Title
Gastrointestinal absorption studies in unanesthetized rhesus monkeys

Author
Nayak, Ramchandra Keshav

Publication Date
1973

Peer reviewed|Thesis/dissertation

GASTROINTESTINAL ABSORPTION STUDIES IN UNANESTHETIZED RHESUS MONKEYS

by

Ramchandra Keshav Nayak
B.Sc., Bombay University, 1963
Bachelor of Pharmacy, Nagpur University, 1966
M.S., Washington State University, 1969

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

PHARMACEUTICAL CHEMISTRY

in the

GRADUATE DIVISION

(San Francisco)

of the

UNIVERSITY OF CALIFORNIA

GASTROINTESTINAL ABSORPTION STUDIES IN UNANESTHETIZED RHESUS MONKEYS

Ramchandra Keshav Nayak

A model has been developed to study the gastrointestinal absorption of drugs and dosage forms in the unanesthetized rhesus monkey. Chronic vascular catheters were implanted in the iliac vein and artery to enable the investigator to withdraw blood samples without disturbing the monkey. The vascular catheters also allow intravenous studies to be carried out so that the kinetic parameters of any drug can be determined. Plastic cannulae were implanted surgically in the stomach and in the duodenum very close to the pylorus. These cannulae provide a means of instilling a drug solution or a dosage form directly into the stomach or the duodenum. A technique was developed using foley catheters to block the pylorus so that a drug solution or drug particles can be maintained in the stomach. With this set-up, absorption of a drug specifically from the stomach can be studied.

The object of this work was to compare the absorption of an ionizable acidic drug, an ionizable basic drug, and a nonionized drug from the stomach and the intestine. Salicylic acid and levo-amphetamine HCl were chosen as representatives of acidic and basic drugs respectively and antipyrine was selected as the nonionized drug. In studying the absorption of these drugs from the stomach and the intestine, an attempt was made to check the predictions of the pH partition hypothesis which proposes that nonionized molecules are the preferred species for the penetration of biological membranes. Based on this proposition, an acidic drug would be absorbed better from the stomach whereas a basic drug would be absorbed preferentially from the intestine. By simultaneous administration of salicylic acid, amphetamine and nonionized antipyrine, the absorption from the stomach of nonionized acidic drug molecules and ionized basic drug molecules was studied relative to the absorption of the nonionized neutral drug molecules. Similarly, in intestinal absorption studies, a comparison was made between the absorption of ionized acidic drug molecules, nonionized basic drug molecules, and nonionized neutral drug molecules.

The results of this work indicate that absorption of all three drugs was faster from the intestine than from the stomach even if the drug was ionized at the absorption site. The large absorptive surface area of the intestine as compared to that of the stomach plays an important role in the absorption of drugs. In fact, the surface area differences can override the effect of the degree of ionization on the rate of absorption. In contrast to the rate, the extent of absorption of the three drugs from the stomach was almost equal to that from the intestine. In view of these results, the pH partition hypothesis has been

restated as follows:

The nonionized form of a drug will be absorbed faster than the ionized form of the drug at any particular site in the gastrointestinal tract. However, the rate of absorption of a drug from the intestine will be greater than the gastric absorption rate of that drug even if the drug is ionized in the intestine and nonionized in the stomach.

Thus in the monkey, if a normal gastric emptying pattern is maintained, it would appear that the small intestine is the chief absorptive site for all drugs, although some absorption can take place from the stomach.

Dedicated to

Sri Sathya Sai Baba

ACKNOWLEDGEMENTS

I wish to express my gratitude to:

Dr. Leslie Z. Benet for his excellent counseling as my graduate advisor, and for giving other non-academic advice and help as a great friend.

Dr. Malcolm Rowland for his helpful suggestions during the course of the experimental work, and in the preparation of the final manuscript.

Dr. Ralph P. Forsyth for helping in the surgical preparation of the monkeys, and for providing constructive criticism to improve the content of this thesis.

Dr. S. B. Matin for providing the sample of levo-Amphetamine HCl.

My parents for their continuous encouragement and support.

My wife, Sheela, for her patience and for making the difficult days of graduate work happier.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (Continued)

I. INTRODUCTION

A. BIOPHARMACEUTICS AND PHARMACOKINETICS

The recent advances in the fields of pharmaceutical science and clinical pharmacology have led to the emergence of two terms--Biopharmaceutics and Pharmacokinetics. The Academy of Pharmaceutical Sciences' "Guidelines for Biopharmaceutical Studies in Man" (1) define biopharmaceutics as a "study of the factors influencing the bioavailability of a drug in man and animals and the use of this information to optimize pharmacologic or therapeutic activity of drug products in clinical applications." Pharmacokinetics is defined as "the study of the kinetics of the factors involved in the absorption, distribution, metabolism, excretion, and hopefully the time course of pharmacological or therapeutic response of the drug in animals and man." Pharmacokinetic studies inherently involve the development of mathematical models which are conceived in an attempt to interpret the kinetic phenomena. Absorption and bioavailability are two key phrases used in pharmacokinetic studies. Various authors define these two terms in different ways. In the present text, absorption refers to the loss of a drug from the lumen of the gastrointestinal tract whereas the term bioavailability will be used to indicate the rate and extent of the appearance in the measurable blood circulation of the unchanged form of the drug.

B. GASTROINTESTINAL ABSORPTION OF DRUGS

Per oral dosing is the most frequently used route of administration for drugs. However, because this is not the most

direct route of drug administration (i.e. intravenous injection), oral dosage forms, designed with the convenience of the patient in mind, may often lead to biopharmaceutical problems in drug availability. A drug has to dissolve in the fluids of the gastrointestinal tract before it can be absorbed. When a drug is not administered in solution, the dissolution step very frequently becomes the rate limiting step in the absorption process. The various physicochemical factors affecting the dissolution pattern of a drug in a solid dosage form have been reviewed (2-4). For example, Gibaldi (3) lists the following factors: particle size, crystal form, salt form, and state of hydration or nonhydration of the compound. In addition to the intrinsic properties of the drug, formulation factors of the dosage form such as the addition of so-called inert additives, the compression pressure exerted in the making of a compressed tablet or the viscosity of a suspension, can modify the dissolution characteristics of the drug. The formulation factors influencing absorption from suspension and emulsion dosage forms (2,3) and tablet and capsule dosage forms (5,6) have been reported in detail in the literature.

1. Gastric Emptying and Motility:

When a dosage form is swallowed, the drug first reaches the stomach. The acidic pH of the gastric contents may cause acidic drugs to precipitate out of a solution dosage form, or contribute to the slow dissolution of acidic drugs from solid dosage forms, or it can slightly increase the rate of dissolution of basic drugs (7). The relaxation of the pyloric sphincter and the emptying of the gastric contents introduces the drug

into a different pH environment more favorable for the dissolution of acidic drugs. The small intestine appears to be the chief absorptive site due to its extensive absorptive area and the various carrier mechanisms for the active transport of nutrients and structurally related drugs (7). The process of gastric emptying is thus an important factor in determining the length of time a drug remains in a favorable or unfavorable dissolution medium and also the "lag time" before the drug reaches the main absorptive site. Gastric emptying is a very complex phenomenon affected by many factors. Hunt has pioneered in elucidating the mechanisms which govern stomach emptying and his results are presented in numerous articles (8-14). Thomas (15,16) has also reviewed the subject of gastric emptying. Several factors that may affect the rate of stomach emptying of liquid meals or dosage forms have been reviewed by Wagner (17). Some of these factors are: a) the volume of the meal--an inverse relationship exists between the volume of a meal and the rate of gastric emptying (18); b) the temperature of the meal--cold meals empty faster than hot meals (19); c) the composition and viscosity of the meal--fats, proteins, and starch in the meals inhibit gastric emptying (20). Levy and Jusko (21) have shown a reciprocal relationship between the viscosity of gastric fluids and the rate of emptying. d) the acidity of the duodenal contents--if the acidity of the duodenal contents is high, there is a reflex chemoreceptor mechanism slowing stomach emptying (22); e) the body position of the subject--a left supine position reduces gastric emptying since the natural curvature of the gastric pouch gives rise to an uphill path leading to the duodenum. Conversely, a right supine posi-

tion facilitates stomach emptying (23). f) the emotional state of the subject--an aggressive mood enhancing and a depressive mood diminishing gastric emptying (17); g) the effect of the drug itself via a central or local mechanism--a drug may inhibit, e.g. acetylsalicylic acid (24), or accelerate, e.g. chloroquine (25), stomach emptying. The rate and extent of absorption of drugs can thus be greatly modified by stomach emptying and biopharmaceutical investigations are now being directed towards studying the effects of giving a drug with or without water, with or without food and in the anesthetized or unanesthetized state, all of which could modify absorption by altering the gastric emptying process. The disintegration and dissolution of solid dosage forms is also influenced by gastric motility.

2. Intestinal Motility:

Intestinal motility is another physiological factor which modifies the absorption of drugs. The normal rhythmic peristaltic movement of the intestine may increase the rate and/or extent of absorption by helping to dissolve solid particles through agitation as well as by bringing the dissolved molecules to the absorbing surface. Cummins and Almy (26) have studied the absorption of methionine and glucose through 45 cm. lengths of upper small intestine from 3 normal human subjects and 2 patients with sprue, under conditions of increased intestinal activity induced by urecholine and physostigmine. They observed an increased absorption rate due to more contact between the drug molecules and the absorbing mucosa as a result of increased peristaltic activity. In this experiment the drugs were retained within the segment of the small intestine by the

use of inflated balloons. Contrary to these experimental findings, in a normal subject, increased intestinal motility can result in malabsorption due to decreased time of contact between the absorbable material and the absorbing mucosa since the drug passes through the intestine more rapidly. Uyeyama and coworkers (27) similarly studied absorption of vitamin A, galactose and methionine in functional hypermotility and found that it could have inhibiting, potentiating or no effect whatsoever on drug absorption. Excessive peristaltic activity would be expected to reduce significantly the biologic availability of drugs that are absorbed by specialized transport mechanisms since with increased motility the drug molecules are carried rapidly past the specific sites responsible for the active transport mechanisms (28). Riegelman (6) has shown that equal doses of griseofulvin when administered in different dosage forms were available to different extents. A greater extent of absorption was seen from the capsule dosage form than from a suspension of the drug due to faster passage of the suspension through the segment of upper intestine which was supposed to be the main absorptive site for this poorly soluble drug. Ether (29) and the barbiturates (30) have been shown to reduce the tone and motility of the small intestine which could alter drug absorption by altering the residence time of a drug in the gastrointestinal tract. Small intestinal motility and pathophysiological alterations in it has been reviewed by Farrar and Zfass (31).

3. Effect of Food on Absorption:

The presence or absence of food in the gastrointestinal tract can have different effects on the rate and extent of

absorption of different drugs. The volume of gastrointestinal fluid available for the dissolution of drug molecules may be decreased by food, and thus result in decreased dissolution of drugs from solid dosage forms. The increased viscosity of the luminal contents resulting from the presence of food may also hinder absorption by increasing the mean diffusion path to the absorbing mucosa. There is an inhibitory effect of food on gastric emptying which in turn delays the entrance of drug molecules into the small intestine. The slow gastric emptying in such a case may cause the drug molecules to trickle into the small intestine and for certain drugs (i.e., a drug absorbed by an active transport mechanism from a selected area of the intestine) may increase the extent of absorption. The slow release of drug molecules from the stomach results in a lower concentration of drug at the absorption site which does not saturate the carrier system thereby resulting in greater absorption. This was found to be true in the case of riboflavin (28) which is thought to be absorbed actively from a site located in the upper region of small intestine. A two-fold increase was seen in the extent of absorption of this vitamin when it was administered with food.

Studies on the effects of dietary components on the gastrointestinal absorption of acetaminophen from tablets in man (32) revealed that carbohydrate test meals significantly impaired the rate of absorption, while no such effect was found with high protein, high lipid or balanced test meals. The reduction in acetaminophen absorption by the carbohydrate meal was thought to be due to adsorption of the drug onto pectin.

Examples of drugs which show faster and/or greater absorption in subjects in the fasting state as opposed to the nonfasting state are: aspirin (33), erythromycin esters (34), sulfadiazine (35), the tetracyclines (36,37) and penicillin (38). Drugs which are better absorbed in the presence of food rather than in the fasted state include riboflavin (28), riboflavin phosphate (39), griseofulvin (40,41) and ethinylestradiol 3-cyclopentyl ether (42).

4. Effect of Blood Flow on Absorption:

Studies in the rat (43), dog (44), and in man (45) have shown that there is approximately a 30% increase in blood flow through the splanchnic area following a meal. More recently Doluisio and coworkers (46) have demonstrated that prolonged periods of inanition cause decreased blood perfusion of the g.i. mucosa and submucosa. They attributed the prolonged absorption half-lives observed for salicylic acid, barbital, haloperidol and chlorpromazine in rats fasted for periods longer than 20 hours to this hemodynamic change. Winne and coworkers (47,48) found that the appearance of aniline, aminopyrine, antipyrine, benzoic acid and salicylic acid in the intestinal venous blood of rats was blood flow dependent. Other rapidly absorbed small molecules such as tritiated water, ethanol, methanol, glycerol, ethylene glycol, urea and erythritol also exhibited blood flow dependent absorption kinetics, but their absorption rates and blood flow dependence decreased in the order presented above. The absorption rate of a slowly absorbed substance such as ribitol was constant at different blood flow rates. Diamond *et al*. (49) have duplicated the findings of Winne and coworkers in their studies on anesthetized dogs, where an

implanted occluder was used to progressively decrease the mesenteric blood flow to different fractional levels of the initial flow.

5. Drug Interaction with Constituents of the Gastrointestinal Tract:

The interactions of drugs with the normal constituents of the fluids of the g.i. tract also play a part in determining the absorption of a drug. The high acidity and enzymatic activity of gastric fluids often catalyzes the degradation of drugs to their pharmacologically inactive forms. In such cases, the extent of degradation of the drug may be limited by decreasing the dissolution rate or facilitating gastric emptying. Enzymes present in the intestine are also capable of causing chemical degradation of a biologically active drug or conversely changing an inactive pro-drug into an active drug. Another component of g.i. fluid, mucin, a viscous mucopolysaccharide, interferes with the absorption of some drugs either by increasing the viscosity of the fluid medium or by complexing with the drugs (50,51). Conjugated bile salts, secreted into the lumen of the small intestine, have been shown to improve the absorption of many poorly soluble drugs by solubilizing the drugs due to the emulsifying properties of the bile salts (52). In certain cases, bile salts may form insoluble, nonabsorbable complexes with some drugs thus markedly restricting their absorption (53).

6. Effect of pH:

The g.i. mucosa is a semipermeable lipoidal membrane across which compounds are normally absorbed by one of the following mechanisms: passive diffusion, pore transport, active or facilitated transport. Most pharmacologically important,

lipid soluble drugs are thought to be transported across biological membranes by a simple diffusion process. The g.i. mucosa is hypothesized to permit the passive absorption of lipid soluble drugs while imposing a barrier to the absorption of lipid insoluble drug molecules. Most drug substances, being weak organic acids or bases, can exist in both the undissociated and dissociated form in an aqueous environment; the degree of dissociation depending on the pH of the medium and the dissociation constant of the drug. Since the undissociated or nonionized form of the drug has the greater lipid solubility, the rate of g.i. absorption of weakly acidic or basic drugs is related to the fraction of total drug in solution in the nonionized form. The rate of absorption is enhanced when pH conditions are changed so that the fraction nonionized is increased. This is the basis for the well-established pH partition hypothesis which relates the drug dissociation constant, lipid solubility and the pH at the absorption site with the absorption characteristics of various drugs throughout the g.i. tract (54-58). The investigations of Schanker et al. (55), in which the pH of the fluids in the rat stomach was varied and similar studies in rat intestine (58) prompted the investigators to suggest that weakly acidic drugs are primarily absorbed from the stomach since weakly acidic compounds would exist essentially in the nonionized state at the low gastric pH values. Conversely, it was stated that the weakly basic drugs would be absorbed preferentially from the small intestine. Discrepancies were observed in the results obtained with some drugs where the absorption did not seem to quantitatively follow the predictions

of the pH partition hypothesis. To explain these discrepancies the virtual pH hypothesis was proposed (58-60), which claimed that the pH at the absorbing surface was different than the pH of the bulk fluid. Wagner (61) has reviewed the experimental evidence which disputes the existence of a virtual pH. Turner et al. (62) have shown that drug ions apparently do pass through the in vitro rat intestine. Kakemi et al. (63,64) have demonstrated that the absorption of various drugs could be correlated very well with the extent of binding of a drug to the mucosa, and that the degree of drug ionization was only of minor importance. They point out that the percent of drug bound to the mucosa and subsequently the degree of absorption, change as a function of pH for both ionized and nonionized drugs.

