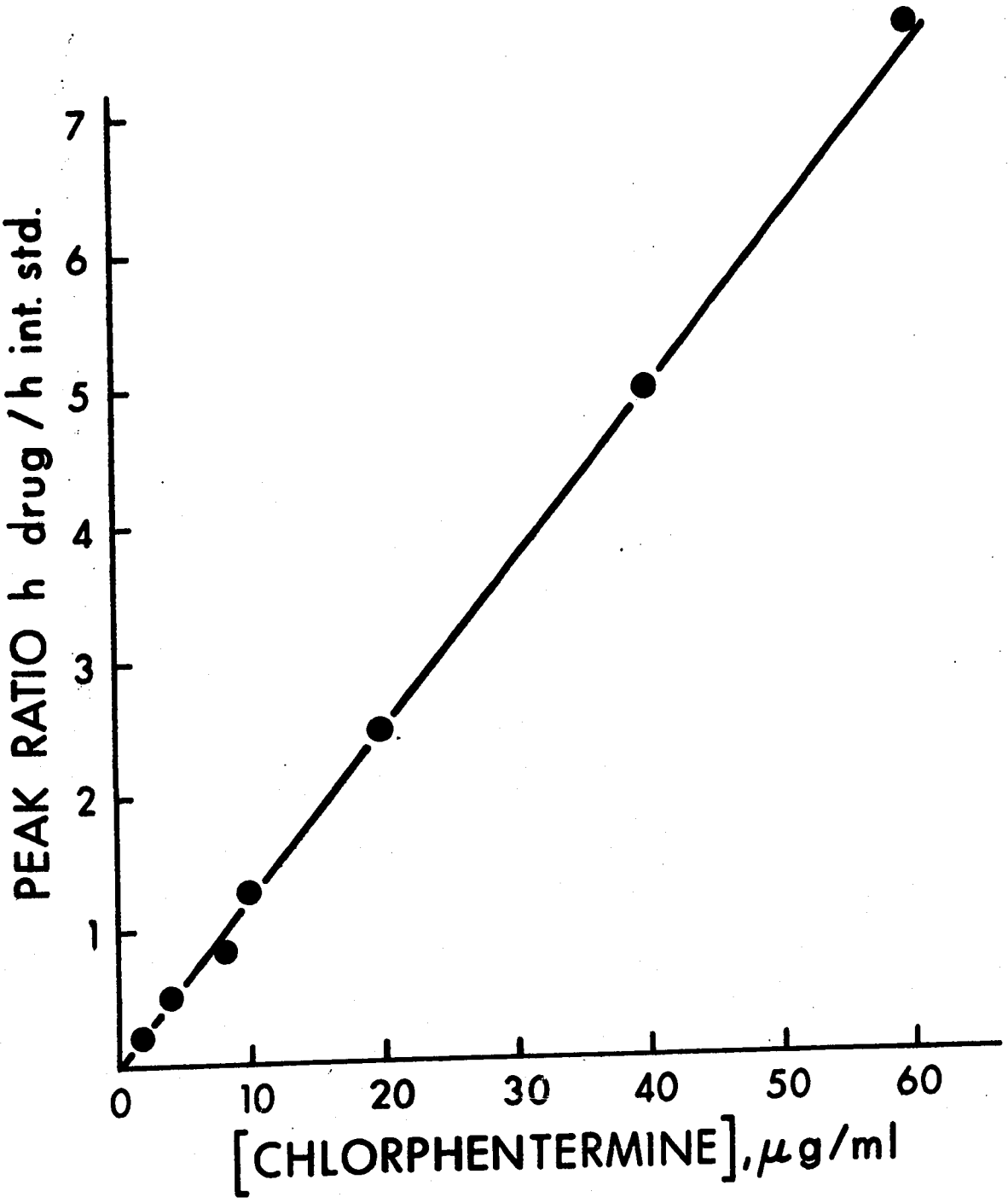

Figure 7. Calibration Curve for Chlorphentermine in Urine

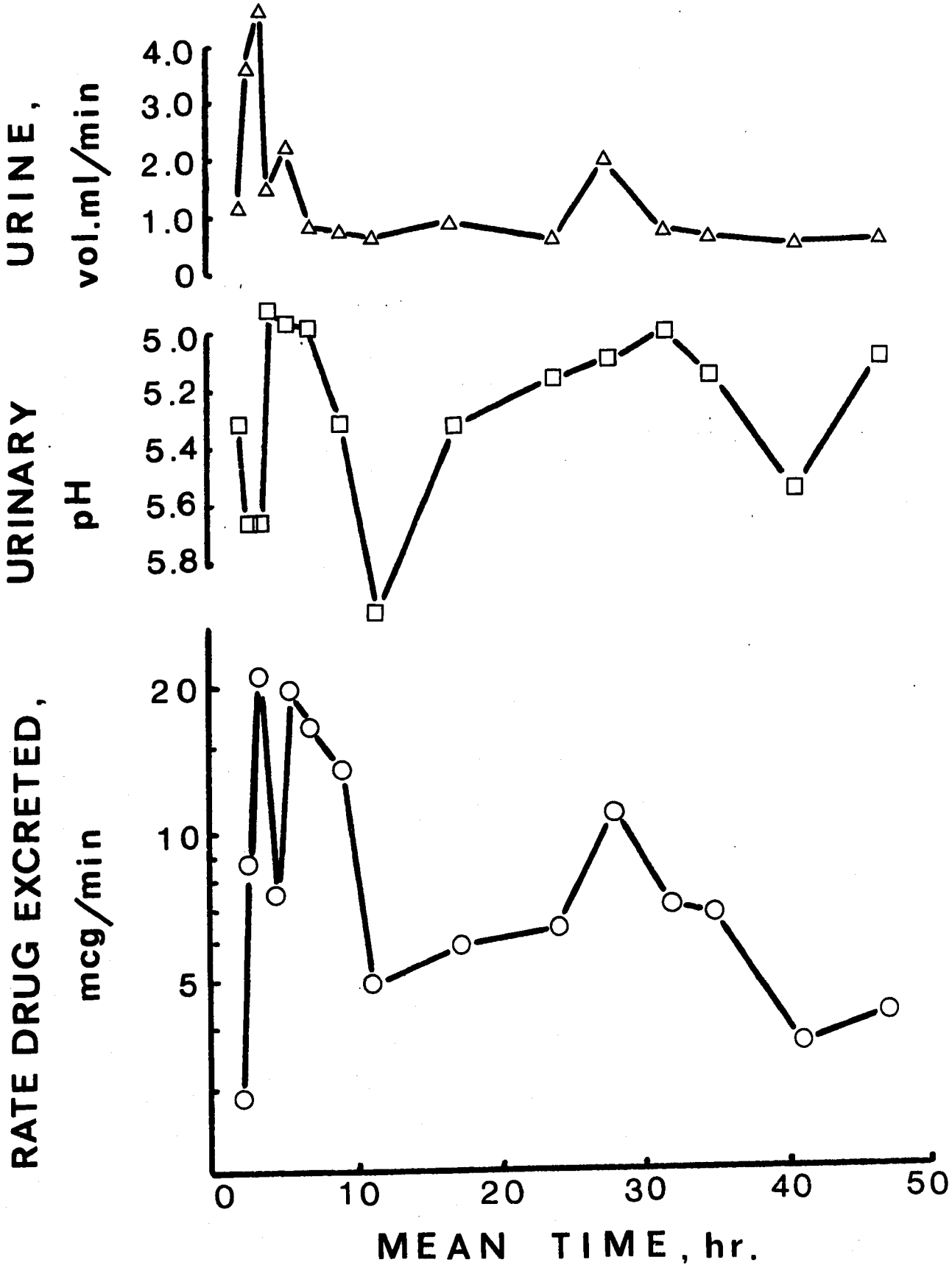

Figure 8. The Urinary Excretion Profile of Chlorphentermine. Oral Dose 78 mg Chlorphentermine Hydrochloride; Urinary pH Uncontrolled; Subject: H.J.

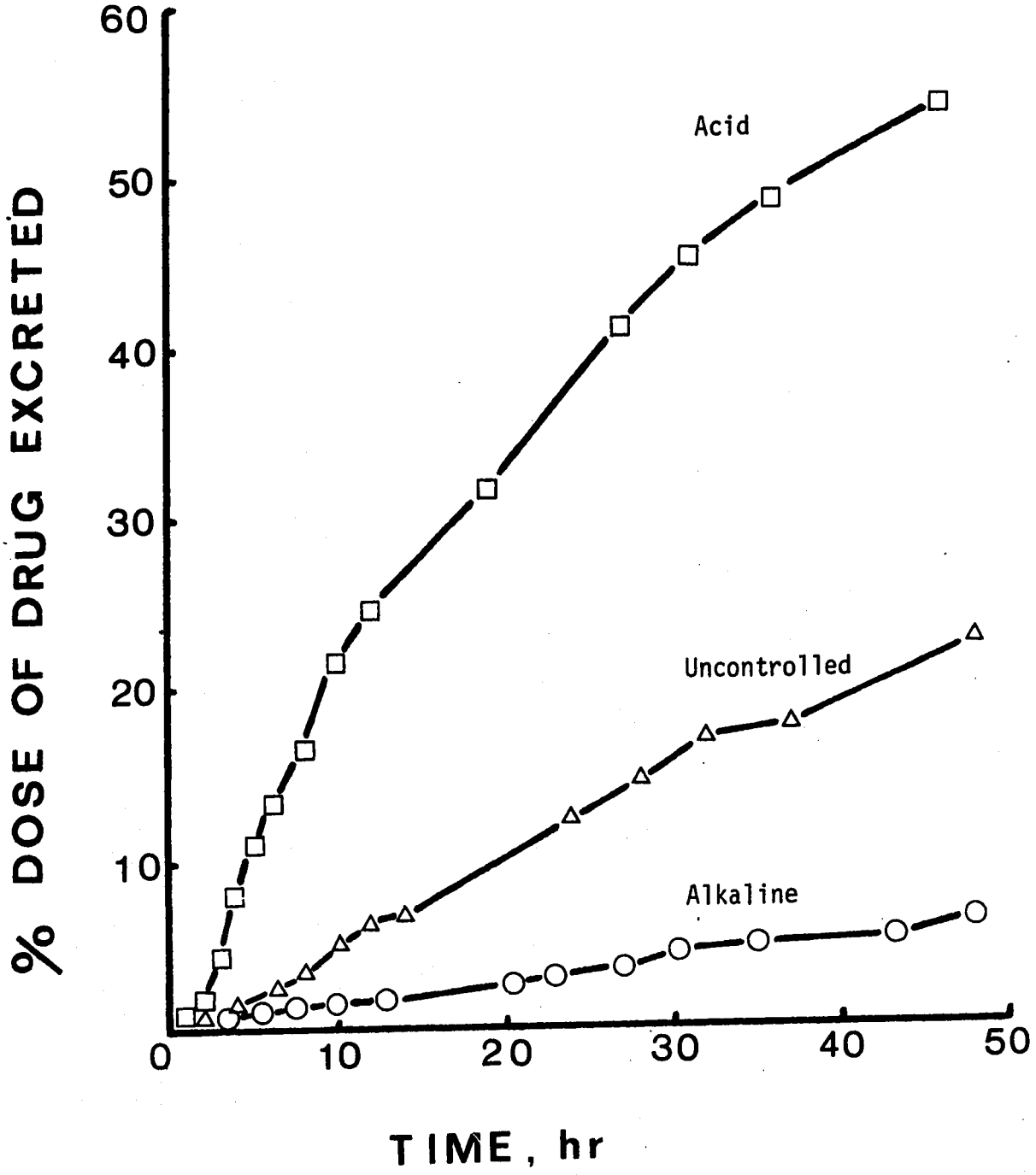

Figure 9. The Cumulative Urinary Excretion of Chlorphentermine Under Uncontrolled, Alkaline, and Acidic Urinary pH Conditions. Oral Dose 78 mg Chlorphentermine Hydrochloride; Subject: H.J.

of the dose of chlorphentermine excreted in 48 hr was found to be 6.1 - 12.7 % (mean 9.4 %). The recovery under uncontrolled pH conditions was 13.4 - 31.3 % (mean 23.7 %), and with urinary pH maintained acidic by oral administration of ammonium chloride it was 51.5 - 67.0 % (mean 57.8%).

The urinary excretion profile of chlorphentermine following oral administration of a 78 mg solution dose under acidic urinary condition is shown in Figure 10. The maximum drug excretion rate occurred at about 3 hours and was followed by a slow exponential decline showing some fluctuations.

Figure 11 illustrates the urinary excretion of the drug following oral administration of a compressed tablet under acidic conditions (subject: A.S.) . The maximum drug excretion rate also occurred at about 3 hours.

Figure 12 shows the urinary excretion rate of drug under acidic conditions following oral administration of the prolonged-release preparation A (subject: K.M.). The maximum excretion rate was delayed and appeared at about 7 hours.

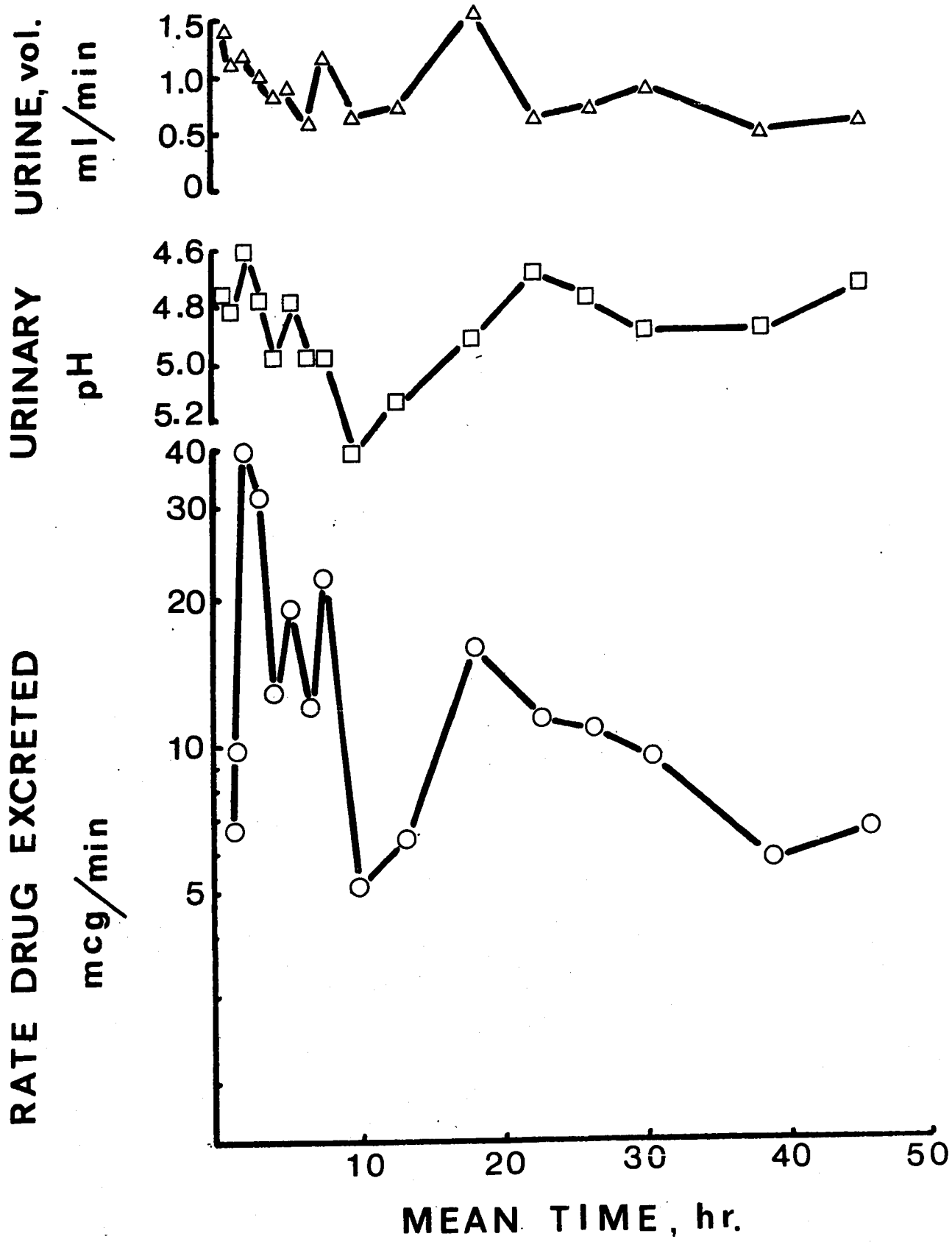

Figure 10. The Urinary Excretion Profile of Chlorphentermine Following Oral Administration of a 78 mg Solution Dose. Acidic Urinary pH Condition; Subject: A.S.