C. METHODS OF STUDYING THE GASTROINTESTINAL ABSORPTION OF DRUGS

Several in vitro and in vivo methods have been used in g.i. absorption studies to learn about one or more of the following aspects of drug absorption: a) the specific site for optimum absorption, b) the mechanism of absorption, c) the kinetics of absorption and d) the physicochemical and physiological factors influencing absorption. The in vitro and in situ techniques employed are often relatively simple, thereby eliminating many of the variables that could effect absorption in vivo but also adding some extraneous variables which may not effect the absorption process occurring in an intact animal. These techniques do not truly approach the conditions of the normal physiologic state of the g.i. organs and the results of such studies, although providing an insight into the mechanisms of drug absorption, cannot be extrapolated to explain drug absorption in a

normal intact animal. The *in vitro* studies are usually carried out on tissues from rats, rabbits, hamsters and dogs. *In vivo* methods for studying drug absorption provide the most useful data and may be applied to both human and animal studies but often require difficult procedures and very careful interpretation.

1. *In Vitro* Methods:

a) Everted small intestinal sac technique:

The method involves isolating a small segment of the intestine of a laboratory animal, everting the segment and filling the sac with a small volume of a drug-free physiologic buffer. Both ends of the segment are tied off and the sac is immersed in a flask containing a relatively larger volume of buffer solution containing the drug. The flask and its contents are oxygenated and agitated continuously at 37° C for a specified period of time, at the end of which the sacs are opened and the inner serosal fluid assayed for drug contents. This technique was developed by Wilson and Wiseman (65) and has been very commonly used for *in vitro* absorption studies. Eversion has been suggested as a means of prolonging the viability and integrity of the tissue since it exposes the mucosal surface to oxygenated mucosal fluid. The small serosal volume also results in large changes in concentration even when a small amount of drug is transported across the mucosa. The disadvantage of this preparation is that each intestinal segment or sac provides only one absorption measurement at the time it is removed from the mucosal solution. This method has been used to study the enteric absorption of pharmacologic substances in rats of different ages (66). It was found that penetration of antipyrine,

sodium salicylate, tetraethyl ammonium and phenolsulfonephthalein to the serosal from the mucosal side of everted sacs was greater in 10 day old rats than in 30 and 120 day old animals. The surface/thickness ratio was found to be optimal for intestinal permeability in 30 day old rats. Using everted rat intestinal sacs, Schanker and Jeffrey (67) showed that two antitumor active compounds, 5-flurouracil and 5-bromouracil, were actively absorbed by the pyrimidine transport system. Dikstein and Sulman (68) have described an inverted rat stomach preparation for use in gastric absorption and gastric acid secretion studies.

b) The Crane and Wilson technique (69):

This is a modified everted intestinal sac technique in which one end of the everted segment is tied off and the other end is tied to a cannula from which frequent serosal samples may be obtained. With this method, a number of different solutions may be tested with a single segment of intestine.

The everted intestinal preparations have been criticized since permeability characteristics of the membrane are significantly altered when the tissue is removed from the animal and the membrane's blood supply. In such a preparation, the drug must traverse the entire thickness of the intestinal wall whereas in vivo a drug only has to cross the epithelial cells to be absorbed into the blood stream and does not have to penetrate through the muscularis. The process of eversion itself is said to make the membrane abnormally permeable (70).

c) Perfusion techniques:

These involve isolating either the entire small intestine or a segment thereof from a laboratory animal and perfusing

oxygenated buffer through the lumen. The outer surface of the intestinal segment is also bathed with oxygenated buffer solution. The intestinal segment can be everted to study both the mucosal to serosal and the serosal to mucosal transfer of drug molecules. This technique has been used by Turner et al. (62) to study the directional permeability of drug ions and by Benet et al. (71) to evaluate the effects of buffer constituents and time on drug transfer through the in vitro rat intestine. Nayak and Benet (72) used perfusion techniques to investigate the transfer of both ionized and nonionized molecules through rat intestinal muscularis. The perfusion or circulation technique was initially used by Wiseman (73) and by Darlington and Quastel (74). Wilson (75) used the method to study the transport of glucose and methionine in segments of the everted small intestine of the golden hamster. Acland (76) using this technique with ^{131}I labelled iodide found that there is no barrier to the passage of iodide ion across the intestinal mucosa. Smyth and Taylor (77) used a slightly modified perfusion technique to study water transport across the intestinal wall. In their experiments the outer surface of the intestine was not bathed in buffer solution. The in vitro methods of Smyth and Taylor (77) and the everted sac technique of Crane and Wilson (69) have been evaluated by Misra et al. (78) with respect to the passage of drugs from the mucosal to the serosal side of the intestine. Dowset and Wingate (86) have described a method for the investigation of the transport of fluids and solutes across human ileum in vitro. The portable apparatus requires only a 2.5 cm segment of normal ileum, which may usually be found in surgical specimens from human ileal resection. The system

permits accurate measurement of water transfer thus enabling net water and solute transfer to be observed simultaneously.

2. In Vivo Methods:

In these methods, the blood supply of the animal remains intact, yet the animal is often surgically prepared for the absorption studies. Because of the intact blood supply, the rates of absorption determined from these studies may be quantitatively more realistic than those determined using in vitro techniques. The in situ perfusion technique used by Schanker et al.(56) allows measurement of the rate of disappearance of a drug from the lumen of the rat small intestine following the perfusion of a drug-containing solution through the lumen at a constant rate. Although in situ, "in place", studies must be included as in vivo methods, we are using the former term to indicate studies where a segment of the g.i. tract is isolated from the rest of the tract and normal passage of drug throughout the tract is interrupted in an acute experiment. Hogben et al. (58) have used this in situ perfusion technique to present evidence in support of the pH partition hypothesis for the intestinal absorption of weakly acidic and basic drugs. Koizumi et al. (79) used a similar technique to study the rate of intestinal absorption of sulfonamides in the rat and found an excellent correlation between the absorption rates and the chloroform-water partition coefficients of the sulfonamides. Barr and Riegelman (80) evaluated the effects of metabolism, tissue accumulation and blood flow on the transport of salicylamide across the basal membrane of the rabbit intestine using both the in vitro Crane and Wilson method and the in situ intestinal loop method. The absorption of ion-pair complexes has been studied in rats by

means of an *in vivo* rectal perfusion technique (81). Doluisio *et al.* (82) have reported a new method for studying the g.i. absorption from isolated gut segments of the anesthetized rat *in situ*. The method involves exposing the small intestine of anesthetized rats by a midline abdominal incision. Two L-shaped glass cannulae are inserted into the intestine through small slits at the duodenal and ileal ends. Each cannula is connected through a 4 cm. long piece of Tygon tubing and a 3-way stopcock to a hypodermic syringe containing the perfusion fluid. The arrangement enables the investigator to pump the lumen solution into either the ileal or the duodenal syringe, remove an aliquot for assay, and return the remaining solution to the intestine in a short time. To assure uniform drug solution concentrations throughout the gut segment, aliquots are removed from the two syringes alternately. It is claimed that the method yields closely reproducible data and absorption rates which are realistic in terms of the known absorption behavior of drugs in humans and intact animals. Using this technique, these workers have studied the effect of fasting on intestinal drug absorption (83), and the influence of pH on the absorption kinetics of weakly acidic drugs in the rat (84).

Levine and Pelikan (51) have described a technique for studying the intestinal absorption of drugs from single or multiple intestinal loops. The technique involves isolating 4 inch long segments of the small intestine by ligatures. The drug solution is introduced into the lumen of the loop by means of a syringe fitted with a 26-gauge needle which is secured by the proximal ligature. After injection, the needle is removed, the proximal ligature is tightened, the intestinal

loop is replaced into the abdominal cavity, and the incision is closed. In the multiple loop preparation, a distance of approximately one-half inch is left between successive loops. After a predetermined period of time, the animal is once again anesthetized, sacrificed by decapitation, and a blood sample is taken if desired. The intestinal loops are rapidly excised and homogenized, and the amount of drug unabsorbed in each loop is determined. Feldman et al. (70) have used this technique to study the influence of sodium deoxycholate on phenol red absorption. Schanker and coworkers (55) have also studied the in situ gastric absorption of drugs in rats. The results of these studies led to the proposed pH partition hypothesis for the absorption of drugs. Doluisio et al. (82) have used a similar in situ technique for studying the gastric absorption of drugs but their technique differs from Schanker's in that both the cardiac and the pyloric ends of the stomach are cannulated. Lanman et al. (85) studied the in situ intestinal absorption of organic anions in rats and found a correlation between the absorption rate and chloroform-water partition coefficient determined at pH 7.4, the pH of solutions used in the absorption experiment.

3. Studies in Man:

Perfusion studies using transintestinal tubes and nonabsorbable markers were initiated by Schedl and Clifton in 1961 (87). The use of a multilumen tube has been described by Holdsworth and Dawson (88). The reliability and accuracy of the double lumen tube perfusion method for measuring intestinal absorption in man has been assessed by the use of a proximal occlusive balloon in 9 normal volunteers by Sladen and Dawson

(89). They found that luminal occlusion significantly reduced the variability of individual glucose and water absorption rates as well as the variability of triplicate marker concentrations within individual studies. The triple lumen tube has been used by Siurala and coworkers (90-92) to study the absorption of warfarin and acetylsalicylic acid from the human stomach and small intestine. Beermann et al. (93) have similarly studied the fate of orally administered ^{3}H-digitoxin in man. They found that approximately 15, 30 and 70% of the radioactivity administered had been absorbed when the test solution passed the stomach, the duodenum and the upper jejunum respectively. The intestinal tube has also been used for studying gastrointestinal absorption of: anticholinergic drugs (94), ^{3}H-butylscopolamine (95), ^{14}C-methyl scopolamine (96), atropine (97), and a quaternary compound (98).

Gibaldi and Kanig (98a) have studied the effect of body position and "test solution" pH on the g.i. absorption of a weakly acidic and a weakly basic compound in human volunteers. Relative absorption rates were evaluated by means of urinary excretion kinetics. Gastric retention was attained by positioning the subjects in a left lateral position during the absorption phase. Gastric retention appeared to promote salicylate absorption but depress the absorption rate of creatinine. It was observed, with both test agents, that the coadministration of a "test solution" which affected an increase in the fraction of the undissociated species at the absorption site, produced an increase in the absorption rate. Conversely, the administration of a "test solution" which enhanced ionization of the test agent at the absorption site, resulted in a

decrease in the rate of absorption. Overall, the results were in agreement with the pH partition hypothesis for drug absorption.

D. OBJECTIVE AND RATIONALE OF THE PROPOSED RESEARCH

The aim of the present work was to develop a model using the unanesthetized rhesus monkey (Macaca mulatta), to study the g.i. absorption of drugs. The model should allow the investigator to carry out an absorption experiment in an unanesthetized animal. It was expected that with such a model it should be possible to administer a drug in any dosage form thus allowing the investigator to evaluate the effect of formulation factors on the bioavailability of the drug. The rhesus monkey model is ultimately planned to be used for studying variables like water loading, starvation, food and anesthesia, all of which can modify the absorption profile of a drug. The rationale for planning this research project was that most of the hypothesis on drug absorption phenomenon have been based on results obtained with the *in vitro* and *in situ* methods described earlier. Although these studies are useful for exploratory investigations and can differentiate between the absorption characteristics of the various regions of the g.i. tract, the extrapolation of these results to explain absorption phenomena or bioavailability problems in the unanesthetized animal is often impossible. The *in vitro* techniques utilize tissues that are removed from their blood supply and a drug has to cross additional barriers which do not exist *in vivo*. The *in situ* techniques usually equate absorption with loss of drug from the gastrointestinal lumen. The accumulation or metabolism of a drug in the gut wall can

give an overestimate of the amount of drug absorbed.

The absorption process in an anesthetized intact animal could be significantly different from a normal animal due to effects of anesthesia on blood flow, g.i. motility and stomach emptying. Kramar and coworkers (99-102) have studied the effects of nonspecific stress due to surgical trauma, ether anesthesia, cold, muscular exercise and emotional upset on the capillary response in the rat. They found an initial rise in resistance, a critical drop, a period of abnormally low resistance and restoration to normal capillary resistance. Unless a physiologically stabilized model is utilized, such acute changes in capillary circulation could significantly interfere with the reliability of results found in drug absorption studies. As described previously, ether and sodium pentothal anesthesia depress myotonic and myokinetic functions of the dog stomach (29,30). The effects of alterations in blood flow on rate and extent of drug absorption have been described earlier (47-49). With antipyrine, it was found that diminishing the blood flow by a factor of 9 reduced the absorption rate to 1/3 of the maximum value, while the blood concentration of the drug increased 3 fold (47). The converse relationship was found with increased blood flow when comparisons were made starting at low initial flow rates. The in vitro methods cannot assess the influence of blood flow on drug absorption. The importance of many factors that may influence drug absorption such as gastric emptying, intestinal motility, the direct effects of drugs on the g.i. tract, as well as dosage form variables can best be assessed by means of in vivo studies.

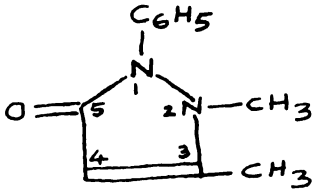
Zeman (103, 104) has developed a versatile device which is applicable for the cannulation of a variety of organs in long term animal experiments. Modifications of the device for cannulation of the gall bladder, urinary bladder, stomach and intestine in dogs are described. The utility of this technique in dogs was demonstrated in a comparative study (105) of the absorption of 16-β-methylprednisone and its 21-phosphate ester after oral, duodenal and jejunal administration of the drugs. Using the same device, permanent cannulation of the gall bladder is described (106) as a physiologic model suitable for long term bile excretion studies. Kennedy (107) has described simple gastric fistula tubes for use in rats and rabbits. The rhesus monkey was preferred over the dog for the proposed research since monkeys can be restrained in chairs for extended periods of time whereas dogs cannot be restrained in straps for more than 6-12 hours.

There has been a steady increase in the number of biomedical investigations done on the rhesus monkey. Reviews have been published on the use of nonhuman primates in evaluation of hematotoxicity (108) and their role in assessing drug toxicity in man (109). Rhesus monkeys have been used for the study of: the enterohepatic circulation of bile (110), biliary lipid secretion (111), vitamin B_{12} absorption (112), gastric secretory physiology (113, 114), structural and physiologic alterations of the esophagus (115), corticosteriod clearance by the gut (116), hemodynamic effects of angiotensin (117), the blood pressure response and the occurrence of duodenal lesions in long term avoidance schedules (118,119). Based on kidney function, body fluid compartments, water and electrolyte

metabolism, the monkey is said to resemble man more than any other experimental animal (120). The biochemical and physiological features of normal rhesus monkeys have been determined by many investigators (120-123). Forsyth and coworkers (124) have studied the normal distribution of cardiac output in the unanesthetized restrained rhesus monkey and have shown that there is a redistribution of cardiac output after pentobarbital anesthesia (125). The gastric secretory response to histamine in the rhesus monkey resembles that observed in man (126). In the basal state the gastric juice obtained through a gastric fistula had a pH above 3.5 and a high pepsin concentration. However, repeated histamine injection led to a rise in free acid and to an increase in pepsin output. This is in contrast to dogs where no such response with histamine is observed. Diurnal variations in the volume and acid concentration have been seen in 24 hour collections of fasting gastric contents in spider monkeys (127). A rise in pH was observed after 6 p.m. while a return to pH 1-2 was seen between 7 and 8 a.m. Brooks and coworkers (128) have studied the effect of restraint on fasting gastric contents of spider monkeys during 3 and 24 hour experiments. In the 3 hour restraint experiments, the volume and acid concentration were significantly reduced. The pepsin concentration was increased but not to a statistically significant level. However, in the 24 hour experiments, there was little change noted in the volume and acid concentration when the animals were restrained. Free moving animals showed a greater gastric fluid volume during the period of daytime activity than when they were restrained but showed a marked decrease in the volume and acid concentration during the night.