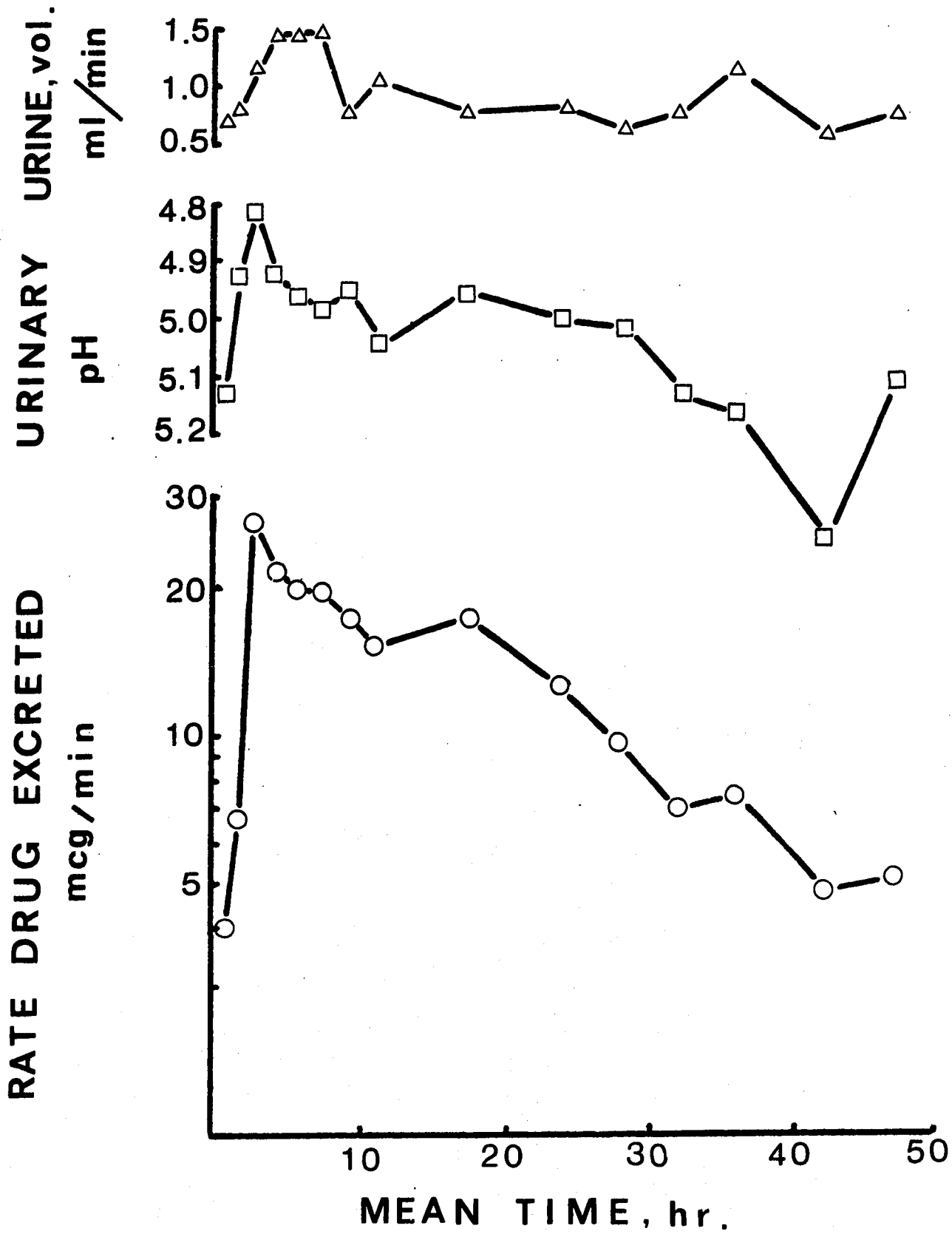

Figure 11. The Urinary Excretion Profile of Chlorphentermine Following Oral Administration of a 78 mg Compressed Tablet. Acidic Urinary pH Condition; Subject: A.S.

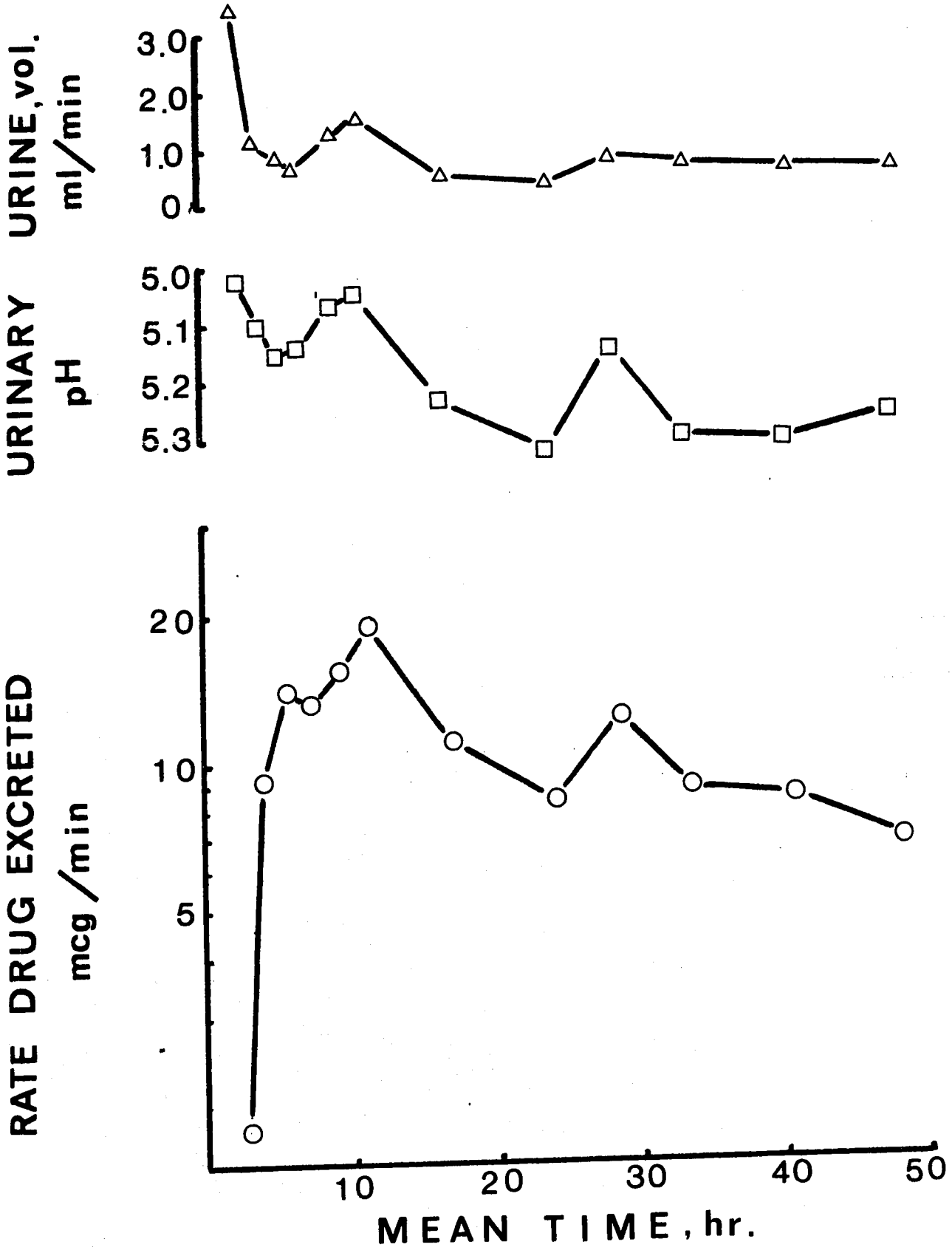

Figure 12. The Urinary Excretion Profile of Chlorphentermine Following Oral Administration of Prolonged-release Preparation. Acidic Urinary pH Condition; Subject: K.M.

B. ANALOG COMPUTER SIMULATIONS

Computer Simulation Techniques

'Solution' and 'Tablet' forms, ie. 'Free' drug (Model I).

The experimental data and typical fitted cumulative excretion curves, together with the derived curves for the amount of chlorphentermine in the gastrointestinal tract, blood compartment, and tissue compartment are shown in Figures 13 and 14. The kinetic parameters obtained using the above method for each subject are shown in Table II.

An excellent fit was found between data calculated by the computer and that determined experimentally in two of the three subjects who received the 'free' forms of chlorphentermine. With subject K.M., however it was not possible to fit the experimental cumulative urinary excretion data obtained after administration of the drug in solution at times greater than 30 hr. Data points for this individual were slightly higher (+2 to +5 % dose/hr) over this period compared with the computer curve. The reason for this discrepancy was believed to be due to a sudden increase in the subject's urinary flow rate 30 hr after drug administration, as urinary flow rate has been shown to markedly influence the rate of urinary excretion of the drug (see p. 68).

Inter- and intra-subject variations in the elimination half-life in most cases were apparent when chlorphentermine was given in different dosage forms as a 78 mg dose. It did appear, therefore, that the elimination half-life of the drug for a subject could vary at each trial, and that this variation was probably due to changes in the subject's urinary flow rate and not as a result of the different dosage forms used.

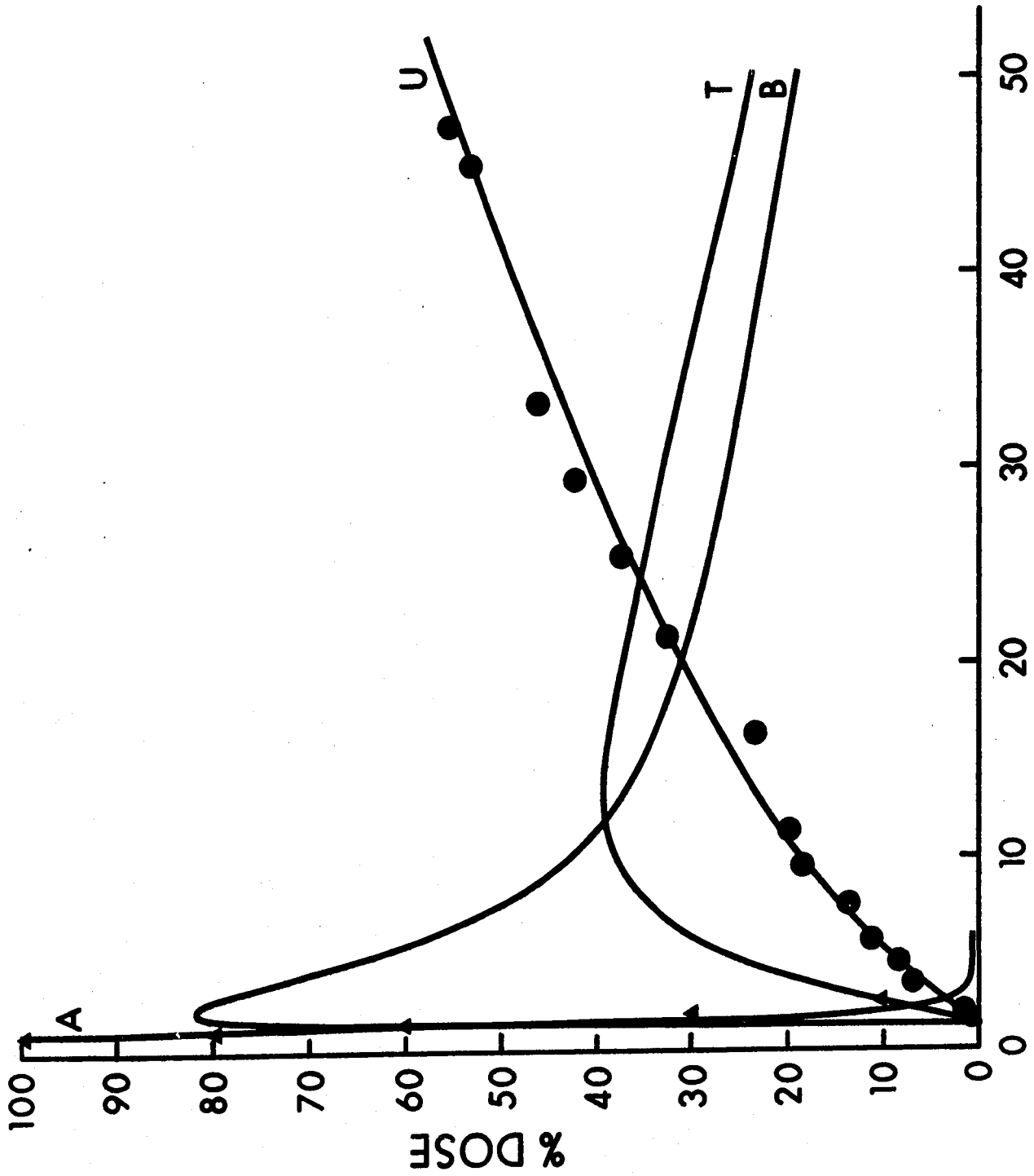

Figure 13. Computer Curves and Experimental Data Points for the Urinary Excretion of Chlorphentermine after Oral Administration of a Solution Dose. (Solid lines: computer predictions; Points: experimental data. A; B; T; and U; see Appendix A. Subject: A.S.)

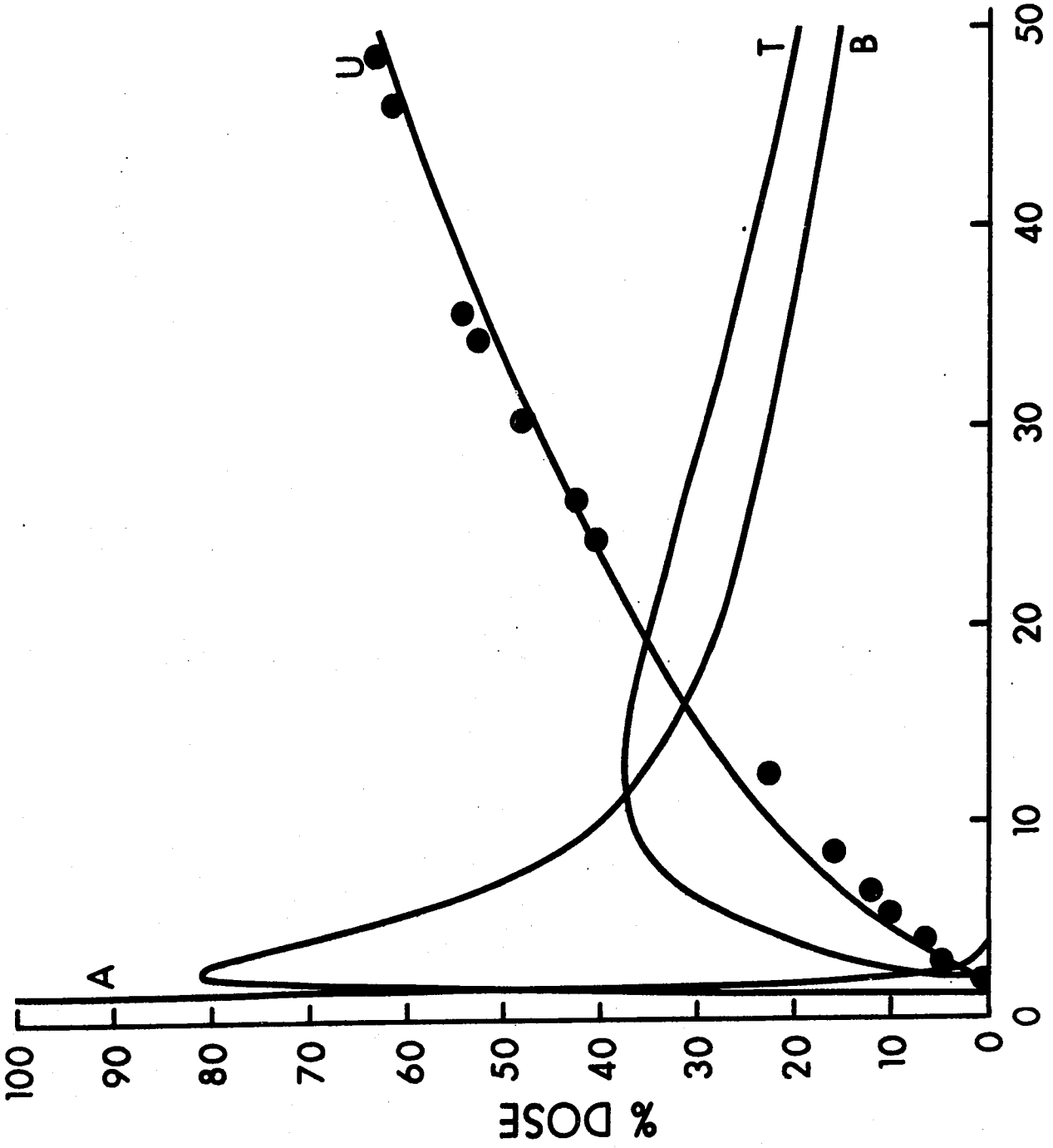

Figure 14. Computer Curves and Experimental Data Points for the Urinary Excretion of Chlorphentermine after Oral Administration of a Compressed Tablet. (Solid lines: computer predictions; Points; experimental data. A; B; T; and U; see Appendix A. Subject: H.J.)

Table II. Kinetic Parameters for the Release, Absorption, Distribution, and Excretion of Chlorphentermine after 78 mg Doses in Various Dosage Forms.

Subject	Dosage form	Dose (mg HCl)	Lag Time (hr)	k_{r1} (hr^{-1})	k_{r2} (hr^{-1})	Break Time (hr)	k_a (hr^{-1})	$k_{B\rightarrow T}$ (hr^{-1})	$k_{T\rightarrow B}$ (hr^{-1})	k_u (hr^{-1})	k_e (hr^{-1})●	Elimination $t_{1/2}$ (hr)+
H.J.	Solution	78	1.0	-	-	-	2.8	1.50	1.40	0.036	0.017	39.8
A.S.	"	78	1.0	-	-	-	2.8	1.50	1.40	0.032	0.016	43.3
K.M.	"	78	3.0*	-	-	-	2.8	1.50	1.40	0.036	0.017	39.8
H.J.	Tablet	78	1.25	-	-	-	2.8	1.50	1.40	0.040	0.019	35.9
A.S.	"	78	1.0	-	-	-	2.8	1.50	1.40	0.036	0.017	39.8
K.M.	"	78	1.5	-	-	-	2.8	1.50	1.40	0.030	0.014	48.6
H.J.	Prepn A	78	1.0	0.10	0.75	5.0	2.8	1.50	1.40	0.040	0.019	35.9
A.S.	"	78	1.0	0.20	0.69	3.75	2.8	1.50	1.40	0.029	0.014	49.3
K.M.	"	78	1.0	0.20	0.69	3.75	2.8	1.50	1.40	0.030	0.014	48.6

Values of k_a, $k_{B\rightarrow T}$ were chosen on the basis of "best values" of solution and tablet data, and were considered invariant for preparation A.