E. THE TEST COMPOUNDS

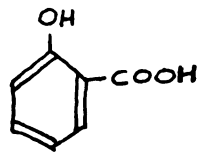
1. Antipyrine: 2,3-Dimethyl-1-Phenyl-3-Pyrazolin-5-one



Antipyrine and the closely related compound aminopyrine have been prescribed for antipyresis and analgesia. The clinical use of antipyrine has been sharply curtailed because of its structural similarity to aminopyrine which causes severe and often fatal agranulocytic angina. Brodie and Axelrod (129) were the first to study the fate of antipyrine in man. Their results indicated complete absorption of antipyrine from the gastrointestinal tract and an equal distribution throughout the body water. About 5% of antipyrine was exreted unchanged; 30-40% oxidized to 4-hydroxyantipyrine which is quickly conjugated with glucuronic acid and sulfuric acid and then excreted; the remaining 55-65% was thought to be metabolized through an unknown route. Recently, the metabolism of antipyrine was investigated in rats, mice, guinea pigs, rabbits and humans (130). All of these species including humans were found to excrete a considerable amount of a new metabolite together with 4-hydroxyantipyrine and the unchanged drug. This new metabolite was characterized as 3-hydroxymethyl, 2-methyl, 1-phenyl, 3-pyrazolin-5-one and was excreted in the urine to a greater extent in the free state than in the conjugated form. The complete metabolism and equal distribution of antipyrine throughout the body water spaces with negligible binding to plasma proteins has made this compound one of the few used to estimate total body water (131,132). Also, since it is

completely metabolized, changes in the plasma half-life of this compound are used to indicate the relative activity of the drug metabolizing enzymes in an animal before and after exposure to a drug or other foreign compounds. The application of this antipyrine test has been helpful in determining whether a drug causes enzyme induction even before it is possible to determine whether a drug affects its own metabolism (133-136).

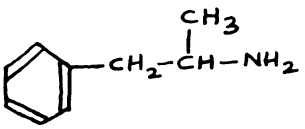
2. Salicylic Acid: o-hydroxybenzoic acid



This compound has been studied by many investigators and its rapid absorption from intestinal perfusates at high pH values seems to have been the driving force for proposing the virtual pH theory. Orally ingested salicylates are absorbed rapidly, both from the stomach and the upper small intestine (136). Although significant gastric absorption of salicylates normally occurs, the upper small intestine is the major site of absorption (136). After absorption salicylate is rapidly distributed throughout all body tissues and most transcellular fluids. At concentrations encountered clinically, from 50-80% of the salicylate in human plasma is bound to plasma proteins. Kucera and Bullock (137) studied binding of salicylate to plasma proteins from several animal species. In man and monkey, the percentage bound was almost identical. McArthur and coworkers (138) have also found an appreciable amount of salicylate bound to red blood cells. Practically all of a given salicylate dose can be recovered in the urine as free

unaltered salicylate and as salicyluric acid, salicyl phenolic and acyl glucuronides and as gentisic acid. The concentration of metabolites in plasma is quite low and is rarely more than 7% of the total plasma salicylates.

3. levo-Amphetamine: levo-α-methylphenethylamine



Amphetamine has been used as an analeptic, antidepressant, anorexigenic and as a nasal decongestant. The levo isomer of the base has no central effects and was used in this work so as to study its absorption without altering any central nervous system functions. Following ingestion of a single oral dose of amphetamine, 33-35% of the dose was recovered in 24 hours in the urine of the rat (139) and man (140). Amphetamine is excreted as the unchanged drug and as 4-hydroxyamphetamine. A new metabolite, benzyl methyl ketone oxime, both the syn and anti forms, has been isolated from the urine of animals treated with amphetamine (141,142). The extent of metabolism of amphetamine is affected by the urinary pH. In acidic urine, amphetamine is largely excreted unchanged or unconjugated but in alkaline urine, much of it is deaminated or conjugated (143). The amount of amphetamine excreted and thus its plasma half life, is significantly influenced by changes in the urinary pH (143,144). The half-life of tritiated amphetamine in the plasma of persons with acid urine (pH 6.0) varied from 8-10.5 hours, whereas in persons with alkaline urine (pH 7.5) a 16-31 hour range was found.

II. EXPERIMENTAL

A. SUMMARY

Male rhesus monkeys were the test animals. The animals were surgically prepared to enable the collection of blood samples and instillation of drug solutions into the stomach or the duodenum. The drugs to be studied were administered intravenously in individual experiments to determine the kinetic parameters for their distribution and elimination. They were also given simultaneously in another intravenous study to investigate if there was any drug interaction among them which would alter their kinetics. In gastrointestinal absorption studies the three drugs in solution were introduced either singly or simultaneously into the stomach or the duodenum. Blood samples were drawn from the venous catheter at different time intervals into heparinized tubes. After centrifugation, the plasma was analyzed for the respective drugs. The analytical methods employed included liquid scintillation counting, spectrophotofluorometry and gas liquid chromatography. The investigative compounds were antipyrine, salicylic acid and levo-amphetamine HCl.

B. MATERIALS

Polyvinyl tubing was used for the chronic venous and arterial catheterizations. (Available from Borden Chemical Co. as Resinite Hi Heat 105C vinyl insulation sleeving #20, I.D. 0.034" and wall 0.016"). Polyvinyl tubing was preferred over other conventional types of tubing because of its flexibility, resilience, and low clot induction properties. Siliconized

tubing may also be used in lieu of polyvinyl tubing. Plastic cannulae for the stomach and the duodenum were made to our specifications by the Research and Development Laboratory, San Francisco campus, University of California. The cannulae are depicted in Fig. 1. They consist of a cylindrical tube 4.5 cm long, I.D. 0.6 cm and wall 0.1 cm. At one end the cannula has a flange 0.1 cm thick. For the stomach cannula, the flange was made circular with a diameter of 1.8 cm but for the duodenal cannula, the flange was made into an ellipse with a major axis of 1.8 cm and a minor axis of 0.8 cm. The cannula has threads on the outside from the end distal to the flange up to about half its length. These threads are for holding two star wing nuts which remain against the skin on the outside and thus prevent the cannula from being drawn inside the abdomen. The cannula can be closed with a screw-in insert the tip of which remains flush with the flange.

Sodium salicylate, reagent grade, was obtained from Allied Chemical. Antipyrine, N.F. grade, was obtained from Merck and Co. Inc., N-methyl ^{14}C antipyrine and ^{14}C toluene radioactive standard was obtained from International Chemical and Nuclear Corp. levo-amphetamine HCl was given to us by Dr. S.B. Matin and was chromatographically pure.

C. SURGICAL PREPARATION OF MONKEYS

Male rhesus monkeys weighing between 5-7 kgs were used. The animals were quarantined and were free of any disease at the time of use. Before anesthetizing the monkey, the animal was immobilized with a 20 mg/kg I.M. dose of Vetalar^{R}. (Vetalar^{R}, Ketamine HCl, 100 mg/ml, Parke-Davis and Co., Detroit, Michigan 48232). Anesthesia was accomplished by giv-

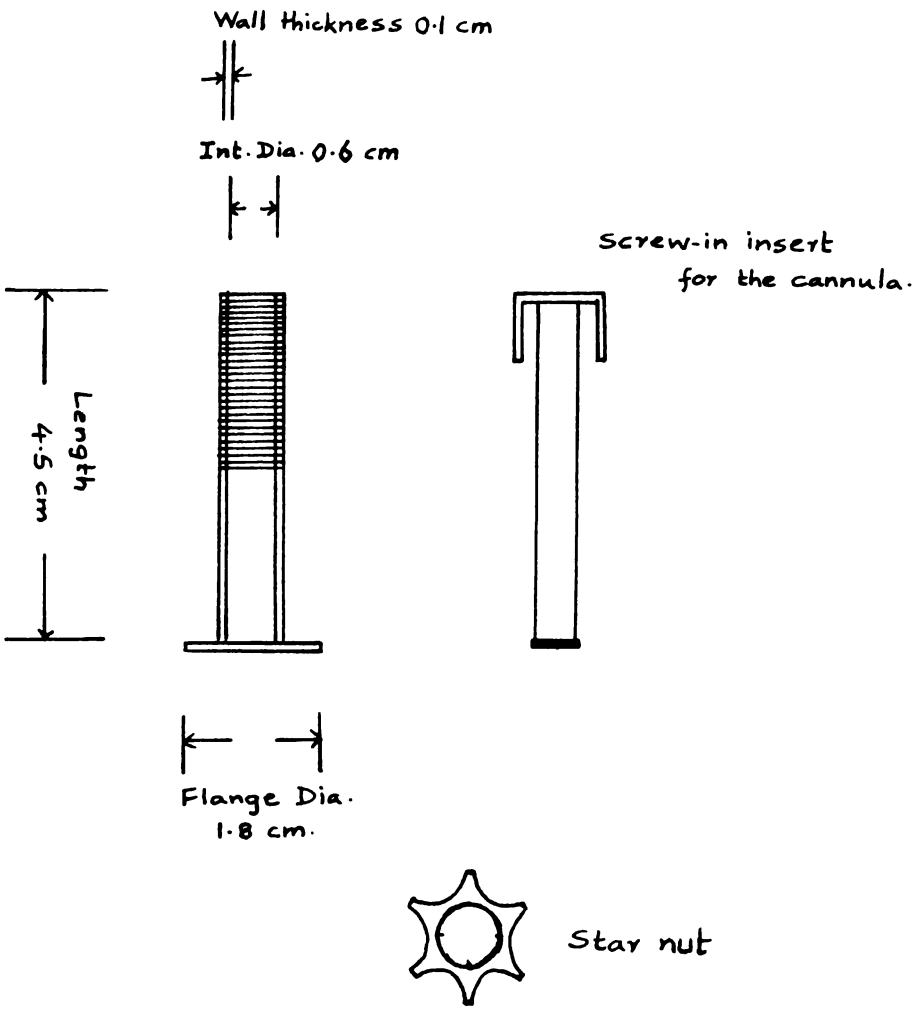


Fig. 1.--Diagram of Cannula Used in Stomach and Duodenal Implantation.

a 30 mg/kg I.V. dose of Diabutal^{R}. (Diabutal^{R}, Sodium pentobarbital, 60 mg/ml, Diamond Laboratories, Des Moines, Iowa 50304). The polyvinyl catheters were sterilized by soaking them overnight in 1:500 solution of Antiseptic No. 3. (Antiseptic No. 3, Antiseptic cyanide, Tablets of Mercury cyanide, Eli Lilly and Co., Indianapolis, Indiana 46206). The catheters were flushed with sterile normal saline just prior to aseptic intravascular implantation. The surgical approach for the catheterization is similar to that described by Werdegar, Johnson and Mason (145). A left flank incision was made that ran obliquely from the midinguinal region to the iliac crest. The left external iliac vein and artery were exposed. Catheters were introduced and passed 12-14 cms cephalad in the vein and 6-7 cms cephalad in the artery placing the tips of the catheters in the inferior vena cava and the abdominal aorta distal to the renal arteries. Several ligatures were made around the vessel and the catheter. The distal end of each catheter was curved gently in an 180^{o} turn, sutured onto fascia and tunneled under the skin to the desired exit point near the umbilicus. The incision was closed and Panalog^{R} ointment applied on and around the wound to prevent infection. (Panalog^{R}, Nystatin, Neomycin Sulfate, Thiostrepton and Triamcinolone acetonide ointment, E.R. Squibb and Sons, Inc., New York, N.Y. 10022). After recovery from anesthesia, the monkey was placed in a restraining chair which has been described in detail (146). The chair is housed in an environment limiting booth which can be closed during an experiment to maintain constant external conditions. The distal ends of the vascular catheters were connected through a luer stub

adapter and a threeway stopcock to an infusion pump (Harvard infusion pump, model #975, Harvard Apparatus Co., Millis, Massachusetts 02054) which was set to infuse 1 ml/hr of a heparinized saline solution containing 5 units of heparin per ml of saline into each catheter. During the recuperation period following the first operation, 1 ml of Streptillin^{R} was given intramuscularly every day alternately in each leg for about a week. (Streptillin^{R}, each ml contains Procaine Penicillin G 200,000 i.u. and Dihydrostreptomycin sulfate equivalent to 0.25 gm Streptomycin, Trico Pharmaceuticals, San Carlos, California 94070). One ml of Imferon^{R} was given intramuscularly once a week to help the monkey recover any lost blood volume. (Imferon^{R}, Iron dextran injection, each ml contains 50 mg iron, Lakeside Laboratories, Inc., Milwaukee, Wisconsin).

About a week after the vascular catheters were implanted, the monkey underwent a second surgery, this time for the insertion of the stomach and the duodenal cannulae. The cannulae were sterilized by soaking them in a 1:500 aqueous solution of Antiseptic No. 3. A mid-abdominal incision was made just below the rib-cage and along the linea alba. The stomach was exposed and an incision large enough to introduce the flange of the cannula was made in the mid region of the stomach along the greater curvature. The cannula was made secure by means of a purse-string knot with a polydek 3.0 suture. The duodenal cannula was similarly implanted in the upper duodenum 1-2 cms distal to the pylorus. A tycron 4.0 suture was passed through the two cannulae and its ends tied to the tips of the screw-in inserts of the two cannulae. The suture helped to pull a foley catheter

through either of the cannulae to block the duodenum. The cannulae were exteriorized through the skin on either side of the incision about 4 cms lateral to it. The star wing nuts were screwed onto the cannulae which were stoppered with the inserts. The incision was closed and the monkey replaced back in the chair after recovery from anesthesia. Post surgical infection was prevented by giving a 0.25 gm I.V. injection of Keflin^{R} twice a day for about 3 days. (Keflin^{R}, Sterile sodium cephalothin, Eli Lilly and Co., Indianapolis, Indiana 46206). Supportive nutritional care was provided during the recovery period by giving a slow 5 ml/kg I.V. infusion of Ambex^{R} twice a day for about 4 days. (Ambex^{R}, Amino acid solution with electrolytes, vitamin B complex and 5% dextrose, Elanco Products Co., a division of Eli Lilly and Co., Indianapolis, Indiana 46206). The monkeys were allowed to recuperate for a period of about ten days before any experiments were done. The monkeys have been shown to have normal blood pressure, heart rate and catechol amine levels for as long as nine months after introduction of vascular catheters(147). The condition of each monkey was monitored by taking a complete blood count and determining the serum protein level once a month. The hematocrit values were determined more frequently.

D. EXPERIMENTAL PROTOCOL

1. Placement of the Foley Catheters:

In studying drug absorption from the stomach a double lumen Bard foley catheter #12 French with a 5 ml balloon was inserted into the duodenal cannula and the balloon inflated with distilled water, blocking the duodenum just below the

pylorus. The effectiveness of this block was checked by fluoroscopic observation of a barium sulfate suspension introduced into the stomach. No leakage of the suspension past the inflated balloon proved that a complete block was accomplished in this way. The drug solution was instilled into the stomach through another foley catheter introduced into the stomach cannula. The balloon of this catheter is inflated inside the wall of the cannula to prevent the gastric contents from escaping out through the cannula.

In studying intestinal absorption, the stomach cannula remains closed. A foley catheter is inserted into the duodenal cannula with its balloon inflated inside the wall of the cannula and the drug solution is introduced into the duodenum. In these studies regurgitation of some of the drug solution into the stomach may occur since the pylorus is not blocked. Figures 2 and 3 schematically represent the positioning of foley catheters for the gastric and intestinal absorption studies, respectively. The balloons were deflated and the foley catheters removed 6 hours after the start of an absorption experiment.