*The lag time for this subject appeared unduly long and is probably the result of the subject having had a heavy breakfast shortly before drug administration.

●The rate constants for elimination are calculated from the rate constants k_u by the expression

$$k_e = \frac{k_u}{1 + \frac{k_{B\rightarrow T}}{k_{T\rightarrow B}}}$$

+The elimination half-lives are obtained from the k_e values by the expression $t_{1/2} = \ln 2/k_e$

Beckett and Tucker (101) showed no appreciable difference in the elimination half-life of amphetamine, a drug whose excretion is unaffected by urinary flow rate, when the drug was given in solution, free pellet or capsule forms.

Prolonged-release Drug Preparation (Model II).

The experimental data and typical fitted cumulative excretion curves, together with derived curves for blood level, tissue level and release of chlorphentermine from the dosage form are shown in Figures 15 and 16. The kinetic parameters for each individual subject are shown in Table II. By assuming two sequential release rates from the dosage form, governed by k_{r_1} and k_{r_2}, good computer fits to the data were obtained for all three subjects. The values for the release constants for prolonged-release preparation A were obtained from the data supplied to us by the manufacturers, and were used as a first estimate in computer fit.

In Vitro Data

Figure 17 illustrates the effect of ammonium chloride on the rate of *in vitro* drug release from prolonged-release preparation A. Due to the very small differences obtained between the rates of drug release, in the presence and absence of ammonium chloride, it did appear that any effect of ammonium chloride on drug release was negligible.

In Vivo / In Vitro Correlation

In Figure 18 the percentage of drug release *in vivo* from the dosage form preparation A is plotted against the percentage of drug released *in vitro* after the same time intervals. If complete correlation between *in vivo* and *in vitro* results were obtained the experimental points would be on the solid line shown.

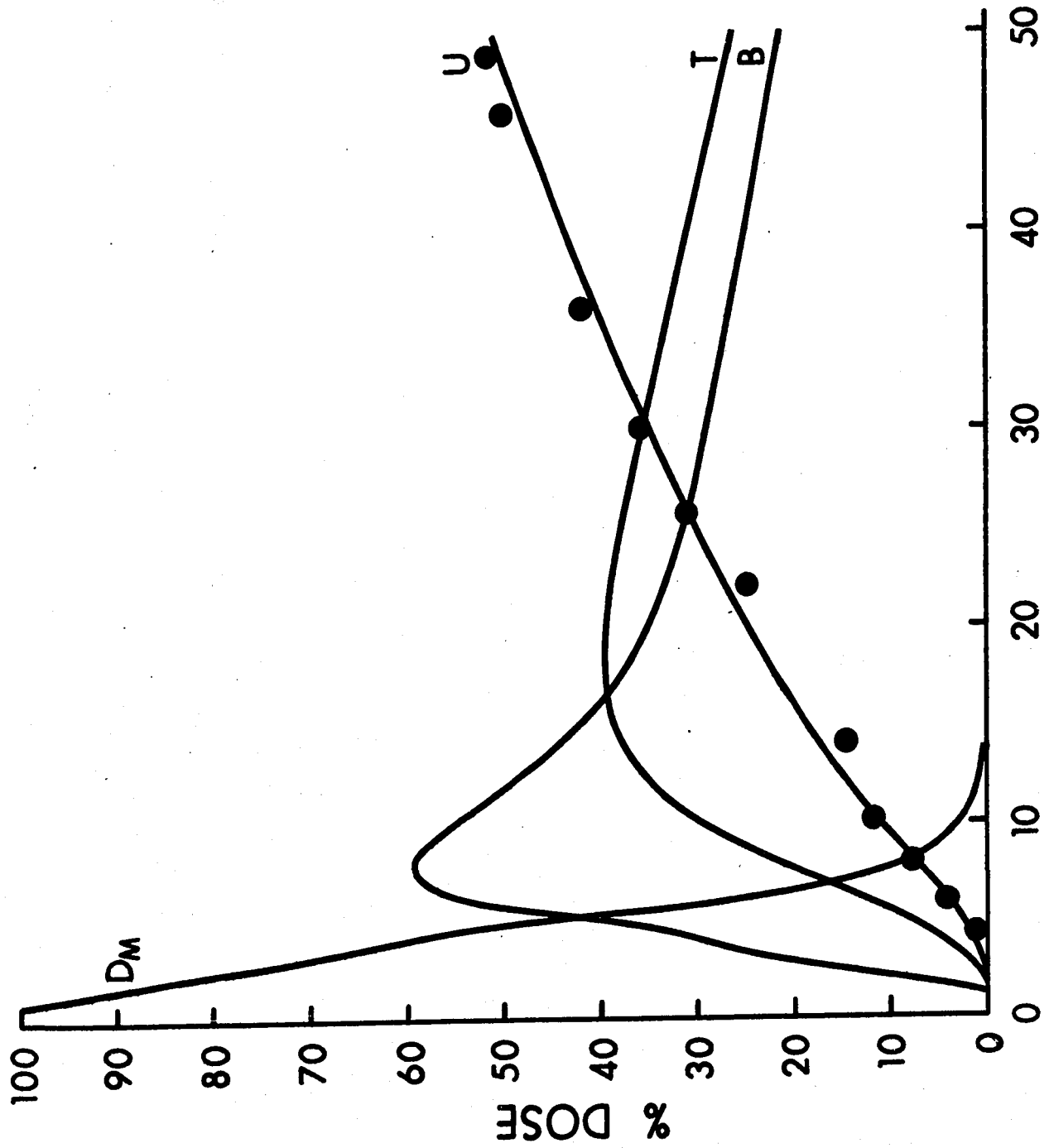

Figure 15. Computer Curves and Experimental Data Points for the Urinary Excretion of Chlorphentermine after Oral Administration of Prolonged-release preparation. (Solid lines: computer predictions; Points: experimental data. D_M; U; T; and B; see Appendix A. Subject: K.M.)

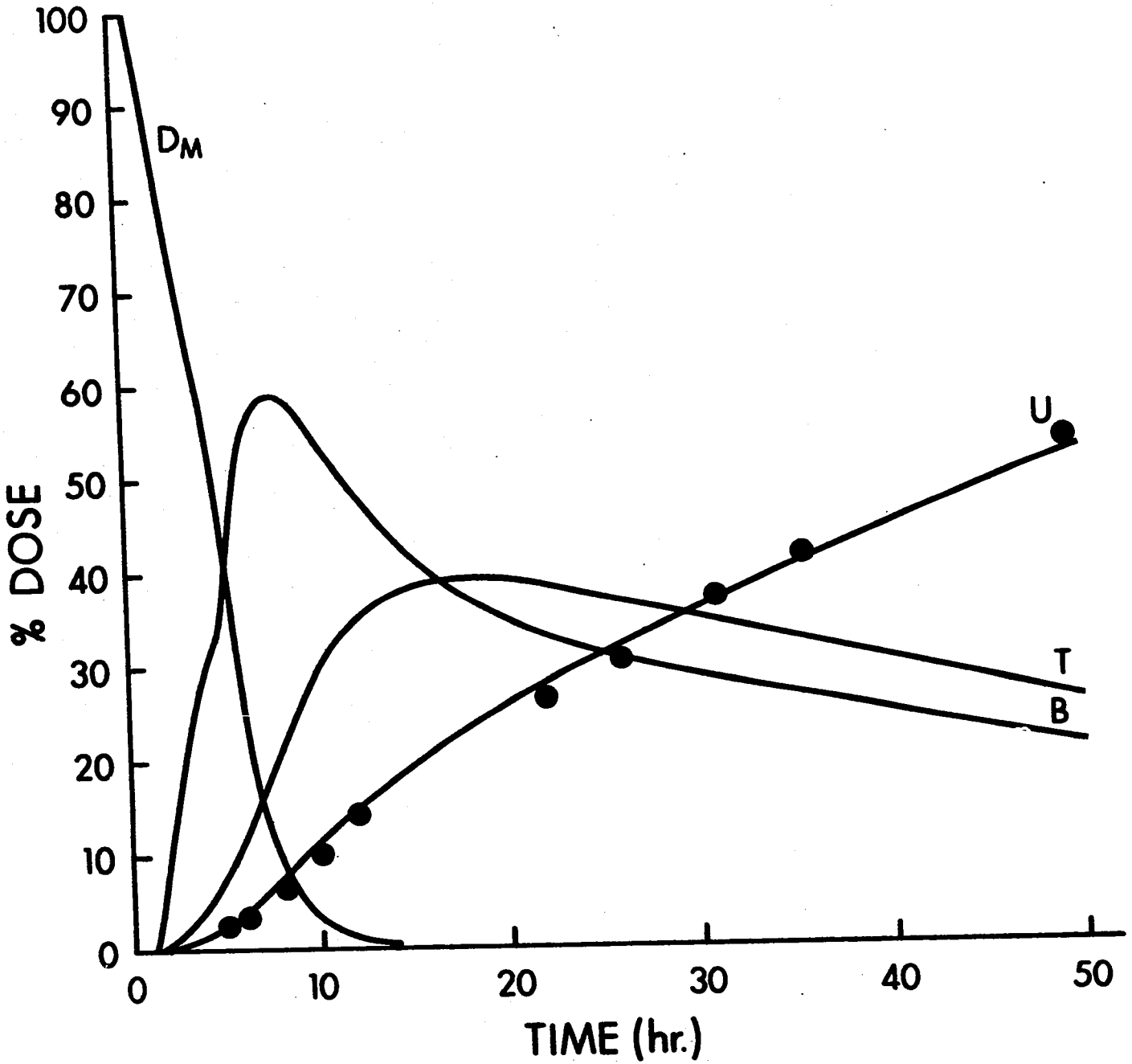

Figure 16. Computer Curves and Experimental Data Points for the Urinary Excretion of Chlorphentermine after Oral Administration of Prolonged-release Preparation. (Solid lines: computer predictions; Points: experimental data. D_M; U; T; and B; see Appendix A. Subject: A.S.)

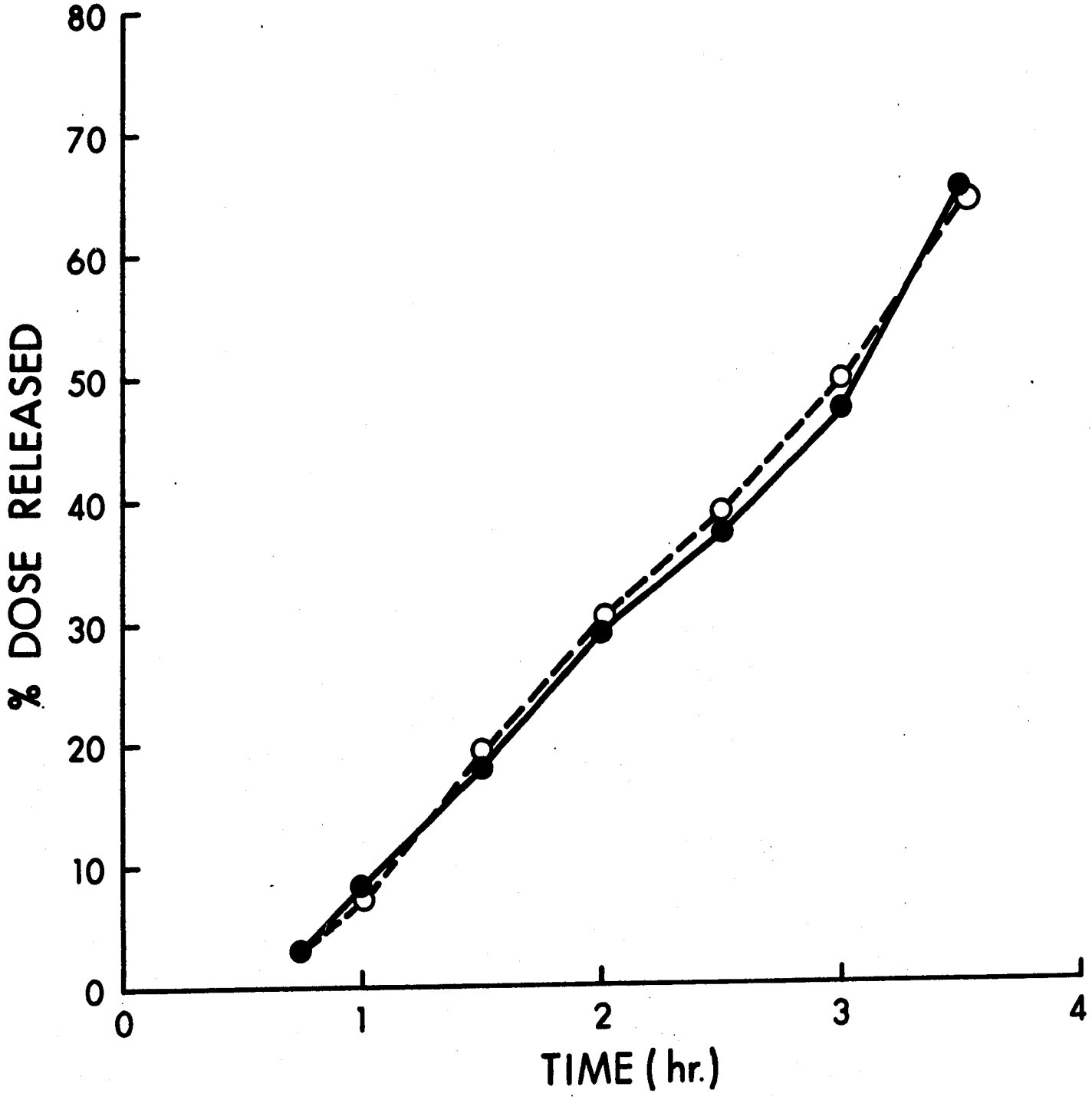

Figure 17. Effect of Ammonium Chloride on the Rate of Release of Chlorphentermine from Prolonged-release Preparation. (Solid line: absence of NH_4Cl; Dotted line: presence of NH_4Cl. Each data point represents the average of two determinations)

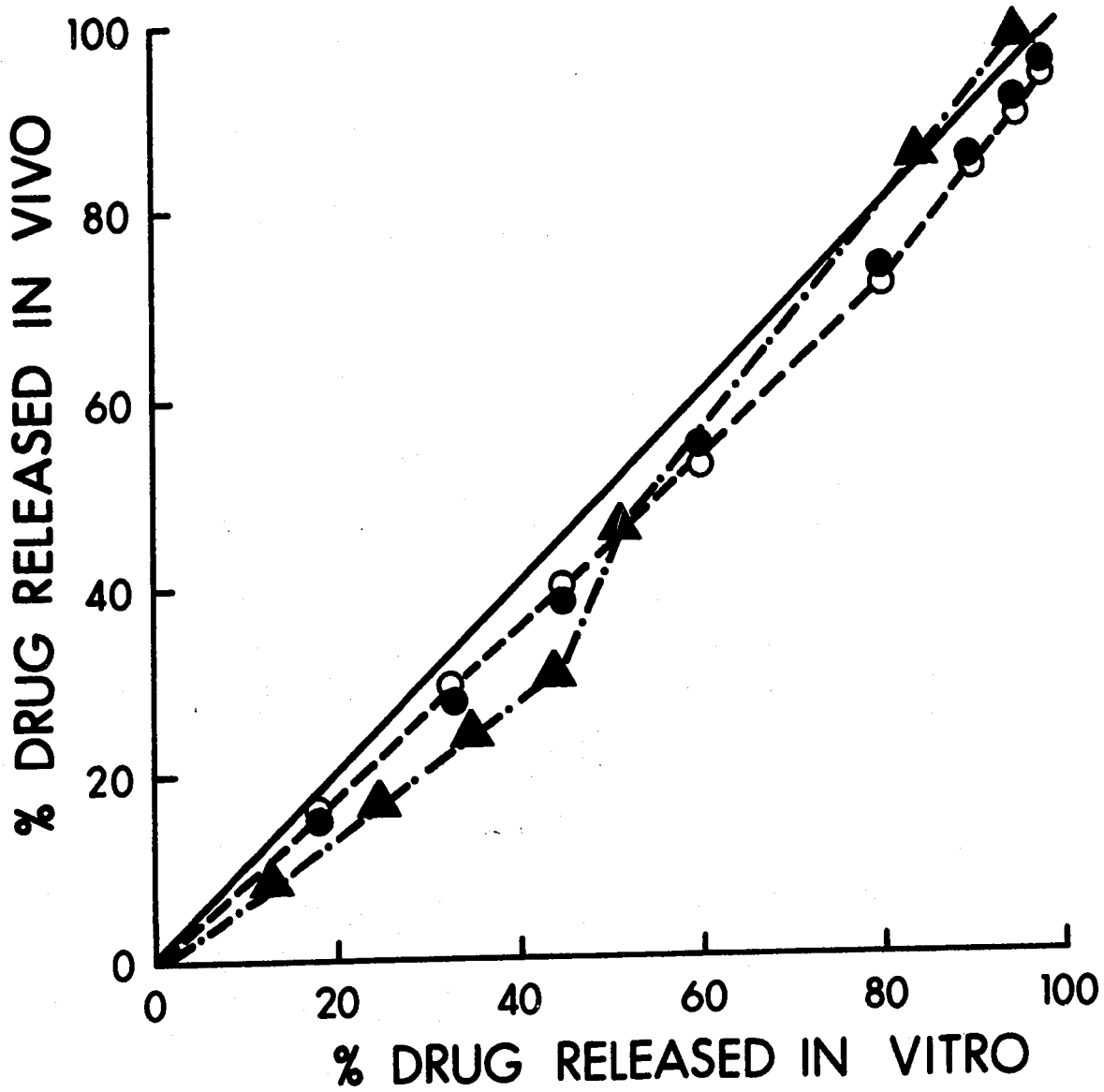

Figure 18. In Vitro/ In Vivo Drug Release Correlations with Prolonged-release Preparation containing Chlorphentermine.
(○: Subject-K.M.; ●: Subject-A.S.; ▲ : Subject-H.J.)