In one of the monkeys, the fluoroscopic examination of the duodenal block 8 months after implantation of the cannulae revealed that the barium sulfate suspension introduced into the stomach passed down into the duodenum and the intestine, even when the balloon was inflated and was thought to be in the duodenum. The cannula had slipped out of the duodenum and was residing in the fistula formed between skin and the duodenum rather than in the lumen of the duodenum. This made it necessary to try another method of blocking the pylorus in a third monkey. A method was developed in which the foley catheter is pulled

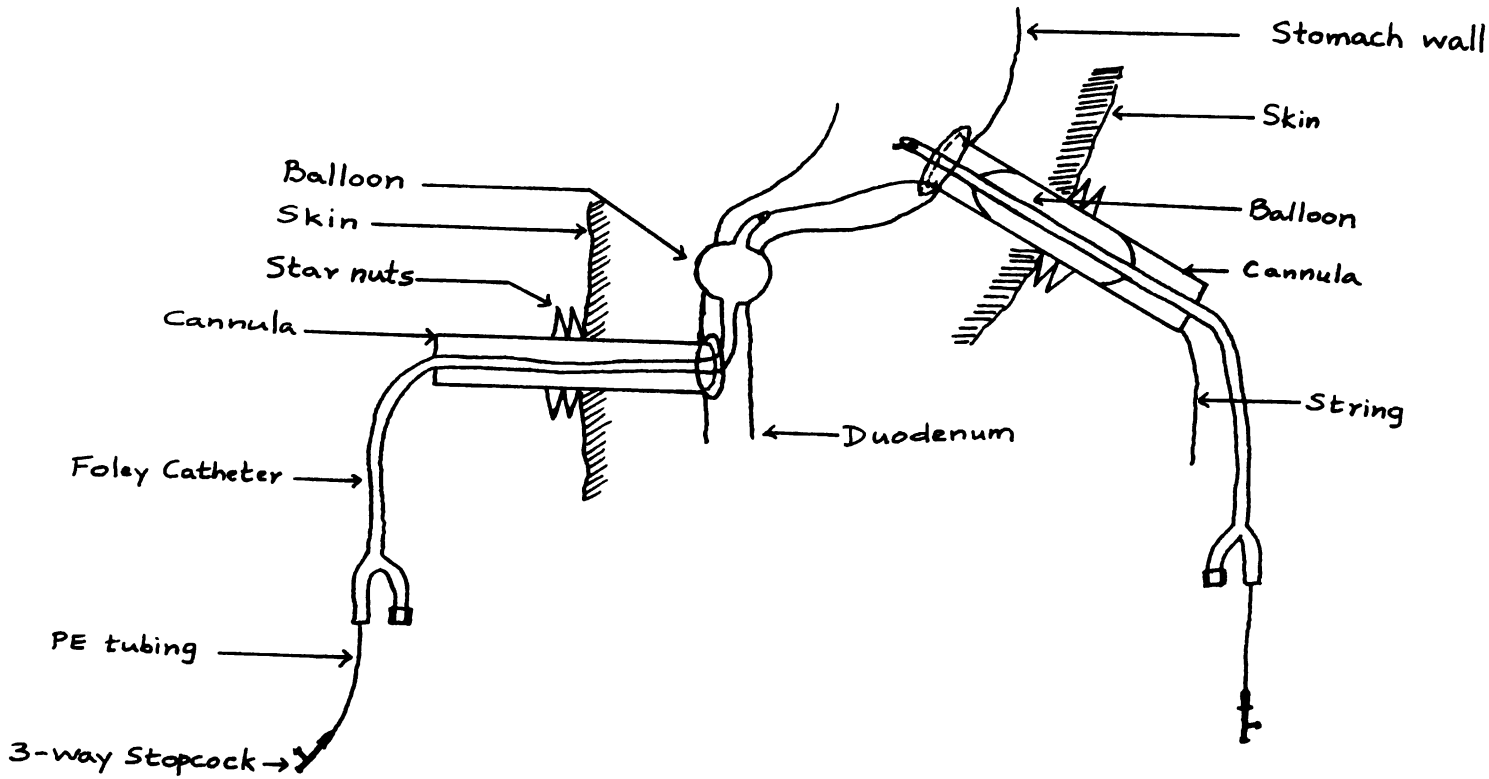


Fig. 2.--Schematic Representation of Positioning of Foley Catheters for Stomach Absorption Study.

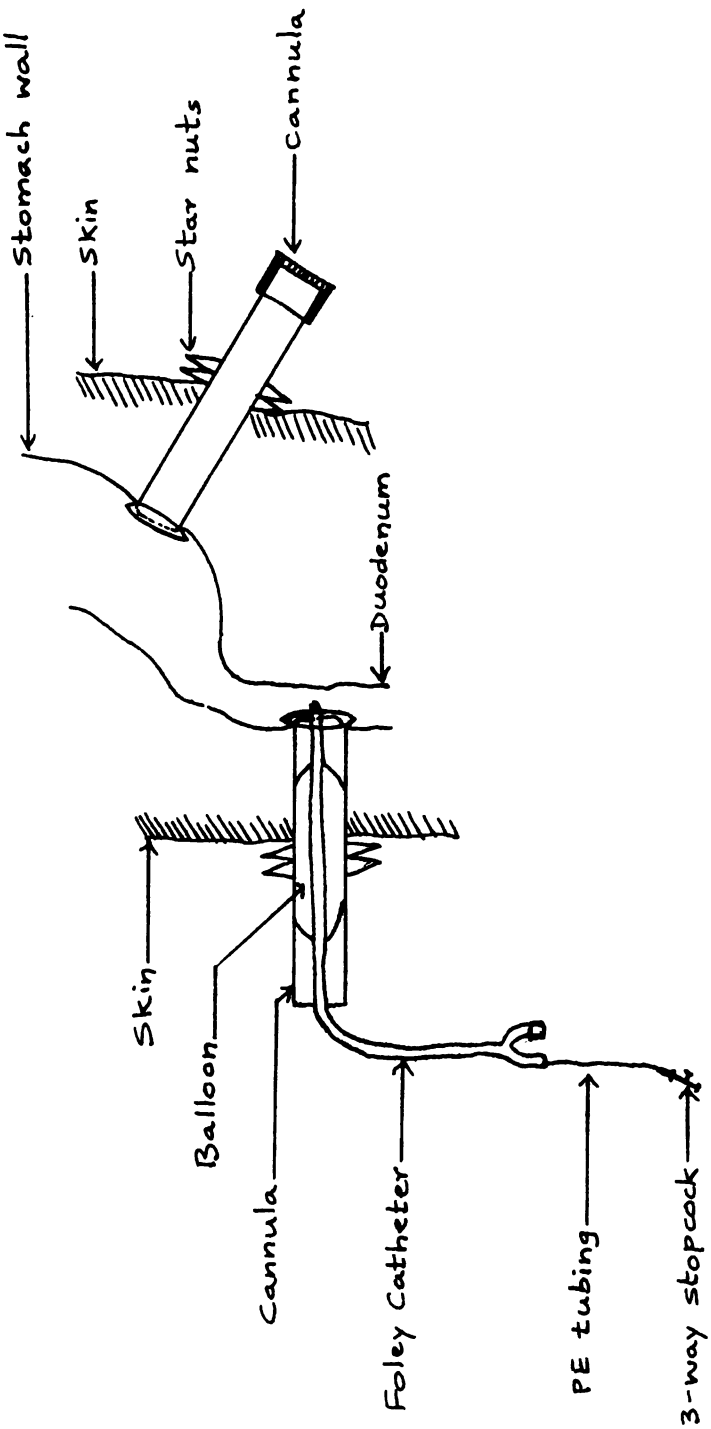


Fig. 3.--Schematic Representation of Positioning of Foley Catheters for Intestinal Absorption Study.

through the stomach cannula by means of the string attached to the duodenal insert. The duodenal cannula is closed and the balloon of the foley catheter inflated with distilled water thus blocking the pylorus. For stomach absorption studies, a drug solution was administered through a polyvinyl tube attached to the outside of the foley catheter with its tip on the stomach side of the balloon. For intestinal absorption studies, the drug solution was given through another polyvinyl tube going through the foley catheter with its tip on the intestinal side of the balloon. Figure 4 schematically shows the placement of the foley catheter for the alternate method of blocking the pylorus.

The number of studies that can be carried out on each monkey is only limited by the time necessary to allow the monkey to recover following an intensive blood sampling protocol. Blood sampling studies were normally carried out every 10 to 14 days and from 12-15 samples were taken during each study. Between these times, studies are run which only involve sampling of the duodenal or stomach contents.

Figure 5 is a photograph of the complete experimental model. Figure 6 is a photograph of the monkey abdomen with the stomach cannula on the right side and the duodenal cannula on the left side. Figure 7 is another photograph of the monkey abdomen and shows the cannulae opened with the foley catheters inserted into them.

2. Intravenous Salicylate Studies:

Salicylic acid 42 mg/kg was weighed out as sodium salicylate. It was dissolved in 2 ml of sterile normal saline

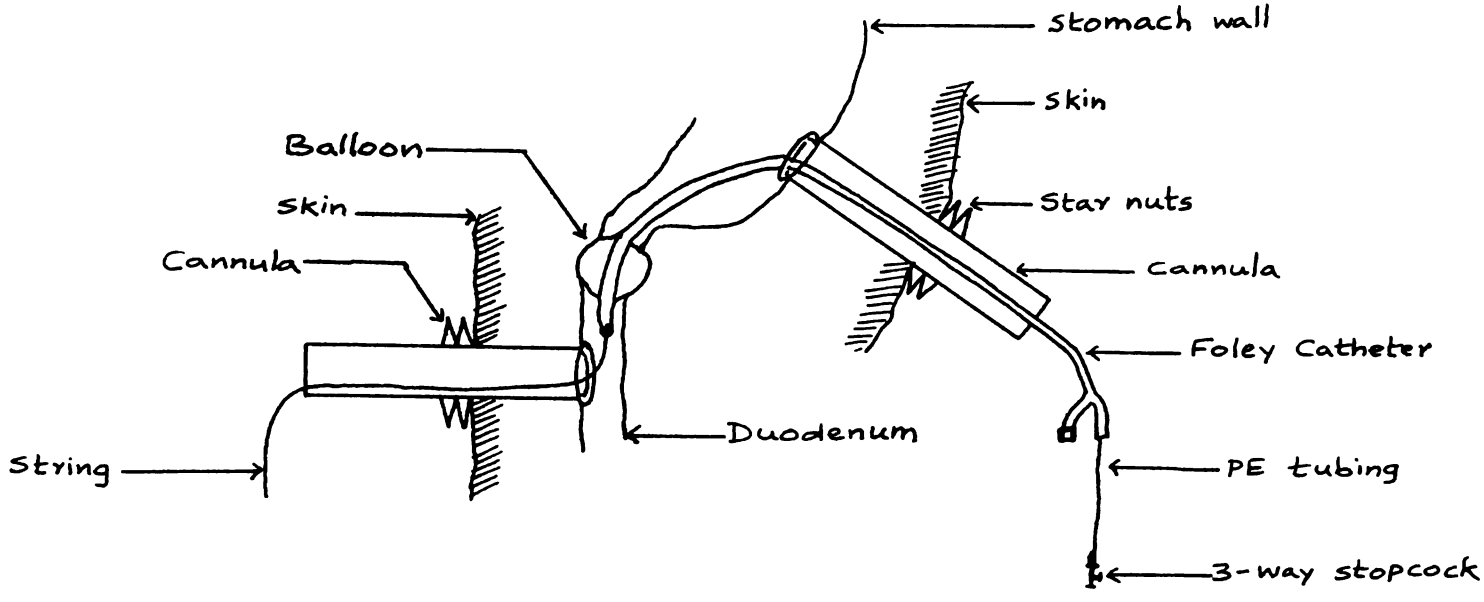


Fig. 4.--Schematic Representation of Positioning of Foley Catheters by the Alternate Method for Stomach Absorption Study.

Fig. 5.--Photograph of Complete Experimental Model.

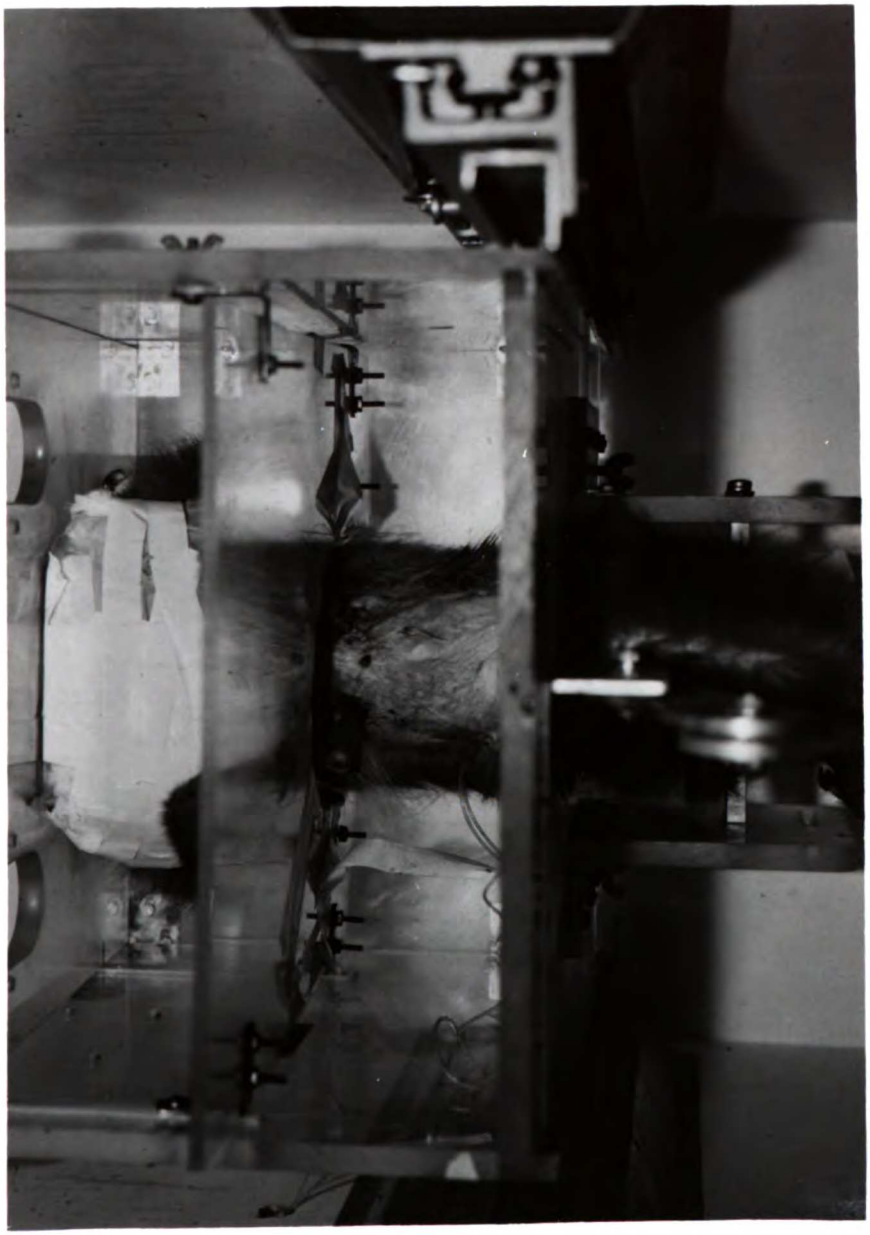


Fig. 6.--Photograph of Monkey Abdomen Showing Stomach and Duodenal Cannulae.

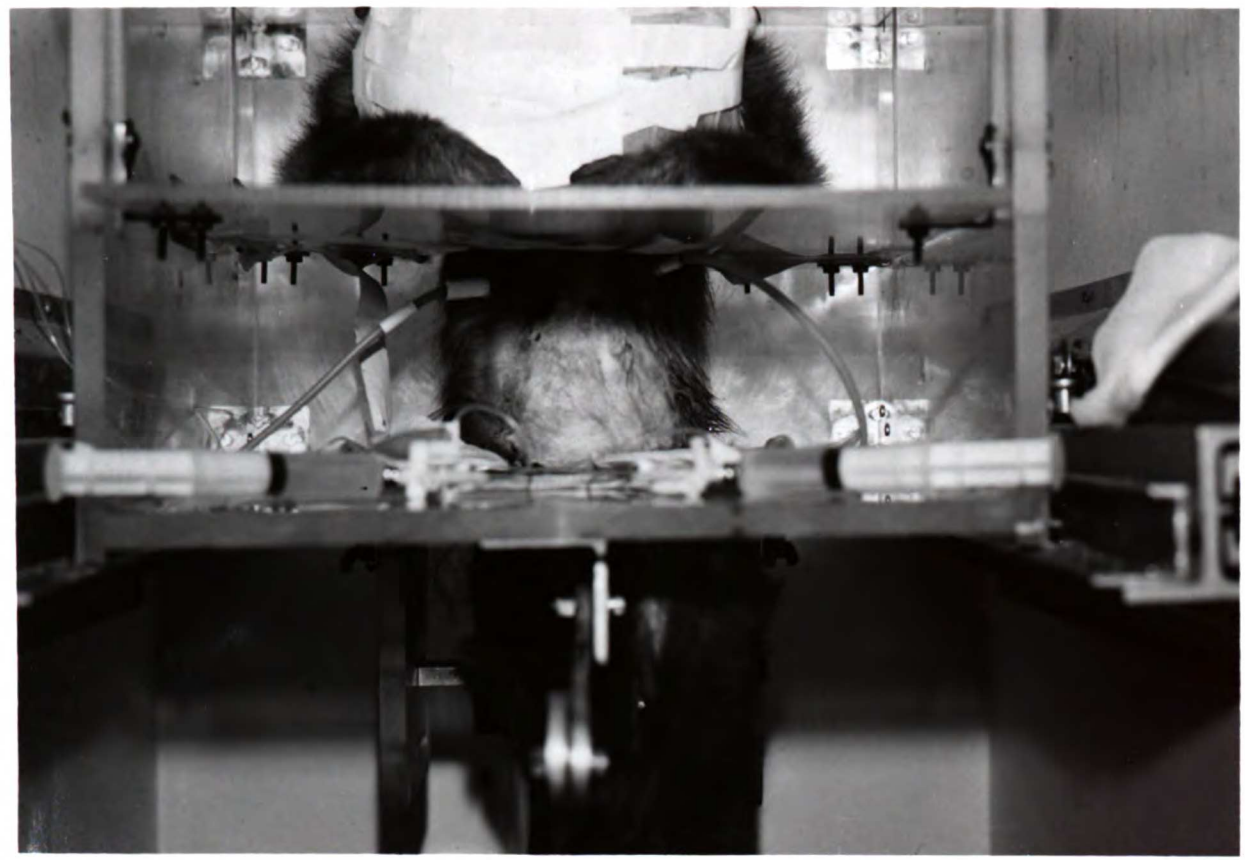


Fig. 7.--Photograph of Monkey Abdomen Showing Cannulae with Foley Catheters Inserted in them.

and administered over a one minute period through the venous catheter. After the infusion the catheter was flushed with 2 ml of sterile normal saline. In one of the monkeys, salicylate kinetics were also studied at 7 mg/kg, 14 mg/kg and 28 mg/kg doses to investigate dose-dependent elimination kinetics. Blood samples were withdrawn at different time intervals into heparinized tubes and centrifuged. Aliquots of plasma samples were used to assay salicylate concentrations.

3. Intravenous Antipyrine Studies:

Antipyrine 14 mg/kg was weighed out and dissolved in 2 ml of sterile normal saline. Solution of antipyrine N-methyl ^{14}C containing 22.93 μc of the ^{14}C isotope per ml of solution was prepared in sterile normal saline. One ml of the radioactive solution was added to the solution of cold antipyrine and the combined solution was administered over a one minute period through the venous catheter. The catheter was then flushed with 2 ml of sterile normal saline. Plasma samples were used for the liquid scintillation counting of the ^{14}C isotope.

4. Intravenous levo-Amphetamine HCl Studies:

A 5 mg/ml solution of levo-amphetamine HCl in sterile normal saline was prepared. An aliquot of this solution was used to give a 0.3 mg/kg dose of levo-amphetamine HCl. The aliquot was added to 2 ml of sterile normal saline and given intravenously as a one minute infusion through the catheter, followed by a 2 ml flush of sterile normal saline. Plasma samples were used for gas liquid chromatographic assay of levo-amphetamine.

5. Simultaneous Intravenous Administration of Salicylic Acid, Antipyrine and levo-Amphetamine HCl:

Salicylic acid (42 mg/kg), antipyrine (14 mg/kg) and levo-amphetamine HCl (0.3 mg/kg) were dissolved in 3 ml of sterile normal saline. One ml of radioactive antipyrine solution was added to the above solution and the 4 ml solution was administered over a two minute period through the venous catheter. The catheter was flushed with 2 ml of sterile normal saline. Blood samples were withdrawn at different time intervals and plasma was analyzed for salicylate, ^{14}C isotope and levo-amphetamine HCl.

6. Stomach and Intestinal Absorption Studies:

The solution was prepared in a manner similar to that used in the simultaneous intravenous administration study, with the exception that for stomach absorption studies only, a dose of 1 mg of levo-amphetamine HCl per kg body weight was used. According to the pH partition hypothesis, basic drugs are not readily absorbed from the stomach. Hence, a higher dose of levo-amphetamine HCl was used in the stomach absorption studies (as compared to the previously described I.V. studies) so that measurable plasma concentrations of the drug would be attained. The solution was instilled into the stomach or the duodenum through the foley catheter. The latter was flushed with 6 ml of distilled water. Blood samples were taken at different time intervals and plasma was used for assaying the three drugs by the methods described.

E. ASSAY METHODS

1. Assay of Salicylic Acid in Plasma:

Salicylate in plasma was assayed by the method of Rowland

and Riegelman (148). Plasma samples of 0.05-0.5 ml were pipetted into a centrifuge tube containing 0.5 ml of 5% potassium bisulfate solution in distilled water. Seven mls of ether was added to the tube and vortexed for about 2 minutes. Potassium bisulfate acts as an acidifying agent and adjusts the pH to 2 where extraction of salicylic acid into ether is quantitative. The tubes were centrifuged for about 20 minutes. One ml aliquots of the ethereal extract were added to 5 mls of 0.1M sodium phosphate buffer pH 7.0. The tubes were again vortexed for about 2 minutes and centrifuged for 20 minutes. Excess ether was removed by suction and nitrogen gas was blown through the phosphate buffer for 30 seconds to remove the dissolved ether, which, if not removed, modified the spectrophotofluorometric readings. The solutions were read on a spectrophotofluorometer. (Perkin Elmer Fluorescence Spectrophotometer, model 203). Fluorescence measurements of standard solutions of salicylic acid in sodium phosphate buffer (0.1-1.0 mcg/ml) were made at the same time. The sample concentrations were calculated from the standard curve by means of a CPS computer program executed through a remote terminal.

2. Assay of ^{14}C Isotope in Plasma:

Antipyrine and its metabolites were assayed in the plasma by liquid scintillation counting of the ^{14}C isotope. A 0.2 ml sample of plasma was pipetted into a polyethylene counting vial. Ten mls of Aquasol^{R} (Aquasol^{R}, Universal Liquid Scintillation Counting Cocktail, New England Nuclear, Boston, Massachusetts 02118) was added to each vial. The vials were shaken vigorously and then equilibrated at a low temperature in a refrigerator. Each vial was counted twice, for a period of 10 minutes each time, on a Packard Liquid Scintillation Counter. (Packard Tri Carb

Liquid Scintillation Spectrometer, model 3375). Quenching was corrected for by the method of internal standardization. To each vial, 50 µl of ^{14}C toluene internal standard (417000 dpm/ml) was added and the vials counted again with the same settings on the spectrometer as previously used. The counts per minute (cpm) due to the addition of the internal standard was divided by the stated disintegrations per minute (dpm) in a 50 µl aliquot to give the counting efficiency ratio for each sample. The respective ratios were used to convert cpm values of the samples to the dpm values. The efficiency of counting was usually 75-80%.

3. Assay of levo-Amphetamine HCl in Plasma:

Plasma concentrations of levo-amphetamine HCl were determined by the method of Rowland (149). In principle, the method involves extracting amphetamine from plasma into hexanes with sodium hydroxide. Amphetamine is again converted to its hydrochloride and the aqueous layer re-extracted with sodium hydroxide and hexanes. An aliquot of the hexane phase was used for injection into the chromatograph.

Preparation of standard curve: 0.2 ml of blank plasma was pipetted into culture tubes. To each tube was added 0.1 ml of the internal standard solution (aqueous solution of α-methylbenzylamine and β-phenethylamine as hydrochlorides at concentrations of about 150 ng/ml and 220 ng/ml respectively), 0.1 ml of standard solution of different concentrations, 8 ml of hexanes and 1 ml of 2 N sodium hydroxide. The tubes were capped, shaken for about 30 minutes and centrifuged. Most of the hexane layer (always >6 ml) was then removed and transferred into another set of special culture tubes which had their ends drawn into narrow tubes. A 0.15 ml portion

of 1N HCl was added to each tube. The tubes were shaken again for about an hour and centrifuged. The hexane layer was removed as completely as possible except for about 0.1 ml and discarded. To each tube, 0.15 ml of 2 N NaOH was added and the tubes were vortexed for about 2 minutes. A freshly prepared solution, 0.1 ml, of pentafluorobenzoyl chloride (20 μl of pentafluorobenzoyl chloride in 10 ml of hexanes) was added to each tube and the tubes left to stand for about 10 minutes. The amphetamine and internal standards in the samples were thus converted to their pentafluorobenzoyl derivatives. The tubes were vortexed for a minute each and centrifuged. The lower aqueous layer from each tube was aspirated and discarded. One to two μl of the hexane layer was used for injection into the chromatograph. The instrument, materials and the conditions of the gas liquid chromatographic assay are described below:

Instrument:	Varian Aerograph 1200 series
Column:	10' glass, 1/8" O.D. (Varian) 6' glass, 1/8" O.D. (Varian)
Column packing:	3% OV-17 on chromosorb W (AW-DMCS H.P.), 100-120 mesh
Gas:	5% CH_4, 95% Argon
Flow Rate:	40 ml/min at R.T. (corresponding to about 20 ml/min at operating column temperature)
Injection port temperature:	215°C
Column temperature:	190°C
Detector temperature:	215°C
Detector:	Tritium foil electron capture
Range:	10^{-10} amp/mv
Attenuation:	32

On the chromatograph, the peak due to amphetamine appeared between those of α-methylbenzylamine and β-phenethylamine. The ratio of peak heights of amphetamine to α-methylbenzylamine was calculated and the ratios plotted against the amount of levo-amphetamine HCl added to each tube, to give the standard curve.

The samples were assayed in a manner similar to that used

in preparing the standard curve. The peak height ratios were calculated and read from the standard curve to give the concentration of levo-amphetamine HCl in each sample.

III. RESULTS

A. INTRAVENOUS STUDIES

1. Salicylic Acid:

Salicylic acid at a dosage of 42 mg/kg was given either alone or simultaneously with the other two drugs, antipyrine and levo-amphetamine HCl. In monkey A, the dose dependent elimination kinetics of salicylic acid was studied by giving the drug at 3 lower dosage levels, i.e., 7 mg/kg, 14 mg/kg and 28 mg/kg. A sample calculation for the salicylate assay in plasma is given in Table A-1 of Appendix A. The data was plotted on semilogarithmic paper. Two sample plots, one obtained from the results when salicylic acid was administered at a low dose, 7 mg/kg, and another obtained from the results when salicylic acid was administered at a high dose, 42 mg/kg, are shown in Figures B-1 and B-2 of Appendix B. At low dosage levels it appears that salicylic acid is eliminated with a biologic half-life of about 40 minutes after an initial distribution phase. A different trend was observed in salicylic acid elimination when the drug was given at higher dosage levels. The initial disposition phase was followed by a rather slow decaying phase with a half-life of about 85-100 minutes. When the plasma salicylate concentration had fallen to about 30 mcg/ml, the elimination became faster approaching the same rate as was observed with the low dose studies. It is known that the formation of salicyluric acid, one of the metabolites of salicylic acid, follows Michaelis-Menten kinetics in man (150). The formation of salicyluric acid from salicylic acid reaches a maximal rate in man when the body content of the salicylate exceeds about

2 mmoles (approximately 300 mg salicylic acid). Salicylic acid when administered in high doses, exhibits simultaneous first and zero order elimination kinetics, since the enzyme system for the formation of salicyluric acid is saturated when a large amount of salicylic acid is given. The half-life of salicylic acid in man varies from 3-20 hours depending on the dose administered (151). Cummings et al. (152,153) have reported that the serum salicylate concentration profile in man closely approaches a simple linear rate of decline when the concentration exceeds 0.3 mM which corresponds to about 30 mcg of salicylate per ml of serum. They suggested that the rate of elimination of salicylic acid becomes predominantly zero order when the rate of formation of salicyluric acid approaches a maximum.

The results obtained from intravenous salicylic acid administration in monkeys A and B, are given in Table I. K_{slow} is the exponential elimination rate constant when the plasma concentration of salicylic acid is 30 mcg/ml or less. (See Figures B-1 and B-2 for K_{slow} designation). The increases in the areas under the plasma concentration versus time curves (A.U.C.) do not correspond proportionately to the increase in the dosage levels, which is characteristic of dose dependent kinetics.

The values obtained for the slow rate constant and A.U.C. when salicylic acid was given alone are not significantly different from the corresponding values obtained when salicylic acid was given simultaneously with antipyrine and levo-amphetamine HCl.

2. Antipyrine:

Antipyrine at a dosage of 14 mg/kg together with 1 ml of radioactive antipyrine solution containing 22.93 μc of ^{14}C

Table I

Intravenous Salicylic Acid Results in Monkeys A and B.

Administration of	Dose	Monkey	K_{slow} hr^{-1}	A.U.C. mcg hr/ml
1. Salicylic Acid alone.	7 mg/kg	A	1.6	40.0
	14 mg/kg	A	1.0	69.0
		A	1.1	71.0
	28 mg/kg	A	1.0	278.0
	42 mg/kg	A	1.20	324.0
		B	1.30	394.0
2. Salicylic Acid with Antipyrine and levo-Amphetamine HCl.	42 mg/kg	A	1.0	383.0
		B	1.3	335.0

isotope was given either alone or simultaneously with salicylic acid and levo-amphetamine HCl. The plasma radioactivity exhibited a triexponential decline when plotted on semilogarithmic paper. The sample calculations for the assay of ^{14}C antipyrine and its metabolites are given in Table A-2 of Appendix A. A sample plot of plasma radioactivity versus time is shown in Figure B-3 of Appendix B. Table II summarizes the results of intravenous antipyrine studies in monkeys A and B. In all of these studies, the terminal exponential phase of the plasma radioactivity versus time plots had a half-life of 3.5-4.5 hours. Welch et al. (133) analyzed plasma antipyrine by the method of Brodie and Axelrod (129) and found the biologic half-life of antipyrine in monkeys to be 109± 18 minutes whereas a more recent study (135) using the same method of analysis, reports a half-life of 70 minutes for antipyrine in monkey plasma. Our values for the half-life are significantly different from those reported by the above workers since our values represent decay half-life of total radioactivity in plasma rather than the elimination half-life of unchanged antipyrine. In both monkeys, a slight increase in the elimination rate constant is observed when antipyrine is combined with the other two drugs as compared with administration of antipyrine by itself.

3. levo-Amphetamine HCl:

levo-Amphetamine HCl by itself or in combination with salicylic acid and antipyrine was administered intravenously at a dosage of 0.3 mg/kg. The sample calculations for the levo-amphetamine HCl assay in plasma is given in Table A-3 of Appendix A. Figure B-4 of Appendix B is a semilogarithmic plot of the

Table II

Intravenous Antipyrine Results in Monkeys A and B.

Administration of	Dose	Monkey	K_{slow} hr^{-1}	A.U.C. dpm hr/0.2 ml
Antipyrine alone	14 mg/kg	A	0.15	12000
		B	0.17	12300
Antipyrine with Salicylic Acid and levo-Amphetamine HCl	14 mg/kg	A	0.17	12000
		B	0.19	12700

plasma concentration of levo-amphetamine HCl versus time, drawn from the results of one of the intravenous administration experiments. A biexponential decline of plasma concentration was observed after intravenous administration of levo-amphetamine HCl. The results of all intravenous studies in both monkeys are given in Table III. The half-life of the terminal log-linear phase varied from 10-12 hours which could be due to variations in the pH of urine as reported earlier (143,144).

B. GASTROINTESTINAL ABSORPTION STUDIES

Solutions of single drugs or combinations of two or three drugs were administered directly into the stomach or into the duodenum by using the foley catheters and the experimental procedures as described in Methods. Sample plots of the plasma concentration of salicylic acid versus time obtained from the results of a stomach absorption study and a duodeno-intestinal absorption study are shown in Figures B-5 and B-6 respectively, in Appendix B. The corresponding plots for antipyrine are shown in Figures B-7 and B-8 and for levo-amphetamine HCl in Figures B-9 and B-10 in Appendix B. Table IV contains the results obtained for gastrointestinal absorption of salicylic acid in monkey A. Values reported earlier for intravenous studies, are included for comparison with the corresponding values obtained from the results of the g.i. absorption studies. The peak concentrations reached in both the stomach and the intestinal absorption studies are almost identical. However, the time when the peak concentration is attained is significantly different when a comparison is made between the stomach and the intestinal absorption experiments.