C. BLOOD LEVEL STUDIES

Analytical Procedure

The absolute recovery of chlorphentermine was found to be 90 - 100 % when known amounts of drug were extracted from 'blank' blood. The percentage recovery of chlorphentermine from 'blank' blood appears in Table III.

Table III.

Recovery of Chlorphentermine added to Blank Blood

Chlorphentermine Hydrochloride added, mcg/5 ml	Recovered[a], mcg/5ml	Recovery, %
0.25	0.22	90
0.50	0.47	94
0.75	0.76	100
1.00	0.91	91
1.50	1.35	90
2.00	1.98	99
3.00	2.76	92

a Average of 4 replicates

The percentage standard deviation of ten analyses of blood samples each containing 0.5 mcg/5ml chlorphentermine hydrochloride was shown to be $\pm$ 4.92 %.

No deterioration of chlorphentermine in blood occurred when refrigerated at $4^{o}C$ for 4 weeks.

Figure 19 shows a typical chromatogram of a blood extract containing chlorphentermine and the internal marker, azobenzene. Symmetrical peaks were obtained for both chlorphentermine and azobenzene. Analysis of 'blank' samples of blood from the subjects used in the studies showed no interfering peaks with the same retention times as chlorphentermine or azobenzene.

Figure 20 represents a calibration curve obtained for chlorphentermine extracted from blank blood for the concentration range 0.25 mcg to 3.0 mcg of drug per 5 ml of blood. The calibration curve was a straight line passing through the origin.

Blood Level Trials

Oral administration.

The average blood level - time curve for chlorphentermine following oral administration of the 100 mg solution dose to four subjects is shown in Figure 21. The pharmacokinetic parameters, apparent plasma half-life, apparent volume of distribution, and the area under the blood level curve were estimated from the blood level - time curves and appear together with the cumulative urine excretion data in Table IV.

Subjects experienced no stimulant effects from the drug at this dosage level. The only effects noticed were pupil dilation and marked anorexia.

The average blood level - time curve for chlorphentermine following oral administration of the 78 mg prolonged-release preparation A to three subjects is shown in Figure 22. Areas under the blood level time curves and urine data are shown in Table IV. No subjective effects were noted.

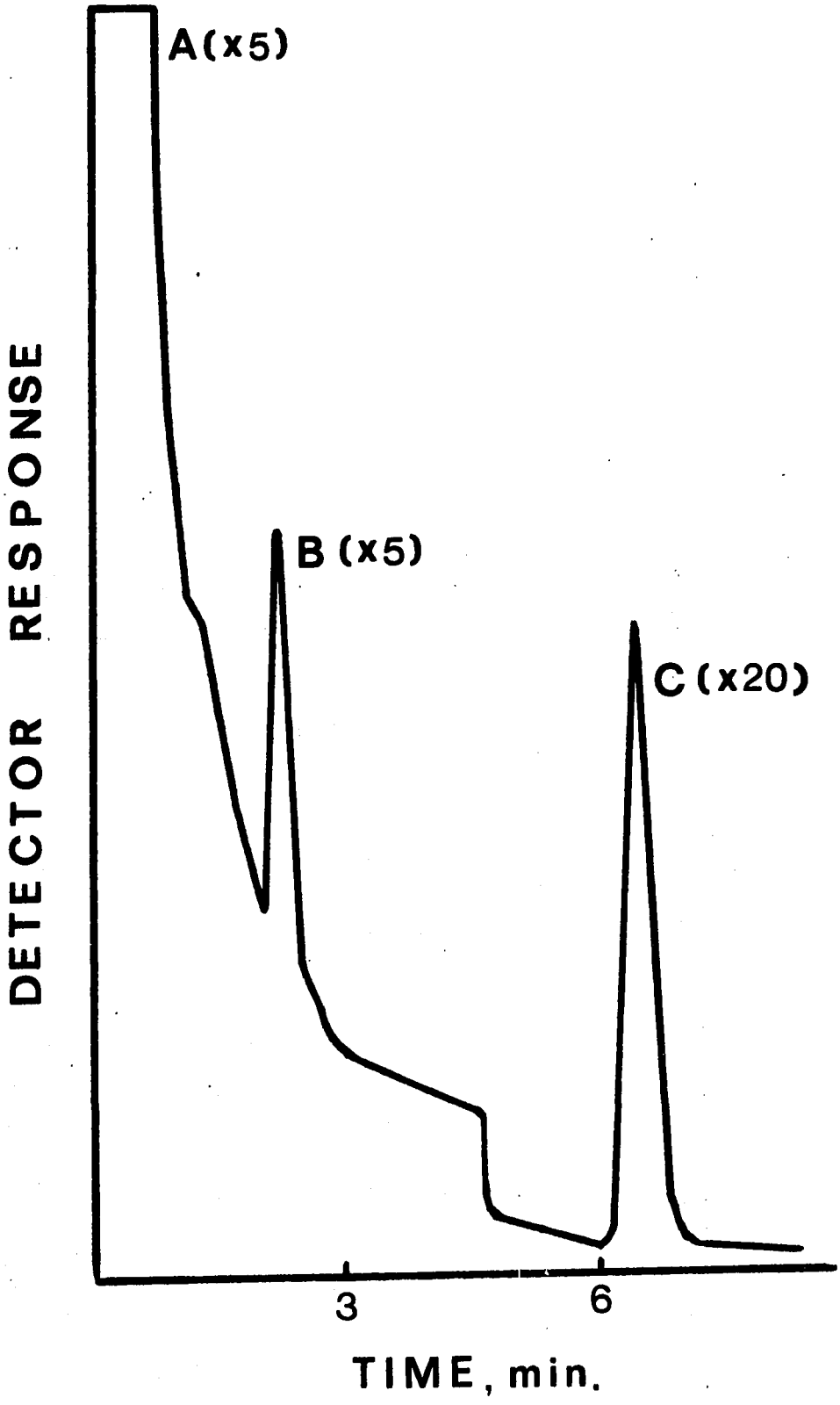

Figure 19. Typical Chromatogram of Blood Extract containing Chlorphentermine A = Solvent Peak (ether), B = Chlorphentermine, C = Azobenzene (internal marker). Figures in parenthesis signify attenuator setting.

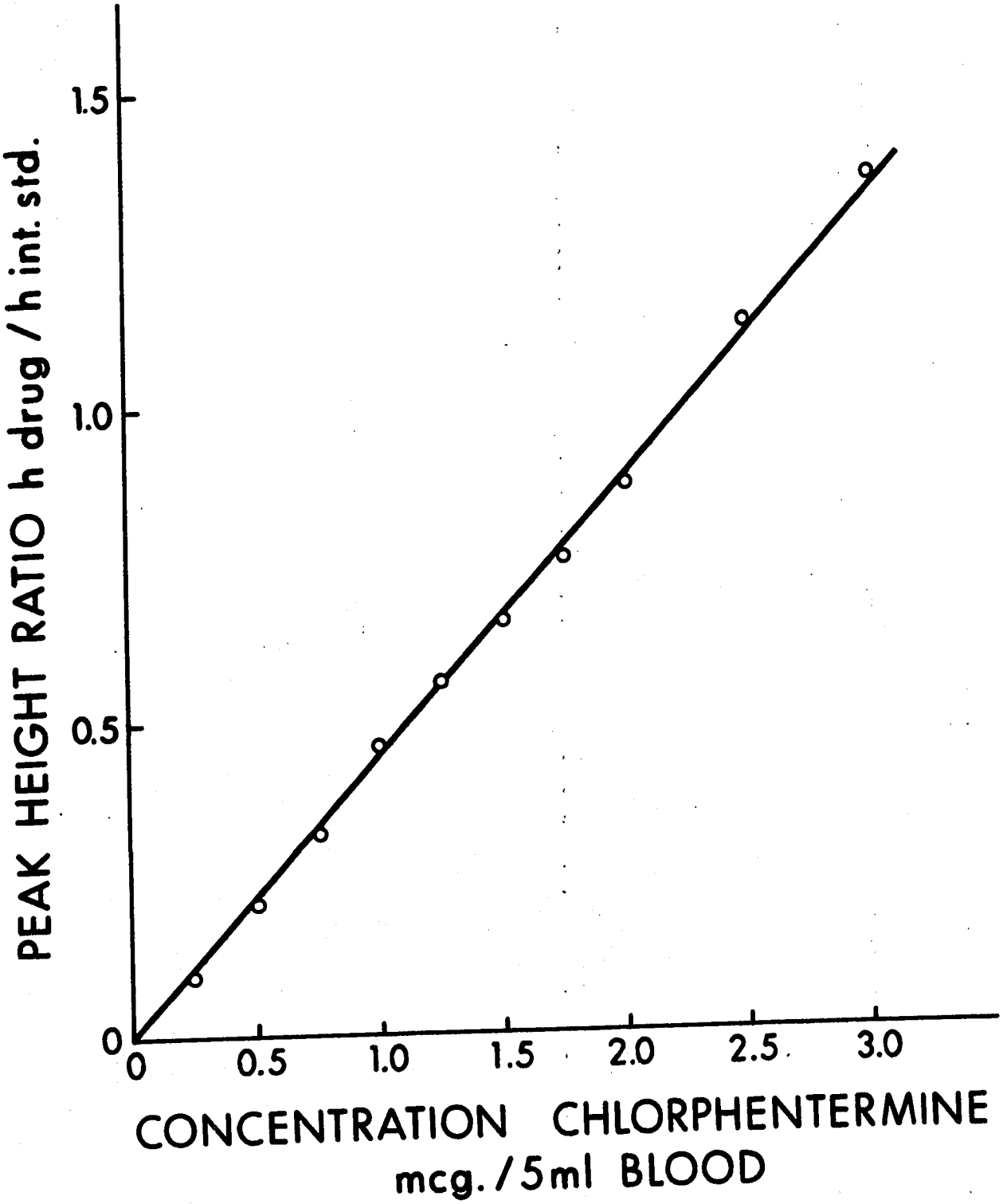

Figure 20. Calibration Curve for Chlorphentermine in Blood.

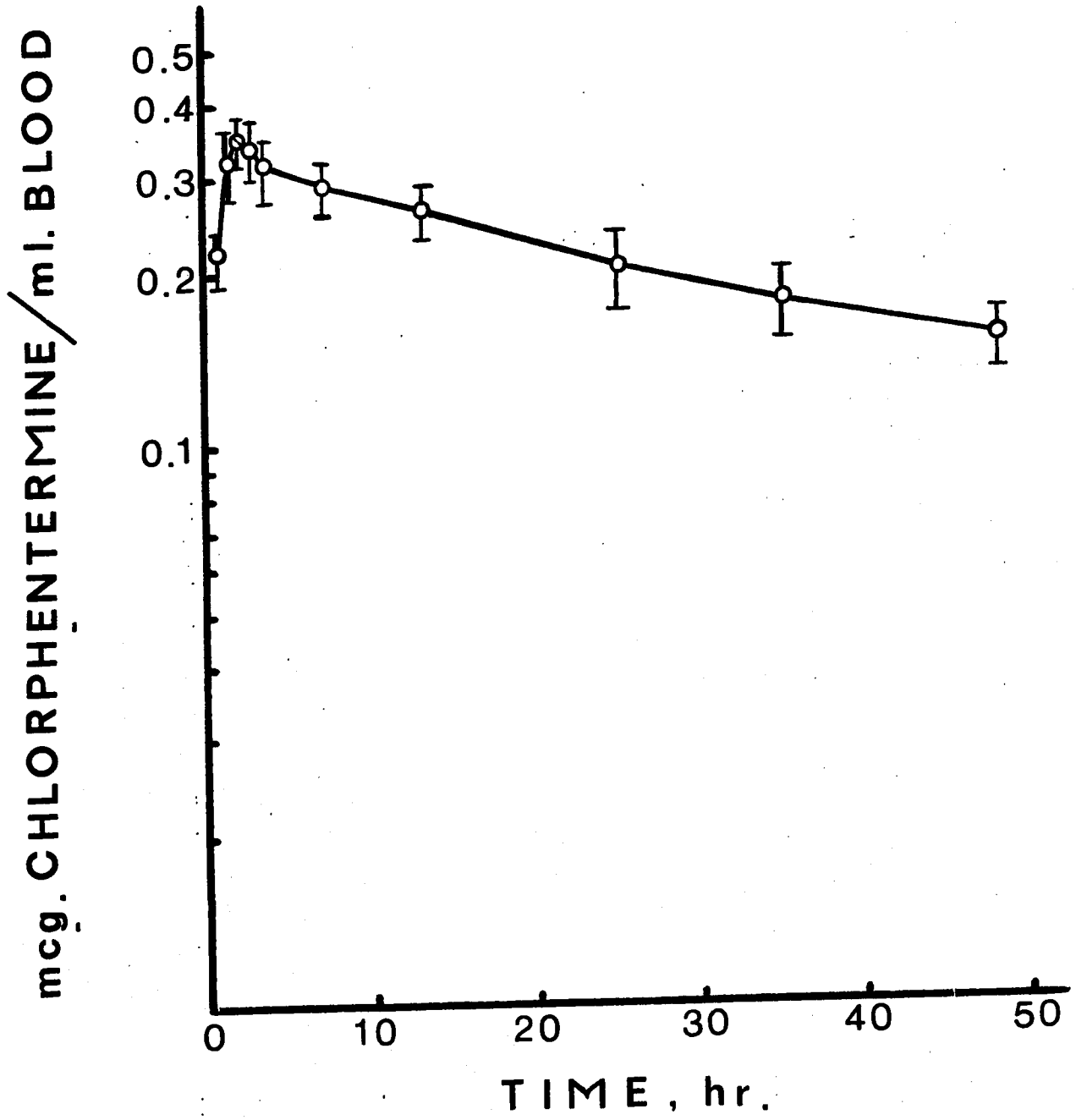

Figure 21. Average Blood Level Versus Time Curve for Chlorphentermine following Drug Administration in Solution (100 mg). ⊕ represents the Average of four Subjects ± Standard Error of the Mean.

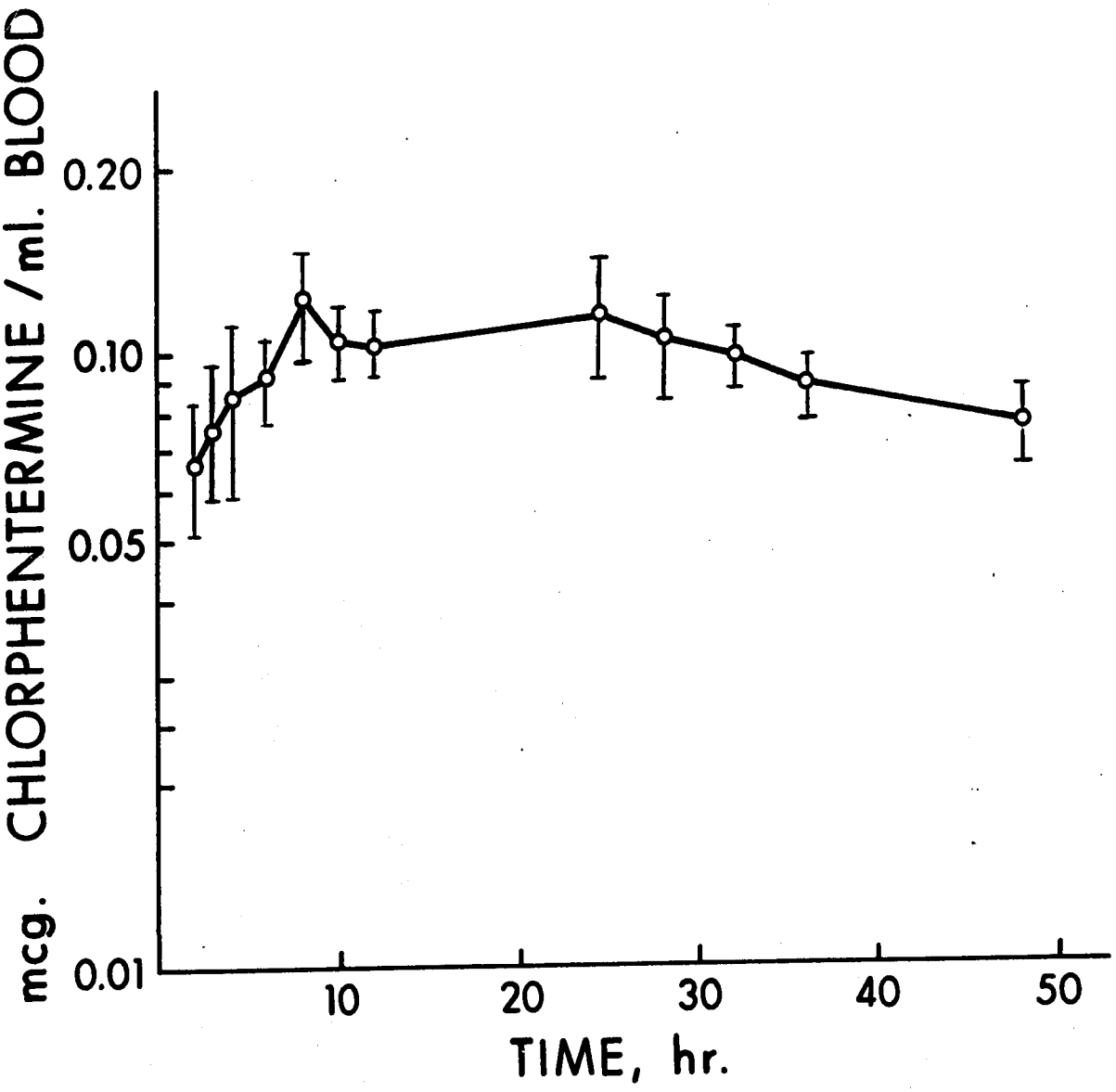

Figure 22. Average Blood Level Versus Time Curve for Chlorphentermine following Drug Administration as Prolonged-release Preparation (78 mg). $\overline{\Phi}$ represents the Average of three Subjects ± Standard Error of the Mean.