Table III

Intravenous levo-Amphetamine HCl Results in Monkeys A and B.

Administration of	Dose	Monkey	K_{slow} hr^{-1}	A.U.C. ng hr/ml
levo-Amphetamine HCl alone	0.3 mg/kg	A	0.06	1160
		B	0.07	1110
levo-Amphetamine HCl with Salicylic Acid and Antipyrine	0.3 mg/kg	A	0.07	950
		B	0.06	1080

Table IV

Salicylic Acid Data in Monkey A.

Administration of	Peak Conc. mcg/ml	Peak Time min	K_{abs} hr^{-1}	K_{slow} hr^{-1}	A.U.C. mcg hr/ml
1. Salicylic Acid in stomach with duodenal block					
a) given with Antipyrine	111	90	1.20	0.920	420
b) Given with Antipyrine and levo-Amphetamine HCl	113	75	1.26	0.920	330
2. Salicylic Acid in duodenum					
a) given with Antipyrine and levo-Amphetamine HCl	124	30	4.60	0.760	320
3. Salicylic Acid intravenously					
a) given alone 42 mg/kg				1.20	320
b) given with Antipyrine and levo-Amphetamine HCl				0.970	380

An early peak in plasma salicylate concentration is observed in the intestinal absorption study. The occurrence of an early peak together with a 4 times greater rate constant for absorption from the intestine as opposed to the absorption from the stomach indicates that salicylic acid is more quickly absorbed from the intestine even though the drug exists almost completely as the ionized form at the pH of the fluid medium in the intestine.

The results obtained for antipyrine in the g.i. absorption studies in monkey A are given in Table V. The peak concentration of radioactivity in plasma is greater in the intestinal absorption study as compared to the peak concentration observed in the stomach absorption study. As was seen with salicylic acid, the peak time in the plasma radioactivity versus time plot was significantly shorter when the drug was instilled into the duodenum as opposed to when the drug was introduced into the stomach. This indicates that antipyrine is absorbed faster from the intestine than from the stomach. A 4 times greater rate constant of absorption was found in the absorption of antipyrine from the intestine as compared to the absorption of the drug from the stomach. The small intestine would thus appear to be the better site for the absorption of this nonionized drug. The slow rate constant of elimination for antipyrine and its metabolites is greater in the g.i. absorption studies than in the intravenous administration experiments.

Table VI contains the data obtained for levo-amphetamine HCl from studies carried out in monkey A. Again, the absorption of l-amphetamine HCl appears to occur faster in the duodenal studies as seen from the occurrence of an early peak in the plasma

Table V

Antipyrine Data in Monkey A.

Administration of	Peak Conc. dpm/0.2 ml	Peak Time min	K_{abs} hr^{-1}	K_{slow} hr^{-1}	A.U.C. dpm hr/0.2 ml
1. Antipyrine in stomach with duodenal block					
a) given with Salicylic Acid	2280	75	2.10	0.20	13300
b) given with Salicylic Acid and levo-Amphetamine HCl	2500	90	1.70	0.18	14200
2. Antipyrine in duodenum					
a) given with Salicylic Acid and levo-Amphetamine HCl	2940	30	8.30	0.29	11700
3. Antipyrine intravenously					
a) given alone				0.15	12000
b) given with Salicylic Acid and levo-Amphetamine HCl				0.17	12000

Table VI

levo-Amphetamine HCl Data in Monkey A.

Administration of	Peak Conc. ng/ml	Peak Time min	K_{abs} hr^{-1}	K_{slow} hr^{-1}	A.U.C. ng hr/ml
1. levo-Amphetamine HCl in stomach with duodenal block a) given with Salicylic Acid and Antipyrine	90	90	1.19	0.09	1100
2. levo-Amphetamine HCl in duodenum a) given with Salicylic Acid and Antipyrine	125	30	8.32	0.09	1200
3. levo-Amphetamine HCl intravenously a) given alone				0.06	1200
b) given with Salicylic Acid and Antipyrine				0.07	950

concentration of the drug and a faster rate constant for absorption.

The results obtained for salicylic acid from experiments done in monkey B are given in Table VII. The results obtained in the stomach absorption studies are comparable to the result of similar studies in monkey A. However, unlike the results in monkey A, the intestinal absorption experiments seem to be different. There is no significant difference between absorption from the stomach and the absorption from the intestine when comparison is made of the peak time and the absorption rate constant values observed in these experiments. In this animal the fluoroscopic examination of the duodenal block 8 months after the implantation of the cannulae revealed that the block was ineffective. This observation still does not explain the slower absorption of salicylic acid from the intestine. When salicylic acid was administered by itself in the stomach without applying the duodenal block, a very rapid absorption was observed, significantly different from the other stomach absorption studies done with the duodenal block. This would indicate the effectiveness of the inflated balloon in preventing the stomach contents from going down into the intestine, which is contrary to the observation of the fluoroscopic examination. It may be speculated that the duodenal cannula might have slipped out of the duodenum and the results obtained with the intestinal absorption studies might be due to peritoneal absorption of salicylic acid. Although no differences were seen between the absorption of salicylic acid from the stomach and from the intestine in this monkey, a significant difference in antipyrine absorption was seen when this drug was given simultaneously with

Table VII

Salicylic Acid Data in Monkey B.

Administration of	Peak Conc. mcg/ml	Peak time min	K_{abs} hr^{-1}	K_{slow} hr^{-1}	A.U.C. mcg hr/ml
1. Salicylic Acid in stomach with duodenal block					
a) given with Antipyrine	106	90	1.39	1.04	339
b) given with Antipyrine and levo-Amphetamine HCl	116	75	1.19	0.83	399
2. Salicylic Acid in duodenum					
a) given with Antipyrine	95	75	1.48	1.19	349
b) given with Antipyrine and levo-Amphetamine HCl	83	75	1.04	0.83	324
3. Salicylic Acid alone in stomach without duodenal block	124	30	8.32	0.97	299
4. Salicylic Acid intravenously					
a) given alone				1.30	394
b) given with Antipyrine and levo-Amphetamine HCl				1.30	335

salicylic acid. The results of such studies are given in Table VIII allowing comparison of the absorption of salicylic acid and antipyrine from the stomach and the duodenum. The data for antipyrine derived from the experiments in monkey B is given in Table IX. As was observed in monkey A, a higher peak concentration, an earlier peak time and a greater rate constant for absorption was observed in intestinal studies as compared to the stomach absorption studies. Table X includes the data for levo-amphetamine HCl derived from experiments in monkey B. The peak concentration of levo-amphetamine HCl attained in the plasma is similar in both the gastric and the intestinal experiments. However, the absorption of this drug occurs faster from the intestine as indicated by the appearance of the earlier peak. The rate constant of absorption is also greater for the intestinal absorption of levo-amphetamine HCl when compared with the value of this rate constant in the stomach study.

The discrepancies observed in the salicylic acid data in monkey B made it necessary to study the absorption of the three drugs from the stomach of another monkey. An alternate technique for blocking the pylorus, as explained earlier, was used in monkey C for the gastric absorption experiment. The results of the experiments carried out in this monkey are given in Table XI. Only two experiments were carried out in this monkey. In one experiment, the three drugs were simltaneously administered intravenously. In the other experiment, the three drugs were introduced together into the stomach using the alternate method of blocking the pylorus. The peak levels achieved for all three drugs in the stomach study are much lower than the corresponding values in the other two mon-

Table VIII

Data for Simultaneous Gastrointestinal Administration of Salicylic Acid and Antipyrine in Monkey B.

Administration of Salicylic Acid and Antipyrine	Peak Time for Antipyrine, min	K_{abs} hr^{-1} for Antipyrine	Peak Time for Salicylic Acid min	K_{abs} hr^{-1} for Salicylic Acid
a) in stomach with duodenal block	75	1.73	90	1.39
b) in stomach with duodenal block and with levo-Amphetamine HCl	75	1.66	75	1.19
c) in duodenum	15	13.9	75	1.48
d) in duodenum with levo-Amphetamine HCl	30	4.16	75	1.04

Table IX

Antipyrine Data in Monkey B.

Administration of	Peak Conc. dpm/0.2 ml	Peak Time min	K_{abs} hr^{-1}	K_{slow} hr^{-1}	A.U.C. dpm hr/0.2 ml
1. Antipyrine in stomach with duodenal block					
a) given with Salicylic Acid	2240	75	1.73	0.28	10300
b) given with Salicylic Acid and levo-Amphetamine HCl	2180	75	1.66	0.22	12000
2. Antipyrine alone in stomach without duodenal block	2650	30	8.31	0.25	12200
3. Antipyrine in duodenum					
a) given with Salicylic Acid	2880	15	13.80	0.22	11900
b) given with Salicylic Acid and levo-Amphetamine HCl	2750	30	4.16	0.22	13900
4. Antipyrine intravenously					
a) given alone				0.17	12300
b) given with Salicylic Acid and levo-Amphetamine HCl				0.19	12700

Table X

levo-Amphetamine HCl Data in Monkey B.

Administration of	Peak Conc. ng/ml	Peak Time min	K_{abs} hr^{-1}	K_{slow} hr^{-1}	A.U.C. ng hr/ml
1. levo-Amphetamine HCl in stomach with duodenal block					
a) given with Salicylic Acid and Antipyrine	81	90	1.10	0.08	950
2. levo-Amphetamine HCl in duodenum					
a) given with Salicylic Acid and Antipyrine	73	45	1.81	0.10	850
3. levo-Amphetamine HCl intravenously					
a) given alone				0.07	1100
b) given with Salicylic Acid and Antipyrine				0.06	1100

Table XI

Data of Experiments Carried Out in Monkey C.

Administration of	Peak Conc.	Peak Time min	K_{abs} hr^{-1}	K_{slow} hr^{-1}	A.U.C.
1. Salicylic Acid, Antipyrine and levo-Amphetamine HCl in stomach with duodenal block					
a). Salicylic Acid	80 mcg/ml	60	1.34	1.26	240 mcg hr/ml
b). Antipyrine	1300 dpm/0.2 ml	60	6.90	0.20	6000 dpm hr/0.2 ml
c). levo-Amphetamine HCl	40 mg/ml	75	1.50	0.10	400 ng hr/ml
2. Salicylic Acid, Antipyrine and levo-Amphetamine HCl intravenously					
a). Salicylic Acid				1.16	300 mcg hr/ml
b). Antipyrine				0.18	9100 dpm hr/0.2 ml
c). levo-Amphetamine HCl			Not assayed		

keys. The peak times, however, compare reasonably well with the respective values obtained in the other two animals. The values for the slow rate constants for the three drugs are also in agreement with those observed in monkeys A and B. However, the extent of absorption of all the drugs was lower in this monkey as is indicated by the comparison of the A.U.C. values. This may be the reason for the lower peak levels attained in the plasma in monkey C.

IV. DISCUSSION

A. MODEL

The experience gained in using the present experimental model has led to the following suggestions for improvement both in the cannulae and the primate chair. On a couple of occasions the intestinal cannula has been dislodged and this may be attributed to the design of the flange of the cannula remaining inside the duodenum. In addition, the length of the cannula used for implantation in the duodenum has been found to be too short. Due to the retroperitoneal position of the duodenum, there is a constant tension exerted on the cannula by the two star nuts in contact with the skin. It is suggested that the duodenal cannula be made at least 1.5 times longer than the present cannula. Additional security can be provided by constructing another flange about 0.4 cm away from the terminal flange which remains inside the lumen of the duodenum. This additional flange would remain on the outside of the duodenal wall. If this flange is made with small holes along its periphery, sutures could be passed through the holes and the duodenal wall thus providing more strength to the whole surgical preparation.

Alterations suggested for making the chair more comfortable for the monkey include elimination of as many nuts and bolts as possible. These have very frequently been the cause of sores and ulcers on the arms, legs and the neck. The other change suggested is the removal of the front half of the top plate which is useless except for some specialized experiments requiring the restriction of the monkey's hands from touching its head. The

elimination of this part of the top panel will result in much more freedom for the monkey, especially when it is feeding itself.

The usefulness of the monkey model described in this work can be extended further to study other biopharmaceutical and metabolic factors that can affect the bioavailability of drugs. For example, it is possible to introduce another cannula at the ileo-cecal junction. With this modification, one can study the effect of specific sites on the extent of drug absorption or the effect of residence time in the g.i. tract on the bioavailability of a drug. The latter may be studied by inflating balloons at the two ends of the intestine to maintain drug particles or drug solution in the intestinal lumen for extended periods of time. The model can also be used for a comparative evaluation of sustained release preparations if the dosage forms are kept in the intestine for identical periods of time by blocking the distal end of intestine. In the absence of this block, different preparations would be in contact with the absorbing surface for variable time periods due to changes in the motility of the g.i. tract.

Catheterization of the hepatic portal vein would enable an investigator to sample the absorbed drug before it reaches the liver, thus allowing measurement of the extent of g.i. degradation and intestinal metabolism of a drug. The hepatic portal venous catheter could be used to isolate the first pass effect of drug metabolism from intestinal degradation processes.

B. RESULTS

As a result of 13 gastric absorption studies and 10 intestinal absorption studies in 3 monkeys, the following generalization of the data was made.

In all stomach absorption studies the rate constant for absorption of antipyrine was greater than the corresponding values for salicylic acid and amphetamine. The rate constant for absorption of amphetamine from the stomach was very nearly equal to the rate constant for absorption of salicylic acid from the stomach.

The intestinal absorption of antipyrine occurred at a faster rate than the intestinal absorption of salicylic acid and amphetamine. The intestinal absorption of antipyrine was faster than its absorption from the stomach. The intestinal absorption of amphetamine was faster than the absorption of the same drug from the stomach.

In monkey A, intestinal salicylic acid absorption was faster than the absorption from the stomach. In monkey B, salicylic acid seemed to be absorbed at nearly the same rate from both the stomach and the intestine. Except for the apparent anomalous salicylic acid data in monkey B, which will be discussed later, these results can be interpreted in terms of pH partition hypothesis.

It has been suggested by Brodie and co-workers (54-58) that the intestinal absorption of drugs which are weak acids and bases can be explained by the pH partition hypothesis, a theory of nonionic diffusion. This theory has been applied often to the movement of various substances across the stomach and the

intestine, and the concept has wide application. The primary principle of the theory is that the nonionized form of a drug is the predominant form absorbed and that ionized drug molecules are only slightly transported. Because a number of discrepancies were found when the intestinal transport of weakly acidic drugs such as salicylic acid (pKa 3.0) were measured, Hogben et al. (58) proposed that a zone or microclimate with a "virtual" pH of 5.3 existed at the site of absorption. The pH of this environment was hypothesized as the intestinal pH influencing the partition of ionizable drugs. The virtual pH hypothesis was necessitated by the fact that Hogben, Schanker, Brodie and co-workers had concluded that drug ions could not be transported at a significant rate through the intestinal membrane. However, one of these authors, in a recent paper (85) on the absorption of organic anions from the rat small intestine, has suggested that these anions are absorbed mainly by simple diffusion through lipoidal regions of the intestinal boundary.

The virtual pH hypothesis has been previously criticized by Barry et al. (154) for the particular case of propionate, and by Benet (155), as discussed by Wagner (17), who presented three general arguments to question the validity of the virtual pH hypothesis as an explanation for the intestinal absorption of ionizable drugs. Benet (155) points out the inability of various workers to demonstrate a pH 5.3 phase as an unbounded microclimate adjacent to the mucosal surface of the gut. He questions the possibility of a membrane bounded virtual pH phase since interposition of a pH 5.3 microclimate between two bulk solutions that are in equilibrium should have no effect in changing the con-

centration of unionized drug next to the absorption surface. He also points out the inconsistencies found in comparing the thermodynamically and kinetically obtained ratios of permeability coefficients. Most recently, Smolen (156) has presented an excellent theoretical treatment which challenges the pH partition and virtual pH hypothesis on the basis of thermodynamic principles. Due to different pH environments in the stomach and the intestine, a basic drug should exist largely as the ionized form in the stomach whereas an acidic drug would be predominantly in the ionized form in the intestine. The degree of ionization can be calculated by using the Henderson-Hasselbalch equation which relates the acid dissociation constant (pKa) and the pH of the fluid medium to the ratio of the activities of the ionized to nonionized moities. For example, at a pH of 1 in the stomach, the ratio of nonionized to ionized salicylic acid (pKa 3.0) molecules is 100:1 whereas amphetamine (pKa 9.7) under the same conditions is more than 99.99% ionized. On the other hand, an intestinal pH of 6.6 causes salicylic acid to exist largely in the ionized form, the ratio of the ionized to nonionized molecules being 4000:1 whereas the increased intestinal pH increases the fraction of amphetamine molecules that exist in the nonionized form (even though the ionized to nonionized ratio is still greater than 1000:1).