Table IV. Some Pharmacokinetic Parameters Obtained from Blood Level Studies for Chlorphentermine Following Solution, Prolonged-release and Intravenous Drug Administration in Man.

Subject*	Mode of Administration	Dose (mg HCl)	Apparent Plasma Half-life(hr)	Apparent Volume of Distribution (l)	Area under Blood Level Curve (mcg-hr/ml)+	% Dose Excreted Unchanged in Urine in 48 hr
H. J.	Solution	100	45	185	14.5	23.3
D. S.	"	"	35	214	10.7	25.8
W. J.	"	"	45	298	8.6	19.6
K. M.	"	"	40	245	8.5	16.1
H. J.	I/V	50	42	139	8.7	20.4
D. S.	"	"	44	198	6.6	21.6
H. J.	Prolonged-release	78	-	-	4.5	16.4
K.M.	"	78	-	-	5.8	12.3
W. J.	"	"	-	-	3.3	14.1

+ Calculated by means of Trapezoidal Rule.

* Incompleted Cross-over Study.

Intravenous administration.

The average blood level - time curve for chlorphentermine following intravenous administration of the drug to two subjects is shown in Figure 23. The pharmacokinetic parameters and urine data appear in Table IV.

Effects noted were light headedness and marked anorexia.

Accumulation study.

Blood level-time curves for chlorphentermine following administration of an oral dose of prolonged-release preparation A daily for seven consecutive days for three subjects appear in Figure 24. Steady state blood levels were approached after three to four days of drug administration for each of the subjects.

No subjective effects were noted during the entire experimental period in all subjects. Anorexia was not significant.

Total amounts of drug left in the body at the times of drug readministration for the three subjects are shown in Figure 25. These values were calculated on the basis of the urinary excretion data with the assumption that the drug was completely absorbed.

D. METABOLISM STUDIES

Tables V and VI show the results of the *in vitro* and *in vivo* metabolism studies of chlorphentermine.

The gas-liquid chromatographic peak for chlorphentermine showed no increase after the analysis of the hydrolyzed human urine samples indicating the absence of any acidic conjugate of the drug itself.

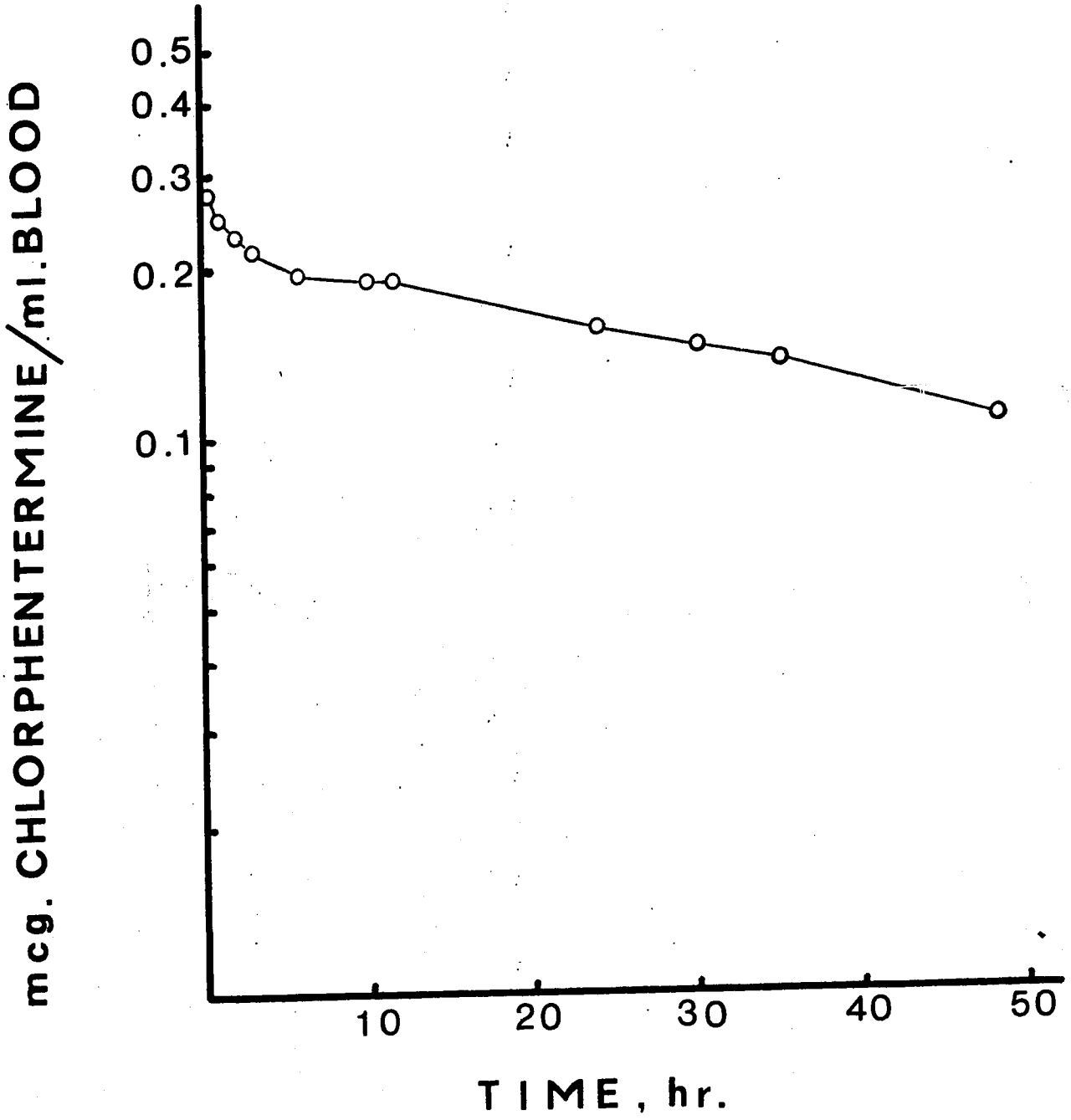

Figure 23. Average Blood Level versus Time Curve for Chlorphentermine following Intravenous Administration (50 mg). Points show the Average of two Subjects

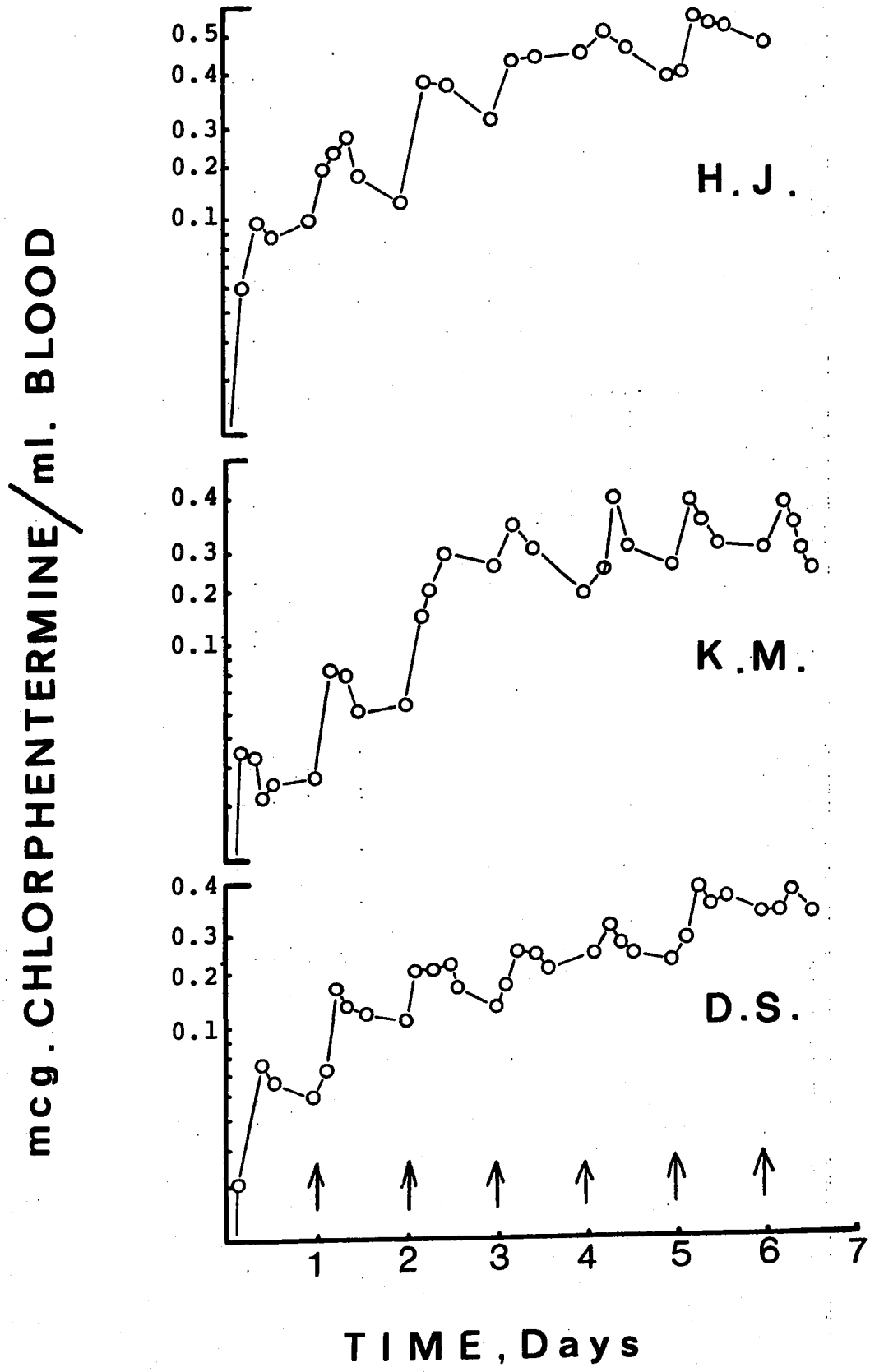

Figure 24. Blood Level versus Time Curves for Chlorphentermine following Administration of Multiple Dosage Regimen of Prolonged-release Preparation (3 subjects). Arrow indicates the time of drug administration.

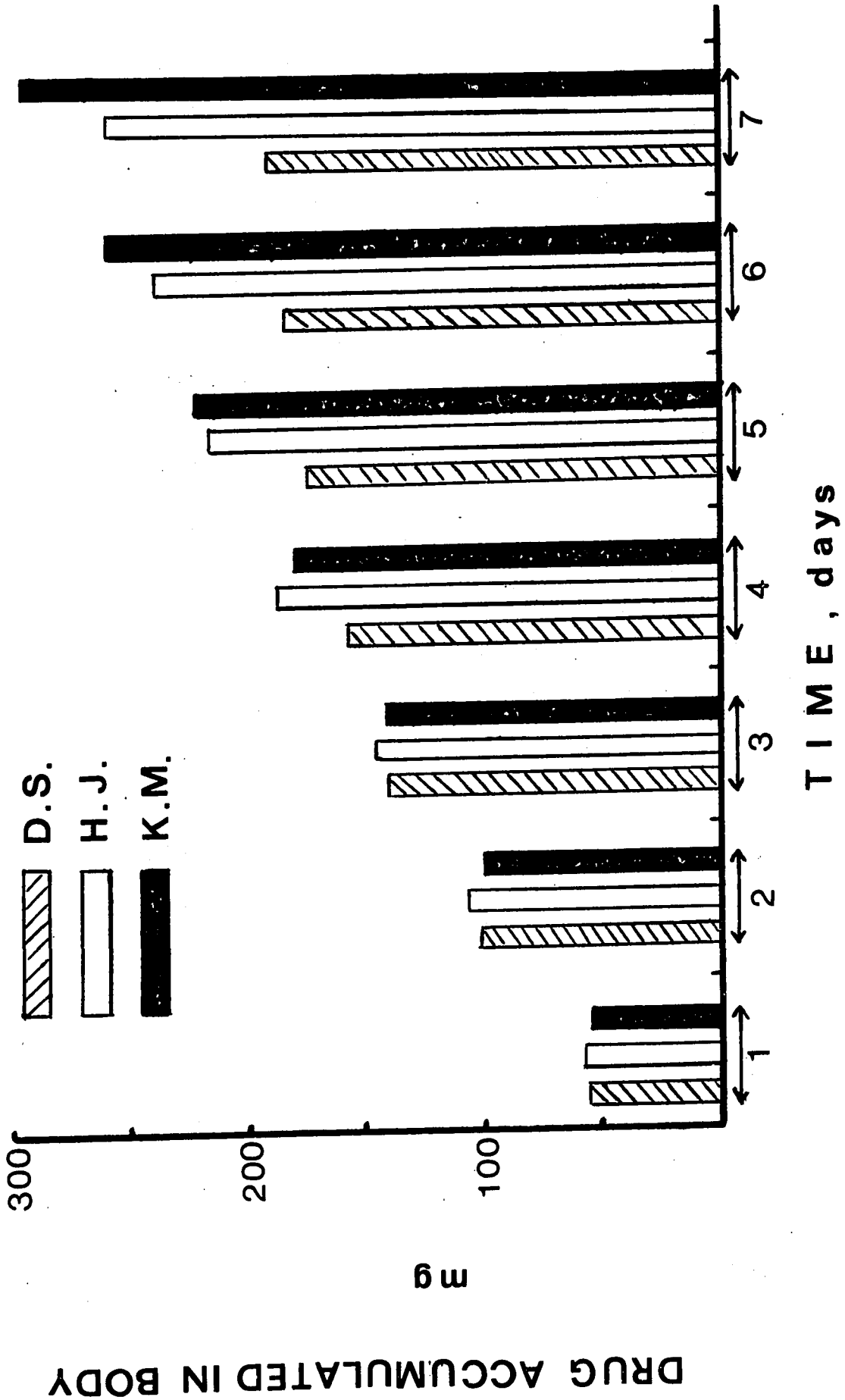

Figure 25. Total Amounts of Chlorphentermine Accumulated in the Body at the Time of Drug Readministration Based on Urinary Excretion Data (3 subjects).

Table V. The Results of In Vitro Metabolism Study of Chlorphentermine.

	Solvent System	Spray Reagent	Expected Color	Chlorphentermine Standard R_f	Blank	Sample	Comments
Base Extraction	Benzene 50 Acetone 40 Ethanol 5 c-NH_4OH 5	1. Dragendorff's Reagent	Yellow-Orange	0.70	-	+ R_f 0.70	No Metabolism Indicated.
		2. Diazotized p-nitro-aniline	Pink	"	-	+ R_f 0.70	Unchanged Chlorphentermine Found.
Acid Extraction	$CHCl_3$ 90 Ethanol 10 gl. HAc 5	1. Bromocresol Purple or Green	Yellow	0.36	-	-	No Metabolism Indicated.
		2. Diazotized Sulfanilic Acid	Pink	"	-	-	"
Neutral Extraction	Benzene 90 Methanol 16 gl. HAc 8	Diazotized Sulfanilic Acid	Yellow-Orange	0.25	-	-	No Metabolism Indicated.
Hydrolyzed Sample Extraction	Benzene 90 Methanol 16 gl. HAc 8	Diazotized Sulfanilic Acid & p-nitro-aniline	Yellow	0.27	-	-	No Metabolism Indicated.
			Pink	"	-	-	

+ indicates positive color reaction

- indicates negative color reaction

Table VI. The Results of In Vivo Metabolism Study of Chlorphentermine.

	Solvent System	Spray Reagent	Expected Color	Chlorphentermine Standard R_f	Blank	Sample	Comments
Base Extraction	Benzene 50 Acetone 40 Ethanol 5 c-NH_4OH 5	1. Dragendorff's Reagent	As Table V	0.63	-	+ R_f 0.63	No Metabolism Indicated.
		2. Diazotized p-nitro-aniline	"	"	-	+ R_f 0.63	Unchanged Chlorphentermine found.
Acid Extraction	$CHCl_3$ 90 Ethanol 10 gl. HAc 5	1. Bromocresol Purple	"	0.41	-	-	No metabolism indicated.
		2. Diazotized Sulfanilic Acid	"	"	-	-	"
Neutral Extraction	Benzene 90 Methanol 16 gl. HAc 8	Diazotized Sulfanilic Acid	"	0.19	-	-	"
Hydrolyzed Urine Extraction	Benzene 90 Methanol 16 gl. HAc 8	Diazotized Sulfanilic Acid & p-nitro-aniline	"	0.17	-	-	"
			"	"	-	-	"

+ indicates positive color reaction

- indicates negative color reaction

E. BINDING STUDY

The possible interaction of chlorphentermine with bovine serum albumin was examined by means of the equilibrium dialysis method. The Table VII shows the chlorphentermine concentrations in compartments A and B of the dialysis cell after the equilibrium time at 37°C. Equilibrium was achieved after 20 hours.