Doluisio *et al.* (82) have shown by *in situ* experiments in rats that when the initial pH of stomach fluids is changed from 3.0 to 6.0, the absorption rates for acids decrease while the absorption rates for bases increase as was observed ten years earlier by Schanker and co-workers (55). When gastric and intestinal

absorption is compared at the same pH (i.e., 6.0), the intestinal first order rates are from 5 to 15 times higher than the gastric absorption rates. However, the most interesting aspect of the data is the comparison of gastric absorption rates at pH 3.0 and intestinal rates at pH 6.0. Taking salicylic acid as an example, the absorption rate from the intestine where the drug is more than 99% ionized is still 5.7 times greater than the absorption rate from the stomach where the drug is only 50% ionized. It is interesting to note that Schanker and co-workers actually observed the same phenomena when data from the entire series of papers is compared. Schanker et al. (55) report that 60% of salicylic acid is absorbed from the stomach in one hour when 1 mg of drug in 5 ml of 0.1 N HCl is placed in the in situ stomach of rats. (This corresponds to about a 1.5 mM solution). In a following paper (56), it is pointed out that 60% of salicylic acid is absorbed from the rat intestine when a 1mM solution of drug in a pH 7.2 weak buffer is perfused through the intestine at a rate of 1.5 ml/minute. (This solution measures pH 6.6 on leaving the intestine). In a later report (60), it is stated that the residence time of the drug in the earlier study (56) is only 7 minutes. Thus when one compares this data, the salicylic acid is absorbed about 8 times faster from the intestine where the drug is essentially completely in the ionized state as opposed to the stomach where the drug is approximately 99% nonionized.

As would be predicted by the pH partition hypothesis, the absorption of nonionized antipyrine in the present work always occurred faster than ionizable salicylic acid and amphetamine

whether these comparisons are made in the stomach or in the intestine. These results are consistent with the greater lipid solubility of the nonionized molecules as compared to that of the ionized molecules and hence their easier penetration of lipoidal biological membranes to reach the vascular bed. The absorption of the basic drug, amphetamine also followed the prediction of the pH partition hypothesis in that the rate of absorption increased in going from the stomach to the intestine with the increase in the fraction of nonionized molecules. The results of this work, however, are in disagreement with the pH partition hypothesis when the absorption of salicylic acid from the intestine is considered. Since, in this case, salicylate absorption increases in going from the stomach to the intestine when the fraction of nonionized drug molecules decreases. Amphetamine which is more than 99.99% ionized in the stomach at a pH of 1 and salicylic acid which remains about 99% in the nonionized state are absorbed at very nearly the same rate from the stomach. This seems to indicate that the degree of ionization may not be the rate limiting factor in the absorption of these two drugs from the stomach. An alternate explanation with respect to gastric blood flow limitations will be presented subsequently.

Absorption of salicylic acid occurred about 4 times faster from the intestine than the stomach in monkey A, although the drug exists largely in the ionized state at the higher pH values of the intestine. As described before, Schanker et al. (55) did observe faster absorption of salicylic acid from the intestine as compared to the stomach which they attempted to explain by

proposing the virtual pH hypothesis . However, it appears that the physiology of the intestine is specifically adapted to facilitate drug absorption as a result of the large luminal surface area and the low electrical resistance of the membrane. This has led some workers to assume that drug ions do pass through the intestine. For example, Crone and Keen (157) have studied the in vitro absorption of quaternary pyridinium compounds. They found that these compounds were transferred across the membrane of rat intestinal sacs and suggested that this transport was due to diffusion through aqueous pores. Turner et al. (62) using an in vitro perfusion technique have demonstrated that drug ions do pass through the in vitro rat intestine and show a difference in directional permeability coefficients.

Therefore, while the difference between the pH of the gastric and the intestinal fluids account to some extent for the difference in absorption rates from these two areas, the primary difference is related to the difference in the absorptive surface areas. The rat duodenal and jejunal mucosal surface area is about 10 times greater than the serosal surface area due to existence of folds in the intestinal mucosa and the numerous villi present in this area of the g.i. tract. Since there are no villi in the stomach, the available surface area in the intestine is markedly greater than that found in the stomach and probably accounts for the increased absorption seen in the intestine regardless of pH considerations (7).

The observations of this work support the concept that a nonionized molecule is a preferred species for absorption, but seriously question the extrapolation of the pH partition

hypothesis by many investigators to predict that a weakly acidic drug would be absorbed exclusively from the stomach while a weakly basic drug would be absorbed mainly from the intestine. The degree of ionization is one of the several factors that could affect the absorption of a drug. However, other factors such as surface area, presence or absence of physiological surfactants and blood flow can overcome the effect of the degree of ionization on the absorption.

The rate constants for absorption of the three drugs studied in this work increased by the same factor when stomach absorption was compared with intestinal absorption. This could very well be explained as a blood flow dependent phenomenon. A greater blood flow in the intestinal membrane as compared to the blood flow in the stomach could cause rapid dilution of the absorbed molecules resulting in a larger concentration gradient of the drug molecules across intestinal membrane. The similar rate constants of absorption observed for highly ionized amphetamine and predominantly nonionized salicylic acid in the stomach, tend to indicate that the gastric blood flow could be the most important factor in the absorption of these drugs from the stomach.

Except for the results from monkey C to be discussed later, the area under curve (AUC) measurements of each of the drugs studied in the other two monkeys are identical when comparison is made of absorption from stomach and the intestine. The identical areas suggest that if a drug was maintained in the stomach for a long enough time, it would be absorbed to the same extent as from the intestine, irrespective of the degree of ionization of the drug in the stomach. These observations contradict the pre-

dictions of the pH partition hypothesis since according to the latter hypothesis a basic drug would not be absorbed from the stomach, in which case the bioavailability of the drug from the stomach would be significantly lower than the bioavailability from the intestine.

On the basis of the foregoing evidence and arguments, and considering the results of the present research project, there seems to be little reason for maintaining the hypothesis that drug ions are not absorbed from the gastrointestinal tract or the elaborately devised hypothesis of the existence of a virtual pH at the mucosal membrane of the intestine. The pH effects on drug absorption described in the pH partition hypothesis have been restated by Benet (7) as follows:

> "The nonionized form of a drug will be absorbed faster than the ionized form of the drug at any particular site in the gastrointestinal tract. However, the rate of absorption of a drug from the intestine will be greater than the gastric absorption rate of that drug even if the drug is ionized in the intestine and nonionized in the stomach."

Thus in the monkey, if a normal gastric emptying pattern is maintained, it would appear that the small intestine is the chief absorptive site for all drugs although absorption can also take place from the stomach.

The absorption rate constants for the three drugs investigated in this work were calculated by the method of residuals. In using this method, one assumes first order absorption kinetics. The magnitude of the disposition rate constants should be

known as a result of I.V. studies, since in absence of such knowledge, it is usually assumed that the slower rate constant obtained by graphical analysis of the data is the elimination rate constant. In some instances (i.e. flip-flop model) this assumption could be wrong as it is possible to have an absorption rate constant smaller than the elimination rate constant, in which case the rate constant obtained by residuals plotting will be the elimination rate constant and not the absorption rate constant.

Computer fitting of the data eliminates the investigator's bias in drawing curves and should give better estimates of the rate constants. However, as in the method of residuals, previous knowledge of the elimination rate constant through an I.V. study is necessary. In the method of deconvolution, equally spaced data points for both the I.V. and the oral studies are needed. This method assumes all processes to be first order but does not require previous knowledge of disposition parameters. The Wagner-Nelson and the Loo-Riegelman methods for calculating the absorption rate constant need I.V. data and assume that disposition kinetics do not change from dose to dose. If there is a change in the terminal half-life, the elimination rate constant of the oral dose can be calculated if the distribution rate constants are assumed not to vary from dose to dose (1). Comparison of peak times gives a relative indication of rates of absorption assuming disposition does not change from dose to dose. In this study peak times and absorption rate constants calculated by plotting residuals were compared since the major objective of the study was to determine relative absorption rates from the stomach and the intestine.

The results of the intestinal absorption studies with salicylic acid in monkey B were different than all other results. Unlike the intestinal studies in monkey A, no difference in the rate of absorption was seen when a comparison was made between the stomach and the intestinal absorption of salicylic acid. As described previously, the fluoroscopic examination in this monkey had revealed that after eight months, the duodenal block by the inflated balloon was ineffective. It appears that the results obtained in the intestinal experiments in monkey B were due to peritoneal rather than intestinal absorption of the drug. If this were true, the peritoneum is as good a site as the duodenum for the absorption of antipyrine since a significant difference was seen in the absorption of antipyrine when a comparison was made between the "intestinal studies" and the stomach studies.

Unlike the results found in monkeys A and B, the extent of absorption of the three drugs from the stomach of monkey C was lower when compared with the availability from intravenous administration. However, since only two studies were undertaken, the significance of this difference cannot be evaluated. At the present time we have no adequate explanation for the lack of equivalent areas since when the stomach block is removed after 6 hours, the drugs should be available for intestinal absorption. In any case, using the alternate method of blocking the pylorus, absorption of levo-amphetamine HCl from the stomach was confirmed in this monkey.

V. SUMMARY

A model has been developed to study the gastrointestinal absorption of drugs and dosage forms in the unanesthetized rhesus monkey. Chronic vascular catheters were implanted in the iliac vein and artery to enable the investigator to withdraw blood samples without disturbing the monkey. The vascular catheters also allow intravenous studies to be carried out so that the kinetic parameters of any drug can be determined. Plastic cannulae were implanted surgically in the stomach and in the duodenum very close to the pylorus. These cannulae provide a means of instilling a drug solution or a dosage form directly into the stomach or the duodenum. A technique was developed using foley catheters to block the pylorus so that a drug solution or drug particles can be maintained in the stomach. With this set-up, absorption of a drug specifically from the stomach can be studied.

The object of this work was to compare the absorption of an ionizable acidic drug, an ionizable basic drug, and a nonionized drug from the stomach and the intestine. Salicylic acid and levo-amphetamine HCl were chosen as representatives of acidic and basic drugs respectively and antipyrine was selected as the nonionized drug. In studying the absorption of these drugs from the stomach and the intestine, an attempt was made to check the predictions of the pH partition hypothesis which proposes that nonionized molecules are the preferred species for the penetration of biological membranes. Based on this proposition, an acidic drug would be absorbed better from the stomach whereas a

basic drug would be absorbed preferentially from the intestine. By simultaneous administration of salicylic acid, amphetamine and nonionized antipyrine, the absorption from the stomach of nonionized acidic drug molecules and ionized basic drug molecules was studied relative to the absorption of the nonionized neutral drug molecules. Similarly, in intestinal absorption studies, a comparison was made between the absorption of ionized acidic drug molecules, nonionized basic drug molecules, and nonionized neutral drug molecules.

The results of this work indicate that absorption of all three drugs was faster from the intestine than from the stomach even if the drug was ionized at the absorption site. The large absorptive surface area of the intestine as compared to that of the stomach plays an important role in the absorption of drugs. In fact, the surface area differences can override the effect of the degree of ionization on the rate of absorption. In contrast to the rate, the extent of absorption of the three drugs from the stomach was almost equal to that from the intestine. In view of these results, the pH partition hypothesis has been restated as follows:

The nonionized form of a drug will be absorbed faster than the ionized form of the drug at any particular site in the gastrointestinal tract. However, the rate of absorption of a drug from the intestine will be greater than the gastric absorption rate of that drug even if the drug is ionized in the intestine and nonionized in the stomach.

Thus in the monkey, if a normal gastric emptying pattern is maintained, it would appear that the small intestine is the

APPENDIX A

Table A-1

Sample Calculations for Salicylate Assay in Plasma.

Intravenous Administration of Salicylic Acid in Monkey A at a Dose of 42 mg/kg.

Least squares fitting of the standard curve by a CPS program. Sample calculations read from the standard curve using the same computer program.

Excitation Wavelength 303 nm
Emission Wavelength 403 nm

Standard solutions mcg salicylate/ml	% Emission	
0.1	11.2	Slope of the least squares line 101.3
0.2	22.0	
0.4	42.5	
0.8	85.0	Y intercept 1.24
1.0	100.0	X intercept -0.012
		Regression coefficient 0.999

Samples at time, min	% Emission	Dilution factor	Net mcg salicylate per ml of plasma
0	0.8	35	-0.75 (0.0)
4	11.2	1750	172.1
7	88.6	175	150.9
10	88.2	175	150.2
15	87.0	175	148.1
20	85.0	175	144.7
30	75.2	175	127.8
40	67.0	175	113.6
50	65.8	175	111.6
60	63.0	175	106.7
75	57.0	175	96.3
90	51.0	175	86.0
120	40.2	175	67.3
150	31.5	175	52.3
180	25.5	175	41.9
240	61.2	35	20.7
300	22.0	35	7.1
360	10.8	17.5	1.7

Table A-2

Sample Calculations for the Assay of Radioactivity in Plasma.

Intravenous Administration of Antipyrine (14 mg/kg), ^{14}C Antipyrine (22.93μc), Salicylic Acid (42 mg/kg) and levo-Amphetamine HCl (0.3 mg/kg) in Monkey B.

Sample at time min	cpm (Average of 2)	% S.D.	cpm with internal standard	% S.D.	cpm due to internal standard	Efficiency	Gross dpm	Net dpm
0	53	4.5	16460	0.2	16407	0.79	67	0
5	5650	0.5	22140	0.2	16490	0.79	7152	7085
7	3625	0.7	19841	0.2	16216	0.78	4647	4580
10	3391	0.7	19762	0.2	16371	0.79	4292	4225
15	3078	0.7	19349	0.2	16271	0.78	3946	3979
20	3035	0.7	19464	0.2	16429	0.79	3842	3775
30	2815	0.7	19118	0.2	16303	0.78	3609	3542
40	2566	0.7	19118	0.2	16552	0.79	3248	3181
60	2380	0.7	18697	0.2	16317	0.78	3051	2984
75	2189	0.7	18375	0.2	16186	0.78	2806	2739
90	2004	1.0	18150	0.2	16146	0.77	2603	2536
120	1770	1.0	17861	0.2	16091	0.77	2299	2232
150	1475	1.0	17809	0.2	16334	0.78	1891	1824
180	1223	1.0	17613	0.2	16390	0.79	1548	1481
240	917	1.5	16257	0.2	15340	0.74	1239	1172
300	547	1.5	16803	0.2	16256	0.78	701	634
360	534	1.5	16736	0.2	16202	0.78	685	618
420	467	1.5	16921	0.2	16454	0.79	591	524
480	381	2.5	16583	0.2	16202	0.78	488	421
540	341	2.5	16579	0.2	16238	0.78	437	370
585	298	2.5	16542	0.2	16244	0.78	382	315

Table A-3

Sample Calculations for the Assay of levo-Amphetamine HCl in Plasma.

Intravenous Administration of levo-Amphetamine HCl (0.3 mg/kg), Salicylic Acid (42 mg/kg), Antipyrine (14 mg/kg) and ^{14}C Antipyrine (22.93 μc) in Monkey A.

Least squares fitting of the standard curve by a CPS program. Sample calculations read from the standard curve using the same computer program.