Table VII.

Chlorphentermine Concentration in Compartments A and B after 20 hours Equilibrium Time at 37°C [a].

Initial Drug Concentration in Compartment A, mcg/ml	Drug Concentration in Compartment A [b] after Equilibrium, mcg/ml	Drug Concentration in Compartment B [c] after Equilibrium, mcg/ml
20.0	10.7	11.1
200.0	94.4	93.8
500.0	241.0	248.8

a Average of 5 replicates

b 1×10^{-4} M Bovine Serum Albumin in 10 ml pH 7.4 Buffer

c 10 ml pH 7.4 Buffer only

F. DETERMINATION OF THE pK_a VALUE OF CHLORPHENTERMINE

Figure 26 shows the pK_a values obtained for chlorphentermine in various concentrations of methanol - water. Extrapolation to 0 % methanol gave a pK_a value for the drug of 9.6.

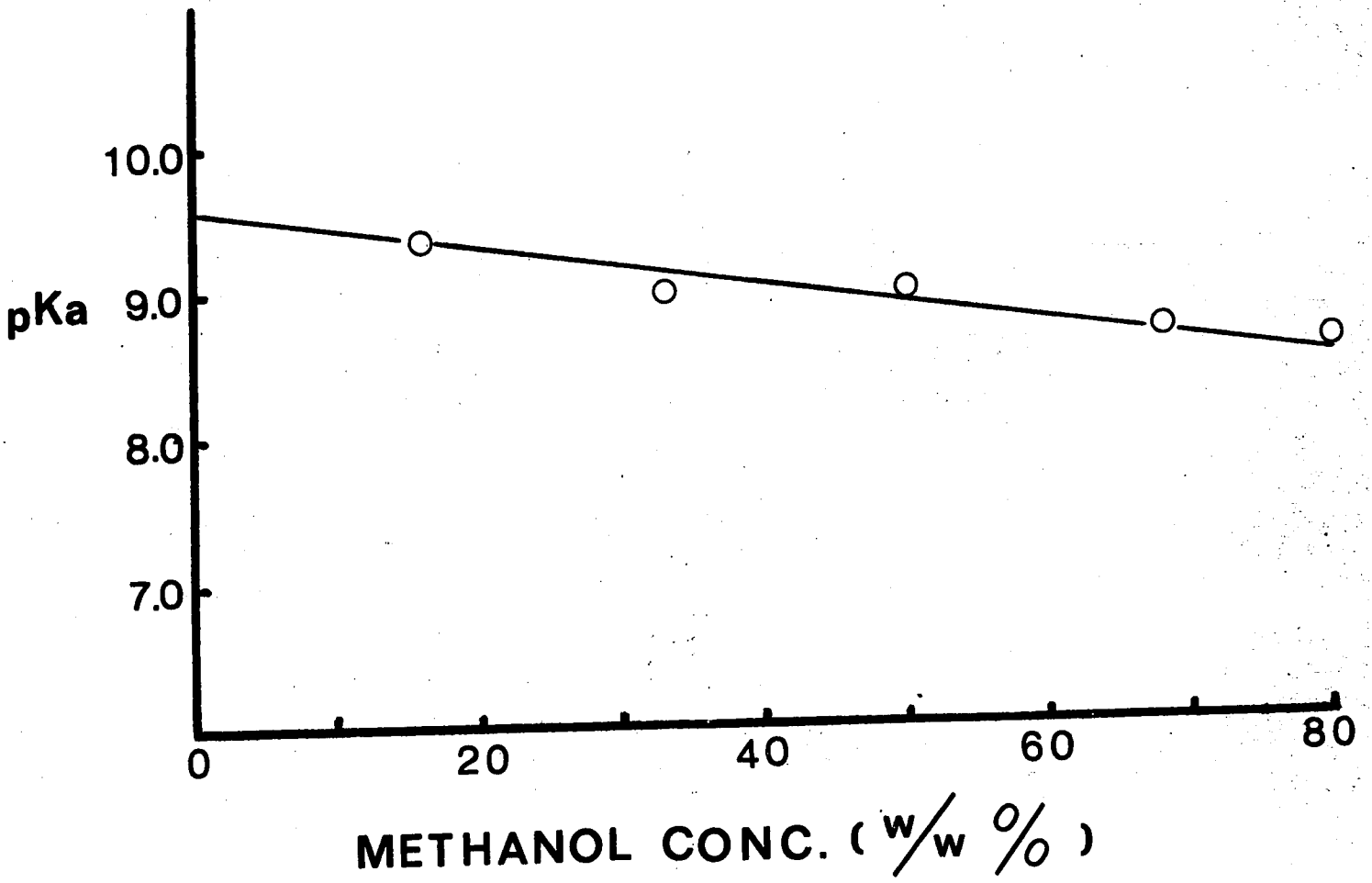

Figure 26. The pK_a values obtained for Chlorphentermine in various Concentrations of Methanol - Water at $20^{o}C$. (Drug Conc. $1x10^{-2}M$).

CHAPTER V. DISCUSSION

A. URINARY EXCRETION STUDIES

Analytical Procedure

Gas-liquid chromatography has been shown to be a very useful technique for the quantitative analysis of drugs in biological fluids (160,161,162). The method developed here has a high degree of accuracy and is relatively simple, sensitive, and specific for chlorphentermine. Good symmetrical peaks were obtained for chlorphentermine and the internal marker, azobenzene. The method requires only a short analysis time per sample (7 min), and showed good reproducibility during several months of continued use.

Although a number of methods are available to quantitate chromatographic peaks, peak heights relative to an internal marker were used in these studies to calculate unknown sample concentrations, rather than by measuring peak area, because of its simplicity (163). Azobenzene was selected as the internal marker since it showed adequate separation from chlorphentermine and appeared as a symmetrical peak.

Under the experimental conditions cited a linear relationship was found to exist between the peak height ratios of chlorphentermine and azobenzene over the expected maximum concentration range of drug excreted in the urine. It was also found that the re-establishment of a calibration curve after a short period of time (less than 2 months) was unnecessary.

The precision of the gas-liquid chromatographic procedure appears to be very satisfactory; a standard deviation of twelve analyses of urine samples was $\pm$ 1.4 %.

The results obtained in this study clearly demonstrated that gas-liquid chromatography is an effective method for the quantitative analysis of chlorphentermine in urine following administration of therapeutic drug doses.

Urine Excretion Trials

The rate of urinary excretion of chlorphentermine was found to be dependent on both urinary pH and urine volume(see Figures 8 & 9). Various authors have shown that the urinary excretion of a number of weakly basic drugs is influenced by urinary pH (164,165), and also, in some cases by urine volume (166,167). The pK_a value (9.6) of chlorphentermine (see p. 96) lies within the optimum pK_a range for organic bases exhibiting pH dependent urinary excretion (pK_a 6.5 - 10.0). The explanation for this phenomenon, based on the theory of non-ionic diffusion has been well discussed (42,43).

The effect of urine volume on the urinary excretion rate of some drugs can be attributed to the dilution of urine, thus changing the concentration gradient for the permeable undissociated form of the drug from the kidney tubular lumen to the peritubular capillaries (167).

Since fluctuations in the urinary pH occurring under normal conditions are well known (168,169), maintenance of a constant acidic urine by administration of ammonium chloride has been advocated for the maximum exploitation of urinary excretion studies for weak organic bases (152, 153). Differences in urinary excretion rates of chlorphentermine under various urinary pH conditions are very significant (see Figures 8 & 9). An attempt was made to reduce these fluctuations in

the drug excretion rate versus time curve by maintaining the pH of the urine acidic (5.0 ± 0.5). However, this was found to be not completely successful in those cases where the subject's urine flow rate fluctuated rapidly.

The rate of urinary excretion of chlorphentermine reached a maximum at about 3 hours following administration of the drug in 'free' dosage forms. Following its administration as a prolonged-release preparation, the maximum excretion rate was lower and occurred 3 - 4 hours later than that of the 'free' forms. This reflects the slow release of the drug from the prolonged-release preparation. There was no significant difference in the total excretion of chlorphentermine when the drug was administered in 'free' dosage forms and as the prolonged-release preparation, indicating efficient physiological availability from the prolonged-release dosage form.

The biological half-life of chlorphentermine in man was found by Brookes (17) to be 13.5 hours. Results obtained in the present study from the excretion profiles of chlorphentermine in three subjects whose urinary pH was maintained acidic, suggested that the biological half-life was in excess of this value. The doses of chlorphentermine administered in the two studies are different; Brookes administered doses of 6 and 12 mg while 78 and 100 mg doses of chlorphentermine were given in this study. Although accurate estimation of the half-life of chlorphentermine from the semilogarithmic graph of the urinary excretion rate versus time curve was not possible owing to fluctuations in drug excretion rate due to urine volume changes in many of the trials, this half-life discrepancy may be explained on the basis of a dose dependent drug excretion rate. Wagner and Damiano (170), and Levy and Nagashima (171) observed increases in the half-lives of novobiocin and warfarin respectively when the

B. ANALOG COMPUTER SIMULATIONS

The basic models used in these analog computer simulation studies to describe the kinetic behavior of chlorphentermine are shown in Figures 2 and 4 (see p. 42, 46). Distribution with regard to the tissue compartment T was found to be necessary to adequately describe the pharmacokinetics of chlorphentermine, as a simpler compartmental model*

$$A \xrightarrow{k_a} B \xrightarrow{k_u} U$$ was not applicable.

Previous workers (11,18) have shown that chlorphentermine is localized extensively in various body organs. These findings, together with our observation of a long elimination half-life (see p. 77) for the drug, suggested the inclusion of a tissue compartment in the pharmacokinetic models.

However, it must be emphasized that although the model apparently described <u>in vivo</u> situations reasonably well, physiological reality remains much more complex. The inclusion of tissue compartment in the pharmacokinetic models of chlorphentermine (see Figures 2 & 4) adds another three parameters (ie. $k_{B\rightarrow T}$, $k_{T\rightarrow B}$, and T) which can not be estimated directly to the simpler one compartmental model. Although the analog computer program for such a model⊕ was programmed, the absolute physiological significance of the calculated rate constants may be doubtful. This would also apply to results obtained by digital computer interpretations and classical mathematical analysis.

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* The body is considered to be a single homogeneous compartment

⊕ This model is referred to a two-compartmental model by some authors

'Solution' and 'Tablet' Data

In general there was good agreement between the computer-generated urinary excretion curves based on Model (I) and the experimental values. Thus, Figures 13 and 14 indicate the suitability of the model to describe the kinetics of absorption, distribution and excretion of 'free' dosage forms of chlorphentermine in man. The results also show the usefulness of the analog computer in analyzing urinary excretion data.

Inter - and intra - subject variation was apparent in the parameters lag time and k_u (see Table II), although such variation was not great with the exception of the k_u values. Lag times have previously been reported (172,173,174), and Beckett and Tucker (101) have listed the various explanations offered for their occurrence. In the post-absorption phase, the elimination of chlorphentermine could be described by a single first-order process, namely urinary excretion. As has been mentioned, k_u values were subject to variation, probably resulting from variation in the subjects' urinary flow rates, pH and kidney functions.

The 48 hr percentage recovery of unchanged chlorphentermine from urine and its elimination half-life ($t_{1/2}$) after single 78 mg doses (3 subjects; 6 trials) ranged from 55 - 64 % of the dose and from 35.9 - 48.6 hours respectively. Both recovery and elimination half-life were variable within a single subject as these values are dependent on the excretion rate constant, k_u.

The rate constant for absorption of chlorphentermine from the gastrointestinal tract into blood was found to be high ($k_a = 2.8\ hr^{-1}$). Inter - and intra-subject variation for this rate constant was not seen probably because of its high value ($> 2\ hr^{-1}$), since a small variation

in absorption rate does not appreciably influence the other drug kinetic constants.

'Prolonged-release' Preparation Data

The release, absorption, distribution and excretion of chlorphentermine after oral administration of prolonged-release preparation A was well described by pharmacokinetic model (II) (see Figure 4). Furthermore, simulations of the experimental data by the analog computer were performed using the parameters for absorption, distribution and excretion essentially consistent with the corresponding values obtained with the 'free' dosage forms.

The dissolution characteristics for the prolonged-release preparation were obtained from the manufacturer and necessitated the use of two sequential rates of release, k_{r_1} and k_{r_2}.

A dosage form including an 'initial' and a 'maintenance' dose to prolong drug blood concentrations has been a widely accepted technique for formulating a prolonged-action product. Rowland and Beckett (175) have presented a quantitative method of formulating such a dosage form on a theoretical basis.

The total dose of chlorphentermine in the prolonged-release preparation A is represented by the amount of the drug released from the outer layer ($D_{o.c.}$) governed by the rate constant k_{r_1}, and that present in the inner core ($D_{I.C.}$) released by k_{r_2}. If the situation is ideal, the $D_{o.c.}$ will initially give the required therapeutic level of drug, while the $D_{I.C.}$ will maintain this level for the prolonged period of time. Figures 15 and 16 indicate the good fit obtained

between the computer simulations for the drug excretion profiles after administration of prolonged-release preparation A.

The curve in Figure 18 indicates a good correlation between the *in vivo* and *in vitro* drug release rates for preparation A. Accordingly, the release of chlorphentermine from preparation A estimated by the *in vitro* procedure described earlier (see p. 48) was found to be a meaningful *in vitro* test, ie. the test could be correlated to *in vivo* data.

C.BLOOD LEVEL STUDIES

Analytical Procedure

The flame ionization detector is an extremely sensitive detector which responds to virtually all compounds with the exception of the permanent inorganic gases (176). Flame ionization gas-liquid chromatography has been used for analyzing blood levels of certain drugs (162,177). This technique has the advantage of simplicity as chlorphentermine can be measured directly without the necessity of derivative formation.

The extraction procedure for chlorphentermine from blood samples was somewhat lengthy, but the percentage recovery (90 - 100 %) of known amounts of drug added to 'blank' blood indicates the efficiency of the method.

In most chromatograms the drug peak occurred on the solvent slope at the low attenuator settings used. However, reasonably accurate peak height measurements could be made as indicated by the size of the standard deviation of ten analyses of blood samples each containing 0.5 mcg chlorphentermine per 5 ml of blood (see p. 83).

The calibration curve was found to be a straight line over the range of 0.25 to 3.0 mcg of the drug per 5 ml of blood and to pass through the origin. Drug concentrations as low as 125 ng/5 ml of blood could be detected. The average blood levels for chlorphentermine obtained in the drug studies were well within the range of sensitivity of the method.

Blood Level Trials

Peak blood levels for chlorphentermine in all subjects occurred at $1^1/_2$ - $2^1/_2$ hr following oral administration of the drug in solution indicating that the drug was rapidly absorbed from the gut, and blood levels after the peak showed a slow exponential decline. The apparent plasma half-life for the drug ranged from 35 to 45 hr (average 41 hr), and was in close agreement with that obtained from predicted blood levels determined by analog computation (see p. 77). The apparent volume of distribution (average 213 l), together with the long apparent plasma half-life suggested extensive distribution of the drug in the body. These findings suggest that the drug may be to a large extent localized. Previous reports (11,18) have shown chlorphentermine to be localized in various body organs of rats and mice.

Peak blood levels for chlorphentermine occurred at about 6 hr following oral administration of the prolonged-release preparation, suggesting a delayed release of drug from the dosage form.

The areas under the blood level curves were compared following intravenous and prolonged-release drug administration and an average of 62 % (maximum 87 %) of the drug was found to be available from the

prolonged-release dosage form. However, it is possible that this average result may be an underestimate since comparisons were made only over the 48 hr time period and ideally should be compared from zero to infinite time (178). Subject variation, which did occur (see Table II), probably also contributed to the spread of the value of the availability term.

The observation that the apparent plasma half-life of the drug was on average in excess of 40 hr, makes it doubtful whether a prolonged-release formulation is indeed clinically necessary for chlorphentermine. It could, however, be argued that tissue levels resulting from administration of the prolonged-release formulation would perhaps follow a more clinically acceptable pattern than those levels following administration of the drug in solution.

A dose of 50 mg of drug was chosen for the intravenous drug administration studies since it was felt that there would be less subjective effects experienced at this dosage level. The initial phase of the blood level curve following intravenous drug administration was indicative of the extensive extra-vascular distribution of the drug (see Figure 23). However, it was not possible with the blood sampling times used to accurately extrapolate this initial drug administration phase to time zero. Reference to the area under the blood level curves (see Table IV) following oral and intravenous drug administration showed that the drug was completely available when administered in solution. This was confirmed by earlier urinary excretion studies in which the cumulative amount of drug excreted in acid pH controlled urine was about 90 % over an extended period of time following drug administration in solution. Also, previous reports have shown the drug to be excreted mainly unchanged in the urine(15, 16).

It is difficult to determine the required therapeutic blood level of a drug largely localized in the tissues by means of blood level studies. Ideally the concentration of drug at its site of action should be measured or a pharmacological response of the drug monitored. However, blood level studies are of importance as they enable the determination of drug pharmacokinetic parameters (179) and comparison between the performance of different dosage forms to be made (74, 180,181).

Accumulation Study

The finding that only a minor proportion of the administered dose of chlorphentermine was excreted in the urine under normal conditions over a period of 24 hr indicated that repeated drug administration at daily or more frequent intervals would obviously result in drug accumulation in the body. Wagner (81) has presented an equation which shows how a drug with a biological half-life of longer than 16.6 hr accumulates in the body if it is administered at 24 hr or more frequent time intervals.

The drug accumulation ratio R_1 defined by Wagner (81) as the maximum plasma concentration at the steady state over the initial maximum plasma concentration was found to be 4.86 for chlorphentermine (average of the three subjects). Since Wagner did not give numerical values corresponding to drug accumulation for the R_1 term it is difficult to positively state whether the experimental value of 4.86 is indicative of drug accumulation or not. However, in the blood level studies obtained following administration of multiple doses, repeated administration at regular time intervals did not produce unlimited building up of the blood concentration, in spite of the fact that a large proportion of the drug administered still remained in the body at the times of

readministration (see Figure 25). This might be explained by one or more of the following: an increased rate of urinary excretion of the drug, increased rate of metabolic transformation, and/or increased localization of the drug in tissues without a corresponding elevation in blood levels. However, increased rates of excretion and metabolism appeared to be only a minor contributing factor as evidenced by the urinary excretion data obtained. No definite conclusion can be drawn, but increased tissue localization of the drug is the most likely to produce the slow progressive increase in the blood level following repeated administration.

The physiological availability of the drug from the prolonged-release preparation was found not to be a contributing factor in producing low drug recovery and low drug blood levels, since the total amount of drug excreted following its administration was comparable to that following oral administration of the drug in solution.

The subjective effects noted following multiple dose administration of chlorphentermine, however, indicated that potential toxicity of the drug is not a problem even though drug accumulation in the body may be occurring.

It is recognized that the site of accumulation of a drug does not necessarily correspond to its site of action. Distribution studies with a number of drugs have shown that drug localization may well occur in body tissues for example, fat and skeletal tissue, which are not part of the drugs biophase (182,183). Thus, chlorphentermine might well accumulate in such tissue where it would be pharmacologically inactive. This drug localization may not only limit the intensity of the pharmacological response to an accumulated dose, but also may tend to prolong

the duration of the effect, since these tissues serve as reservoirs which gradually release the drug. Therefore, the amount of the drug taken up by the tissues may greatly influence the pharmacological effect of chlorphentermine as it limits the concentration of available drug at the site of action.

D. METABOLISM STUDIES

The results of the *in vitro* and *in vivo* metabolism studies correspond well to earlier reports given by Opitz and Weischer (15) and Portnoy, *et al* (16), which showed that chlorphentermine was mainly excreted unchanged in the urine in rat and man respectively. Pletscher, *et al* (19), and Fuller and Hines (21,22) also found that a longer biological half-life and less percentage drug biotransformation occurred with the compounds DL-p-chloro-N-methylamphetamine and DL-p-chloroamphetamine respectively, when compared with their dechlorinated analogs. Thus, the properties of chlorphentermine observed in this study ie., a long biological half-life and minor metabolic transformation, may perhaps be attributed to its chemical structure (see Figure 1), as the major metabolic reactions noted with this class of drug, namely p-hydroxylation and deamination, are unlikely to occur. These observations are further supported by the prolonged anorexigenic effect of the drug noted by various authors. Gylys, *et al* (184), compared the anorexigenic action of chlorphentermine in rats to that of d-amphetamine, and found that chlorphentermine had a more prolonged pharmacological effect than d-amphetamine. Gylys, *et al* (184), and Friedman, *et al* (185), suggested that this prolonged effect of chlorphentermine was possibly related to the slower rate of metabolism of the compound in the body owing to a structural hinderance to drug parahydroxylation and deamination. Holm, *et al* (7),and Kopt, *et al* (186), attributed the prolonged pharmacological activity of chlorphentermine to para-chloro substitution. Dubnick, *et al* (11), also concluded that para-chlorine atom contributes to the high tissue to blood concentration ratio found for the drug

and may be a factor in prolonging its biotransformation and elimination from the body.

Although Dubnick, et al (18), were unsucessful in isolating and identifying a conjugated drug metabolite, they suggested that acidic conjugation might be the significant metabolic pathway of chlorphentermine in female mice since they observed an increased amount of methyl orange reactive base after hydrolysis of mouse urine. However, after careful examination of human urine in our studies the presence of such a conjugated metabolite was felt to be unlikely.

E. BINDING STUDY

The complexation reactions of drugs with body components, such as plasma and tissue proteins, and nucleic acids, etc. has been recognized to greatly influence the pharmacokinetic properties of certain drugs in the body. Burns, et al (187), reported that the prolonged biological half-life (72 hr) and very slow rate of metabolic transformation of phenylbutazone in man was due to extensive plasma protein binding of the drug. The slow biotransformation and negligible urinary elimination of dicoumarol was also attributed to a high degree of plasma protein binding (44). Beisel, et al (188), studied body cortisol elimination and concluded that plasma binding provided a reservoir of readily available steroid which was protected from excretion and biotransformation.

While attempts were made in this study to examine the contribution of plasma binding of chlorphentermine to its long biological half-life and minor metabolic transformation, the results showed no evidence of a significant degree of binding with bovine serum albumin. Thus, the long biological half-life and low blood levels experienced with chlorphentermine may be mainly due to its extensive tissue distribution. According to Axelrod (13), about 15 % of d-amphetamine was found to be bound to plasma proteins at concentrations of 2 and 10 mg per one liter of plasma. However, the chemical structure of chlorphentermine may possibly exert steric hinderance to the complexation reaction observed with plasma plasma proteins and d-amphetamine.

It is possible that chlorphentermine might become bound to tissue components in the body but this was not investigated in these studies.

F. DETERMINATION OF THE pK_a VALUE OF CHLORPHENTERMINE

The pK_a value of chlorphentermine obtained from this study was found to be 9.6 which is in general agreement with the pK_a range established for the amphetamine analogs (189). This value also lies within the range suggested by Milne, et al (190), for weakly basic compounds showing urinary pH dependent excretion.

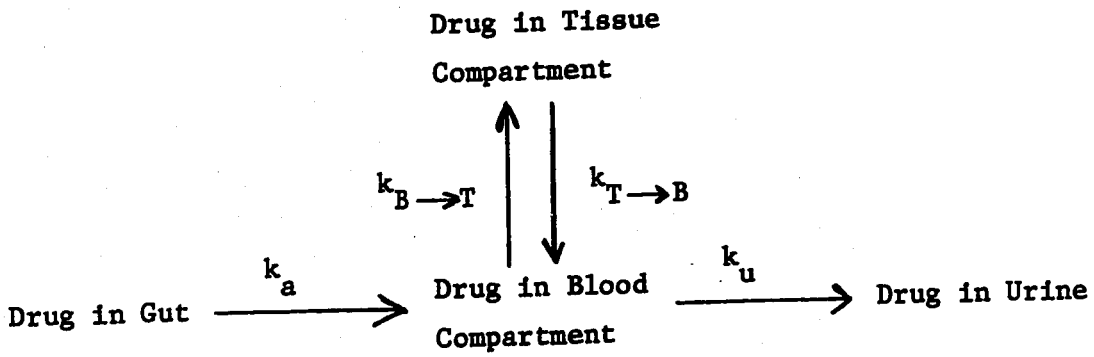
CHAPTER VI. SUMMARY AND CONCLUSIONS

1. Both urinary pH and urine volume were found to influence the excretion rate of chlorphentermine in man.

2. No evidence of chlorphentermine metabolism in rats and humans was found in either in vitro or in vivo studies.

3. Urinary excretion profiles following oral administration of solution, compressed tablet, and commercial prolonged-release dosage forms of chlorphentermine were compared under acidic urinary pH conditions. The excretion profiles were found to be very similar for all of the dosage forms. However, a delay in the time of maximum excretion, and a lower excretion rate was observed for the prolonged-release preparation. The percentage recoveries of the drug in the urine after the three forms of drug administration were nearly the same.

4. The pharmacokinetic parameters for chlorphentermine in man were calculated by an analog computer simulation method. The following compartmental model was chosen for the best fit:



Indications obtained from the results for the drug showed a fast absorption rate, a long elimination half-life, and probable extensive tissue localization.

5. Blood level studies of chlorphentermine in man following oral administration of the drug in solution and as a prolonged-release preparation were compared to those levels obtained after intravenous injection.

   The observed data showed low blood levels throughout the time course of drug in the body, a long plasma half-life, and extensive extra-vascular distribution. The drug was found to be completely available as a solution dose and to the extent of 62 - 87 % as the prolonged-release preparation.

6. Blood and urine levels of chlorphentermine in man following an oral multiple dosage regimen of the prolonged-release preparation were examined.

   The results indicated that a significant amount of the administered drug accumulated in the body.

7. Therapeutically, prolonged-release formulations of chlorphentermine may be unwarranted.

8. Chlorphentermine did not appear to bind in any significant amount to serum albumin.

9. The pK_a value of chlorphentermine was found to be 9.6 .

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APPENDIX A

The terms used in the pharmacokinetic equations;

t — Time in hours after ingestion of the dose.

Lag time — The time interval between ingestion of the dose and zero time.

Zero time — The time at which loss of drug from the gastrointestinal tract may be described as a first-order process.

Break Time — The time after dosage at which k_{r_1} is changed to k_{r_2}.

A — The amount of drug present in the gastrointestinal tract.

B — The amount of drug in the blood compartment.

T — The amount of drug in the tissue compartment.

U — The amount of drug in the urine.

D_M — The amount of drug in "initial" and "maintenance" dosage form ($D_M = D_{O.C.} + D_{I.C.}$).

k_a — The rate constant for the absorption of drug from the gastrointestinal tract into the blood.

$k_{B \rightarrow T}$ — The rate constant for the transfer of drug from the blood compartment into the tissue compartment.

$k_{T \rightarrow B}$ — The rate constant for the transfer of drug from the tissue compartment into the blood compartment.

k_u — The rate constant for the excretion of drug from the blood compartment in to the urine.

k_e — the rate constant for the elimination of drug from the body.

k_{r_1} & k_{r_2} — Rate constants for the release of drug from "initial" and "maintenance" dosage form into the gastrointestinal tract.

The term "maintenance" form refers to dosage forms from which the drug is not immediately available for absorption, ie., formulated fractions of prolonged-release preparations.

APPENDIX B

Table 1.

Subject : K.M. **Dose :** Aqueous Solution (78 mg Chlorphentermine HCl)

Urine pH: Acid **Date :** 3/7/68

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg base)	Urine pH
0.0	Blank	0.0	5.20
0.7	31	65.4	5.25
1.0	16	41.4	5.25
1.5	30	110.7	5.25
2.0	35	181.0	5.20
3.0	20	304.8	5.28
4.0	27	908.6	5.15
5.0	29	1391.4	5.05
6.0	28	1094.8	5.00
8.0	83	2650.2	5.09
10.0	88	2292.4	5.00
12.0	120	2245.2	5.00
22.0	230	5998.4	5.20
25.7	240	4389.6	5.10
30.0	128	2976.0	5.08
34.0	162	3789.2	5.00
36.3	175	1834.0	5.00
46.0	230	3790.4	5.20
49.0	177	2575.4	5.12

Table 2.

Subject : H.J. Dose : Aqueous Solution (78 mg Chlorphentermine HCl)

Urine pH: Acid Date : 6/8/68

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg base)	Urine pH
0.0	Blank	0.0	5.12
1.0	182	87.4	5.22
2.0	175	612.5	5.22
3.0	110	1668.7	5.22
4.0	250	2040.0	5.22
5.1	120	1560.0	5.22
6.1	235	1222.0	5.22
8.1	122	1916.6	5.02
10.0	140	2506.0	4.93
12.0	133	1677.1	5.12
18.0	390	3950.7	5.22
22.0	132	2341.7	4.98
26.5	172	2652.2	5.18
30.0	290	2276.5	5.16
34.5	285	1707.2	5.22
44.5	336	2728.3	5.30
47.5	143	878.0	5.17

Table 3.

Subject : A.S. Dose : Aqueous Solution (78 mg Chlorphentermine HCl)

Urine pH : Acid Date : 27/8/68

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg base)	Urine pH
0.0	Blank	0.0	4.73
0.5	53	13.3	4.88
1.0	42	30.7	4.78
1.5	34	200.9	4.80
2.0	23	302.9	4.72
2.5	36	1131.9	4.62
3.5	63	1959.3	4.78
4.5	50	753.5	4.88
5.8	68	1508.2	4.78
7.5	64	1232.0	4.88
9.5	142	2709.4	4.88
11.5	82	587.1	5.30
16.5	225	1935.0	5.12
21.5	475	5006.5	4.92
25.5	150	2776.5	4.68
29.5	175	2595.3	4.78
33.5	210	2270.1	4.90
45.5	350	3944.5	4.90
47.5	68	813.3	4.75

Table 4.

Subject : H.J. Dose : Aqueous Solution (78 mg Chlorphentermine HCl)

Urine pH : Acid Date : 2/7/68

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg base)	Urine pH
0.0	Blank	0.0	5.30
0.5	36	9.4	5.18
1.0	23	18.6	5.57
1.5	29	109.3	5.34
2.0	30	278.4	5.12
3.0	105	1149.8	5.80
4.0	130	716.3	5.23
5.0	82	601.9	5.75
6.0	79	846.1	5.55
8.0	200	2366.0	4.96
9.7	128	3193.6	4.85
12.0	55	1408.0	5.03
21.7	238	6207.0	5.18
26.2	130	2769.0	5.10
30.0	130	3175.9	4.95
35.5	230	3703.0	5.15
46.0	285	3417.2	5.18
50.0	96	654.7	5.20

Table 5.

Subject : H.J. Dose : Aqueous Solution (78 mg Chlorphentermine HCl)

Urine pH : Acid Date : 5/12/68

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg base)	Urine pH
0.00	Blank	0.0	5.00
0.50	8		5.30
0.45	9	6.7	5.50
1.00	13	35.9	6.00
1.25	11	43.3	5.60
1.50	13	78.4	5.40
1.75	17	154.4	5.90
2.00	17	229.5	5.40
2.25	16	326.4	5.20
2.50	12	284.4	5.10
2.75	22	385.0	5.22
3.00	34	336.6	5.12
4.00	120	912.0	5.15
6.00	220	1553.2	5.30
8.00	174	2025.4	5.27
10.00	93	1833.0	5.13
13.00	180	3065.4	5.20
23.00	570	8749.5	5.24
28.00	255	3406.8	5.22
33.00	155	2847.4	5.17
37.00	140	2478.0	5.20
48.00	275	2200.0	5.23

Table 6.

Subject : A.S. Dose : Compressed Tablet (78 mg Chlorphentermine HCl)

Urine pH : Acid Date : 27/11/68

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg base)	Urine pH
0.0	Blank	0.0	5.06
1.0	47	235.5	5.12
2.0	50	401.5	4.92
3.0	82	1962.4	4.82
5.0	130	2369.0	4.92
6.0	85	1182.4	4.96
8.0	169	2350.8	4.98
10.0	94	2052.0	4.95
12.0	127	1841.5	5.04
22.0	466	10332.0	4.96
26.0	198	2975.9	5.00
30.0	150	2326.5	5.02
34.0	183	1681.8	5.13
38.0	272	1776.2	5.15
46.0	285	2260.1	5.38
48.0	98	679.1	5.10

Table 7.

Subject : H.J. Dose : One Compressed Tablet (78 mg Chlorphentermine HCl)

Urine pH : Acid Date : 27/8/68

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg base)	Urine pH
0.0	Blank	0.0	5.15
0.5	25	0.0	5.08
1.0	19	0.0	5.30
1.5	19	7.6	5.30
2.0	22	29.4	5.28
2.5	35	85.8	5.25
3.0	108	261.4	5.42
4.0	275	1265.0	5.42
5.0	90	449.1	5.05
6.0	132	1184.0	5.08
8.0	105	1980.3	5.08
10.3	110	1894.2	5.25
12.0	68	484.2	5.78
22.3	560	3589.6	5.26
26.0	125	1398.8	5.18
30.0	475	2598.3	5.15
34.0	165	1673.1	5.10
36.0	78	806.5	5.18
46.0	250	2170.0	5.38
48.0	60	499.8	5.15

Table 8.

Subject : K.M. Dose : One Compressed Tablet (78 mg Chlorphentermine HCl)

Urine pH : Acid Date : 5/10/68

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg base)	Urine pH
0.00	Blank	0.0	5.30
0.50	17		5.70
1.50	18		5.60
2.00	33	375.2	5.20
2.50	23	368.0	5.12
2.75	25	387.5	5.08
3.00	29	524.6	5.07
3.25	23	422.5	5.25
3.50	20	417.6	5.20
4.00	23	518.7	5.12
5.00	150	1468.5	5.10
8.50	165	3511.2	5.17
16.50	295	3483.9	5.40
20.50	120	3000.0	5.30
26.50	160	3497.6	5.27
31.00	275	3294.5	5.20
40.50	280	5003.6	5.30
43.00	310	1813.5	5.25
47.00	60	269. 4	5.27

Table 9.

Subject : H.J. Dose : One Compressed Tablet (78 mg Chlorphentermine HCl)

Urine pH : Acid Date : 11/5/68

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg base)	Urine pH
0.0	Blank	0.0	5.05
1.0	18		5.04
2.0	80	288.8	5.04
3.0	400	1864.0	5.67
4.0	108	1375.9	5.27
5.5	68	1669.4	5.05
6.5	32	1103.7	5.18
8.5	125	1968.8	5.30
10.5	143	2123.6	5.31
12.5	63	1592.0	5.30
22.5	235	8476.5	5.23
24.5	77	1494.6	5.23
26.5	72	848.0	5.32
30.5	330	3075.6	5.22
34.5	132	2728.4	5.20
36.0	40	676.8	5.32
46.5	210	4044.6	5.32
49.0	73	797.2	5.20

Table 10.

Subject : K.M. Dose : One Prolonged-release Preparation (78 mg Chlorphentermine HCl)

Urine pH : Acid Date : 1/9/68

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg base)	Urine pH
0.0	Blank	0.0	5.20
1.0	145	0.0	5.29
2.0	50	0.0	5.12
3.0	220	94.6	5.00
5.0	162	1103.2	5.08
6.0	62	837.0	5.13
8.0	105	1596.0	5.12
10.0	165	1815.0	5.06
12.0	195	2281.5	5.04
22.0	405	6642.0	5.22
26.0	135	2025.0	5.31
31.0	270	3679.0	5.14
35.5	225	2408.4	5.29
46.0	420	5292.0	5.30
49.0	130	1365.0	5.26

Table 11.

Subject : A.S.

Dose : One Prolonged-release Preparation (78 mg Chlorphentermine HCl)

Urine pH : Acid

Date : 10/7/68

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg base)	Urine pH
0.0	Blank	0.0	4.87
1.0	106	0.0	4.90
2.0	43	0.0	4.75
3.0	67	160.8	4.70
4.25	65	557.3	4.78
5.0	44	604.8	4.80
6.0	38	738.0	4.75
8.0	80	1885.2	4.73
10.0	138	1620.0	5.20
14.0	144	2520.8	5.25
22.0	650	5472.0	4.90
26.25	173	3386.4	4.80
30.0	133	2803.5	4.85
36.0	134	3027.6	5.05
46.0	438	4404.0	5.25
49.0	63	824.4	4.95

Table 12.

Subject : H.J. Dose : One prolonged-release Preparation (78 mg Chlorphentermine HCl)

Urine pH : Acid Date : 5/4/68

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg base)	Urine pH
0.0	Blank	0.0	5.00
0.5	23	30.6	5.05
1.0	13	14.2	5.15
1.5	14	16.8	5.12
2.0	17	17.3	5.12
3.0	55	57.2	5.10
4.0	120	157.2	5.00
5.0	78	167.7	5.10
6.0	55	229.9	4.95
8.0	104	1447.7	4.92
10.0	122	3739.3	4.80
12.0	170	2267.1	5.00
22.0	810	11380.5	5.05
26.7	123	2104.5	5.05
30.0	130	2164.5	5.02
36.0	210	4311.3	5.00
46.0	270	5292.0	5.15
48.3	52	743.6	5.15
53.5	145	2234.3	5.15
73.0	500	4630.5	5.22
81.0	350	1564.5	5.30
95.0	460	874.0	5.50
106.0	540	934.0	5.40

Table 13.

Subject : K.M. Dose : Aqueous Solution (78 mg Chlorphentermine HCl)

Urine pH : Uncontrolled Date : 4/6/68

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg base)	Urine pH
0.0	Blank	0.0	5.76
2.0	90	89.1	6.72
4.25	118	193.5	6.15
6.0	72	324.0	5.63
8.0	70	663.6	5.04
10.0	108	494.6	5.08
12.0	97	501.5	5.09
14.0	150	348.0	5.40
24.0	468	1127.9	5.50
28.0	135	521.2	5.38
32.75	144	508.3	5.40
37.29	141	454.0	5.65
48.0	290	2030.0	5.48

Table 14.

Subject : D.L. Dose : Aqueous Solution (78 mg Chlorphentermine HCl)

Urine pH : Uncontrolled Date : 22/6/68

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg base)	Urine pH
0.0	Blank	0.0	5.22
2.0	118	1196.5	5.20
4.0	95	1877.2	5.00
6.0	69	872.9	5.30
8.0	54	711.2	5.27
10.0	45	952.2	5.08
12.0	84	873.6	5.42
14.0	66	479.0	5.40
24.0	270	4139.1	5.27
28.0	88	909.9	5.19
32.0	110	1541.1	5.13
36.0	69	701.7	5.29
48.0	313	2673.0	5.42

Table 15.

Subject : H.J.

Dose : Aqueous Solution (78 mg Chlorphentermine HCl)

Urine pH: Uncontrolled

Date : 12/1/69

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg base)	Urine pH
0.0	Blank	0.0	5.15
0.5	25		5.08
1.0	19		5.30
1.5	19	7.6	5.30
2.0	22	29.0	5.28
2.5	35	85.8	5.25
3.0	108	261.4	5.32
4.0	275	1265.0	5.32
5.0	90	449.0	5.05
6.0	132	1184.0	5.08
8.0	105	19803	5.08
10.5	110	1894.2	5.25
12.0	68	484.2	5.78
22.0	560	3598.6	5.26
26.0	125	1398.8	5.18
30.0	475	2598.3	5.15
34.0	165	1673.1	5.10
36.0	78	806.5	5.18
46.0	250	2170.0	5.38
48.0	60	499.8	5.15

Table 16.

Subject : K.M.

Dose : One Prolonged-release Preparation (78 mg Chlorphentermine HCl)

Urine pH : Uncontrolled

Date : 8/5/69

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg base)	Urine pH
0.0	Blank	0.0	6.30
3.0	115	0.0	6.40
6.0	205	340.2	6.60
10.0	90	523.8	5.97
15.0	225	1239.7	5.90
24.0	550	2645.5	5.74
27.0	290	832.3	5.87
31.0	175	624.8	5.50
36.5	265	768.5	6.27
48.0	400	2588.0	5.80
51.0	210	1774.0	6.10
53.5	175	1804.9	6.05
57.0	244	1550.0	5.93

Table 17.

Subject : W.J. Dose : One Prolonged-release Preparation (78 mg Chlorphentermine HCl)

Urine pH : Uncontrolled Date : 3/6/69

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg base)	Urine pH
0.0	Blank	0.0	6.10
3.5	185	561.0	6.00
6.0	135	1705.5	5.93
8.0	150	2094.5	6.30
12.0	315	1987.7	6.22
14.0	139	440.0	6.37
24.0	310	814.2	6.31
32.0	390	1212.9	6.27
39.0	410	1324.0	6.10
48.0	326	854.4	6.32
51.0	227	1135.0	5.90

Table 18.

Subject : H.J.

Dose : One Prolonged-release Preparation (78 mg Chlorphentermine HCl)

Urine pH : Uncontrolled

Date : 3/5/69

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg base)	Urine pH
0.0	Blank	0.0	5.70
2.5	140	49.1	5.47
6.5	600	656.0	5.90
11.0	460	2465.6	5.90
24.0	670	4958.0	5.85
36.5	500	3465.0	5.80
48.0	275	1250.0	6.00
52.0	197	1417.5	5.90
56.0	150	1763.3	5.75
59.0	226	1184.0	5.80
68.0	390	1699.4	6.05

Table 19.

Subject : G.N. Dose : One Prolonged-release Preparation (78 mg Chlorphentermine)

Urine pH : Uncontrolled Date : 22/7/68

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg base)	Urine pH
0.0	Blank	0.0	5.45
1.0	49	0.0	5.36
2.0	35	62.3	5.15
3.0	48	277.0	5.20
4.2	34	870.4	5.28
5.0	38	1178.0	5.30
6.0	38	1148.0	5.30
9.3	108	4374.0	5.32
11.0	63	1650.6	5.50
13.5	92	1384.6	5.80
21.75	387	5054.2	5.68
25.75	110	1862.3	5.52
29.75	175	2660.0	5.53
35.5	165	2673.0	5.52
45.5	665	2453.0	5.94
49.5	112	661.9	5.76

Table 20.

Subject : D.S. Dose : Aqueous Solution (100 mg Chlorphentermine HCl)

Urine pH : Uncontrolled Date : 23/5/69

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg base)	Urine pH
0.0	Blank	0.0	5.92
3.0	300	880.0	5.90
7.5	325	4313.0	5.80
9.0	110	1500.0	5.80
11.0	275	847.2	5.95
13.5	210	1150.1	5.85
26.0	450	3843.5	5.80
35.0	300	2782.4	6.20
39.5	345	2356.3	6.17
48.0	410	3795.9	5.60
51.0	128	2148.0	5.45
54.5	130	1204.0	5.75
58.0	270	1463.0	6.10

Table 21.

Subject : H.J.

Dose : Aqueous Solution (100 mg Chlorphentermine HCl)

Urine pH : Uncontrolled

Date : 7/4/69

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg/ml)	Urine pH
0.0	Blank	0.0	6.00
1.5	80	22.4	6.10
2.0	20	95.6	5.80
3.0	38	385.3	6.30
5.0	67	986.2	6.10
6.0	84	1008.8	6.50
8.0	170	685.1	6.30
10.0	285	1436.4	5.59
16.0	270	1267.3	5.65
30.0	480	5184.0	5.25
40.0	435	5571.0	5.35
46.0	290	1948.8	5.60
48.0	156	750.4	5.59

Table 22.

Subject : K.M.

Dose : Aqueous Solution (100 mg Chlorphentermine HCl)

Urine pH : Uncontrolled

Date : 4/4/69

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg base)	Urine pH
0.0	Blank	0.0	6.10
2.0	130	341.9	6.10
4.0	228	351.1	6.15
8.0	395	821.6	6.30
12.0	178	987.9	6.00
24.0	400	4732.0	5.70
32.0	275	1993.3	5.85
36.0	132	968.9	5.90
48.0	470	3172.5	5.78
51.5	145	1024.0	5.82
55.0	210	1369.4	6.10
58.0	170	1274.0	6.04

Table 23.

Subject : W.J.

Dose : Aqueous Solution (100 mg Chlorphentermine HCl)

Urine pH : Uncontrolled

Date : 29/6/69

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg base)	Urine pH
0.0	Blank	0.0	6.40
2.5	165	513.0	6.44
4.0	205	930.0	6.35
7.5	410	3226.7	6.05
11.5	385	2082.9	6.35
13.0	175	540.6	6.50
23.0	460	3532.8	5.90
32.0	385	1824.9	6.00
37.0	490	2361.8	6.30
46.0	540	1328.4	5.90
49.0	190	772.0	5.85
51.5	115	689.6	5.95
54.5	140	705.0	6.10

Table 24.

Subject : D.S.

Dose : Intravenous Administration (50 mg Chlorphentermine HCl)

Urine pH : Uncontrolled

Date : 7/7/69

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg base)	Urine pH
0.0	Blank	0.0	6.50
2.5	275	95.0	6.77
5.5	400	580.0	6.30
7.5	175	542.5	6.25
13.25	320	1110.4	5.85
24.0	347	1086.1	5.90
35.25	475	1323.0	6.00
48.25	600	4272.0	5.75
51.75	190	1167.7	5.78
55.5	225	1960.3	5.73
59.0	240	1543.2	5.80
62.0	165	1040.0	5.94
71.0	588	2116.4	6.05
75.0	230	1300.0	6.01

Table 25.

Subject : H.J. Dose : Intravenous Administration (50 mg Chlorphentermine HCl)

Urine pH : Uncontrolled Date : 15/7/69

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg base)	Urine pH
0.0	Blank	0.0	6.45
2.0	235	164.5	6.75
3.0	180	342.0	6.15
5.75	270	1196.1	5.82
11.5	360	580.0	5.93
22.5	350	2355.5	5.45
27.5	175	750.6	5.75
37.0	325	1839.5	5.60
46.0	380	1288.3	5.80
49.0	146	920.0	5.73
51.5	129	1203.3	5.70
54.0	150	1040.7	5.92
60.0	270	1260.7	6.05
70.5	410	2145.0	6.03
74.0	149	886.0	5.90

Table 26.

Subject : H.J.

Dose : Aqueous Solution (78 mg Chlorphentermine HCl)

Urine pH : Alkaline

Date : 9/6/68

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg base)	Urine pH
0.0	Blank	0.0	7.60
2.0	140	190.4	8.22
3.5	330	257.4	7.75
5.5	95	168.1	8.10
7.5	56	63.3	7.30
10.0	168	136.1	8.17
13.0	287	249.7	8.00
20.5	460	492.2	7.63
22.5	126	155.0	7.80
26.5	225	353.3	8.00
30.0	163	213.5	7.63
35.5	270	189.0	8.10
43.0	420	327.6	7.44
46.3	183	525.2	6.40

Table 27.

Subject : A.S.

Dose : Aqueous Solution (78 mg Chlorphentermine HCl)

Urine pH : Alkaline

Date : 2/6/68

Time (hr)	Urine Volume (ml)	Chlorphentermine Excreted (mcg base)	Urine pH
0.0	Blank	0.0	4.90
4.0	155	1960.8	5.55
6.0	55	1704.4	5.08
8.0	63	878.9	6.33
10.0	85	482.0	6.22
14.0	215	365.5	6.95
18.0	208	592.8	6.25
20.0	50	167.5	6.70
22.0	93	323.6	7.58
24.0	65	198.3	7.18
26.0	72	218.2	7.40

Table 28.

Blood Levels of Chlorphentermine Following Oral Administration of a 100 mg Solution Dose.

SUBJECT ;	H. J.		K. M.		W. J.		D. S.	
Time (hr)	Blood Level (mcg/ml)	Time (hr)	Blood Level (mcg/ml)	Time (hr)	Blood Level (mcg/ml)	Time (hr)	Blood Level (mcg/ml)
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.5	0.27	0.5	0.22	0.5	0.23	0.5	0.18
1.0	0.39	1.5	0.34	1.5	0.28	1.5	0.42
1.5	0.45	2.5	0.29	2.0	0.28	2.5	0.39
2.0	0.46	3.5	0.29	5.0	0.25	3.5	0.37
2.5	0.43	7.5	0.26	11.0	0.27	7.0	0.32
3.0	0.40	13.0	0.24	24.5	0.18	13.0	0.27
4.0	0.42	25.0	0.16	35.0	0.15	25.0	0.19
7.0	0.37	36.0	0.14	48.0	0.13	35.0	0.18
24.5	0.31	48.0	0.12			48.0	0.16
48.0	0.20						

Table 29.

Blood Levels of Chlorphentermine Following Oral Administration of a 78 mg Prolonged-release Preparation.

Subject;	H. J.		W. J.		K. M.
Time (hr)	Blood Level (mcg/ml)	Time (hr)	Blood Level (mcg/ml)	Time (hr)	Blood Level (mcg/ml)
0.0	0.0	0.0	0.0	0.0	0.0
1.0	0.0	2.0	0.016	2.0	0.108
2.0	0.057	4.0	0.020	4.25	0.140
4.0	0.066	6.0	0.026	6.0	0.121
6.0	0.092	8.0	0.034	8.0	0.150
8.0	0.137	10.0	0.033	10.0	0.114
11.0	0.118	12.5	0.036	12.0	0.110
13.0	0.125	23.5	0.035	24.5	0.170
25.0	0.103	27.0	0.029	32.0	0.123
28.0	0.096	31.5	0.038	36.0	0.110
31.5	0.092	39.0	0.026	48.0	0.098
36.0	0.083	48.0	0.021		
48.0	0.076				

Table 30.

Blood Levels of Chlorphentermine Following Intravenous Administration of a 50 mg Dose.

Subject;	D. S.	H. J.	
Time	Blood Level (mcg/ml)	Time	Blood Level (mcg/ml)
0.0	0.0	0.0	0.0
2.0 min	0.22	20.0 min	0.355
5.0 "	0.21	40.0 "	0.34
10.0 "	0.196	1.0 hr	0.29
20.0 "	0.189	2.0 "	0.27
1.0 hr	0.18	3.5 "	0.26
3.0 "	0.17	6.5 "	0.22
6.0 "	0.168	11.5 "	0.22
10.0 "	0.175	23.0 "	0.17
24.0 "	0.14	35.5 "	0.157
30.0 "	0.13	48.0 "	0.127
35.0 "	0.122		
48.0 "	0.088		

Table 31.

Blood Levels of Chlorphentermine Following Administration of Multiple Dosage Regimen of Prolonged-release Preparation.

Subject;	D. S.		K. M.		H. J.	
Time (hr)	Blood Level (mcg/ml)	Time (hr)	Blood Level (mcg/ml)	Time (hr)	Blood Level (mcg/ml)
0.0	0.000	0.0	0.000	0.0	0.000
2.0	0.000	2.0	0.000	3.0	0.000
5.0	0.046	5.0	0.032	6.0	0.060
7.0	0.046	8.5	0.082	9.0	0.097
10.0	0.032	11.5	0.069	12.5	0.090
12.0	0.035	23.0	0.060	23.5	0.100
24.0	0.037	28.0	0.076	27.0	0.150
29.0	0.087	30.0	0.145	30.5	0.170
31.0	0.083	32.0	0.120	33.5	0.190
33.5	0.062	37.5	0.115	36.5	0.140
36.0	0.060	48.5	0.108	47.5	0.110
48.0	0.064	51.0	0.154	51.5	-
53.0	0.124	55.0	0.152	54.0	0.290
55.0	0.152	59.5	0.161	57.5	-
58.0	0.202	62.0	0.140	60.5	0.280
72.0	0.184	72.0	0.120	71.5	0.210
76.0	0.251	75.5	0.141	75.0	-
79.0	0.352	78.0	0.182	78.0	0.340
82.0	0.205	82.5	0.178	83.0	0.340
96.0	0.143	85.5	0.159	96.0	0.350
101.5	0.173	96.0	0.124	99.0	-
103.0	0.311	99.0	0.179	102.0	0.410
106.0	0.214	102.0	0.212	107.5	0.360
108.0	0.205	106.5	0.189	118.0	0.290
120.0	0.174	109.5	0.179	121.0	0.300
125.0	0.310	123.0	0.166	124.0	0.460
127.0	0.252	127.0	0.193	129.0	0.420
130.0	0.193	130.0	0.290	133.0	0.420
132.0	0.207	134.5	0.258	144.0	0.370
144.0	0.203	136.5	0.266		
149.0	0.282	143.0	0.237		
151.0	0.246	146.5	0.230		
154.0	0.192	150.0	0.270		
156.0	0.172	153.5	0.247		
		156.0	0.235		