Standard solutions ng levo-Amphetamine HCl/ml	Peak height ratio	
0	0	Slope of the least squares line 0.032
10	0.31	Y intercept -0.032
25	0.68	X intercept 1.025
50	1.46	Regression coefficient 0.9951
80	2.72	
100	3.01	

Samples at time, min	Peak height ratio	Dilution factor	Net ng levo-Amphetamine HCl per ml of plasma
0	0.1	2.5	10.5 (0.0)
4	1.17	10	370.0
7	0.39	10	123.5
10	0.38	10	120.3
15	0.74	5	112.4
20	0.67	5	100.7
30	0.57	5	85.5
40	0.48	5	70.3
50	0.66	5	99.6
60	0.47	5	66.8
75	0.41	5	59.0
90	0.47	5	68.2
120	0.75	2.5	51.6
150	0.71	2.5	48.0
180	0.72	2.5	49.0
240	0.70	2.5	47.1
300	0.64	2.5	43.0
360	0.60	2.5	39.1
420	0.57	2.5	37.0
480	0.43	2.5	25.8
540	0.49	2.5	31.2
600	0.37	2.5	21.4

APPENDIX B

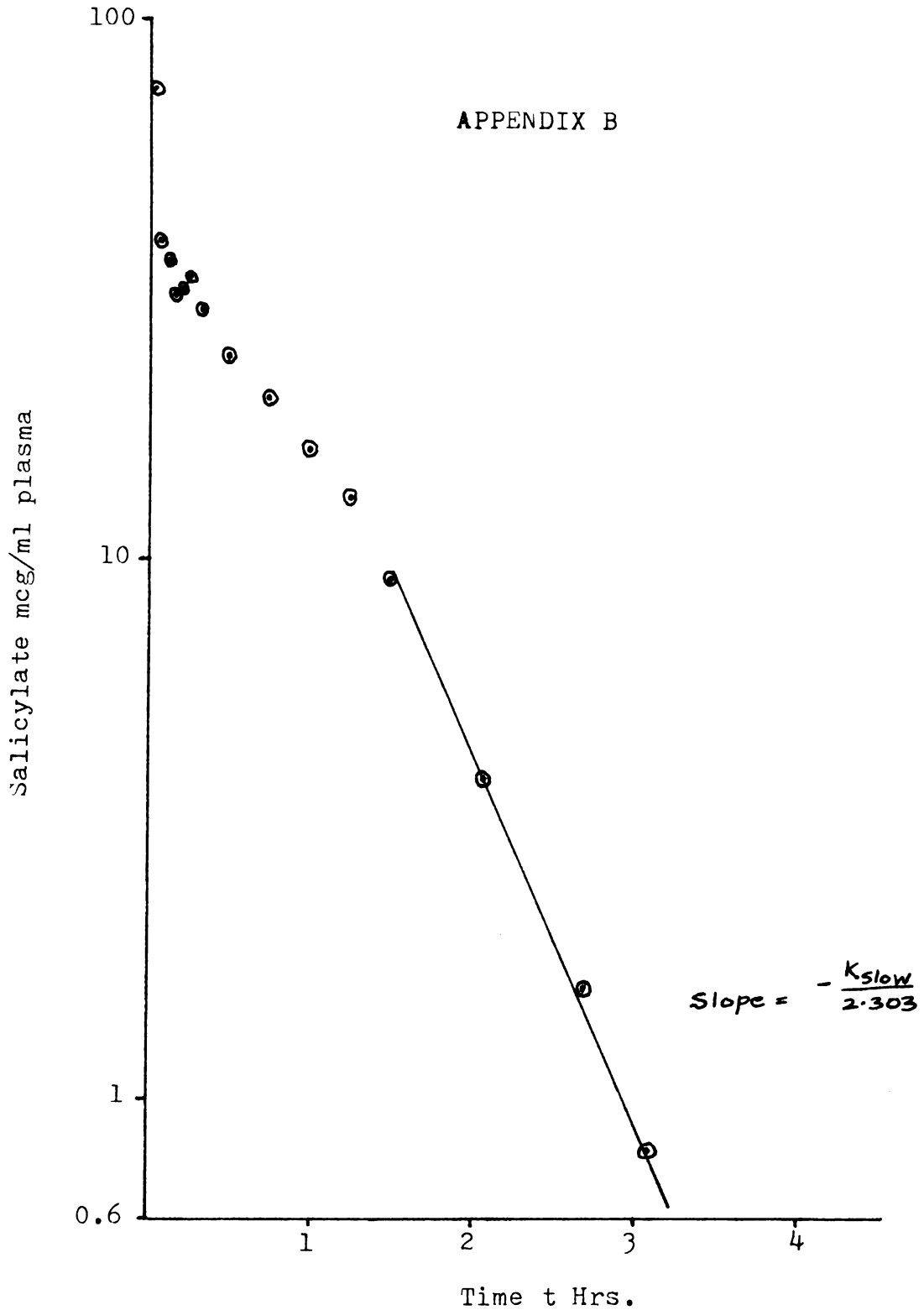


Fig. B-1.--Sample Semilogarithmic Plot of Salicylic Acid Concentration in Plasma Versus Time.

Intravenous Salicylic Acid in Monkey A at a dose of 7mg/kg.

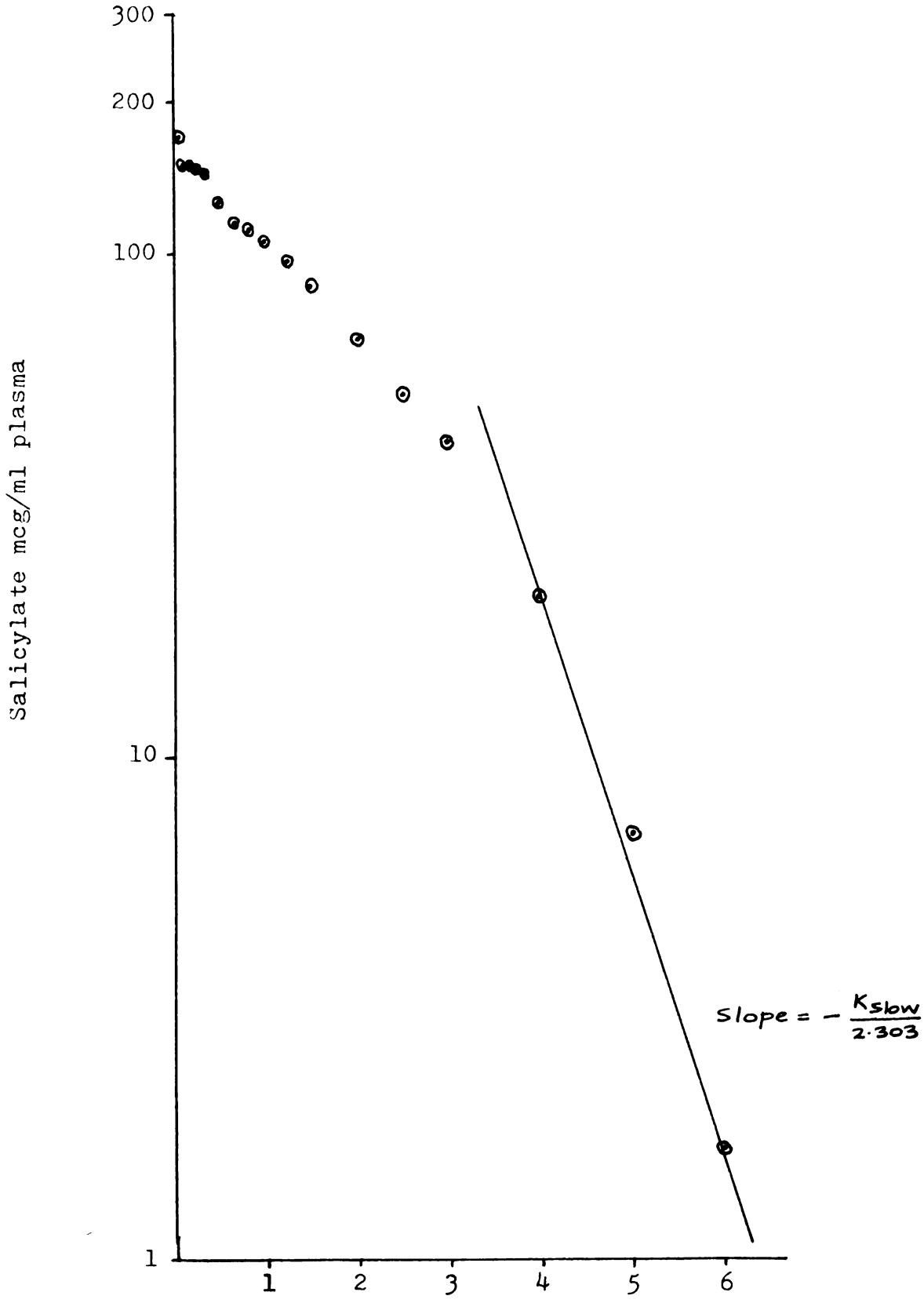


Fig. B-2.-- Sample Semilogarithmic Plot of Salicylic Acid Concentration in Plasma Versus Time.

Intravenous Salicylic Acid in Monkey A at a dose of 42mg/kg.

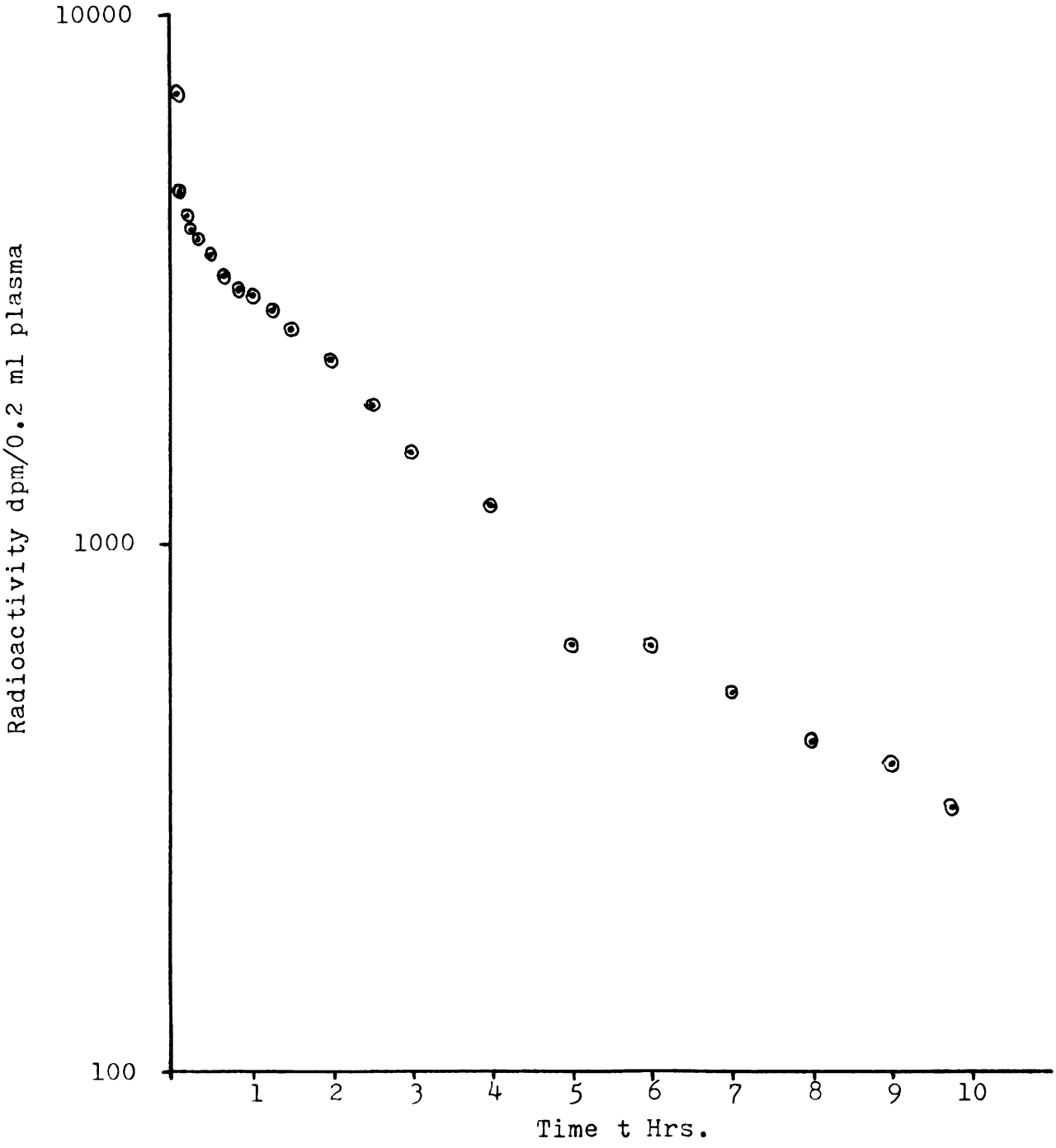


Fig. B-3.--Sample Semilogarithmic Plot of Plasma Radioactivity Versus Time.

Simultaneous Intravenous Administration of Antipyrine (14mg/kg) ^{14}C Antipyrine (22.93μc), Salicylic Acid (42 mg/kg) and levo-Amphetamine HCl (0.3 mg/kg) in Monkey B.

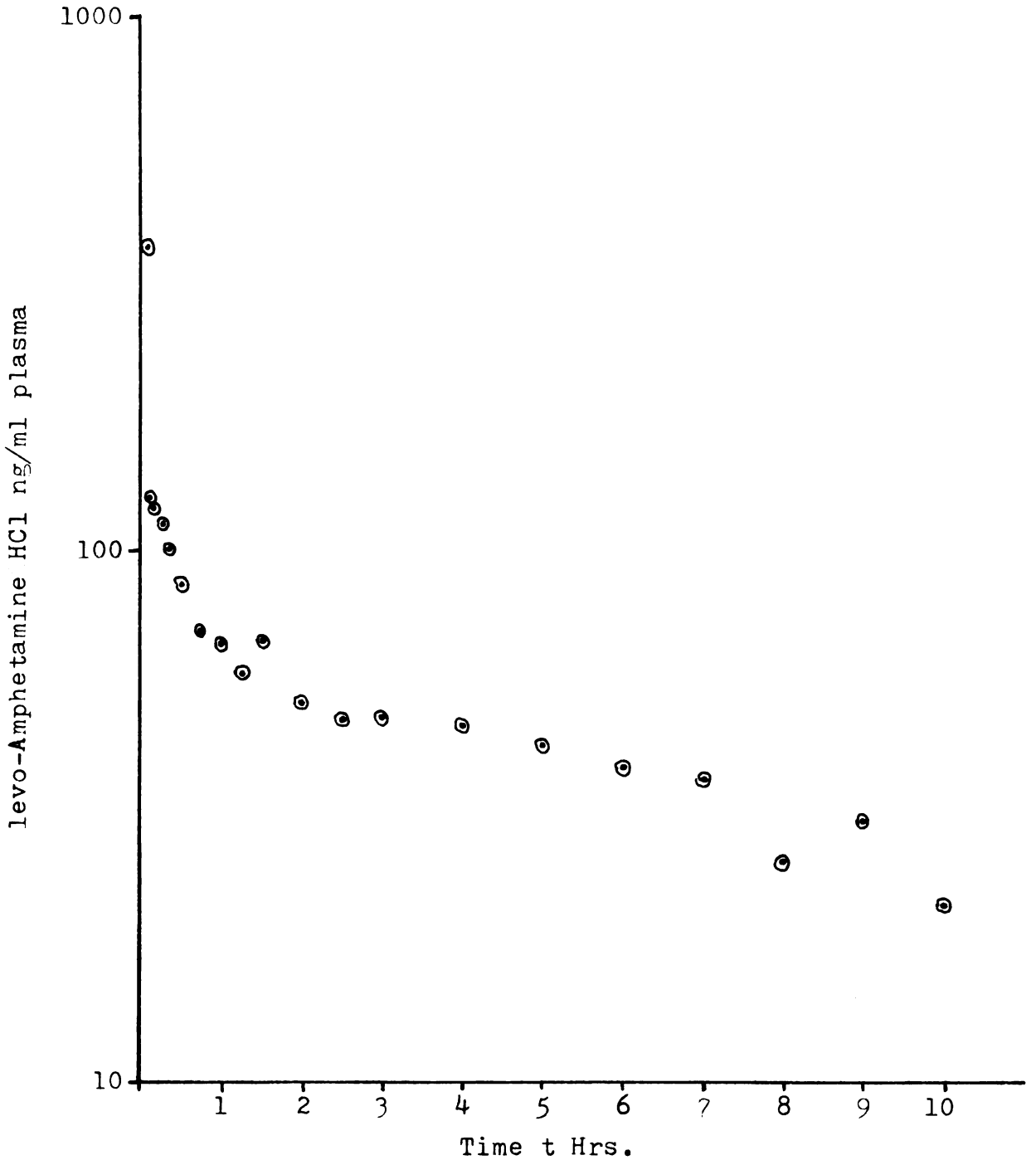


Fig. B-4.--Sample Semilogarithmic Plot of levo-Amphetamine HCl Concentration Versus Time.

Simultaneous Intravenous Administration of levo-Amphetamine HCl (0.3 mg/kg), Salicylic Acid (42 mg/kg), Antipyrine (14 mg/kg), and ^{14}C Antipyrine (22.93μc) in Monkey A.

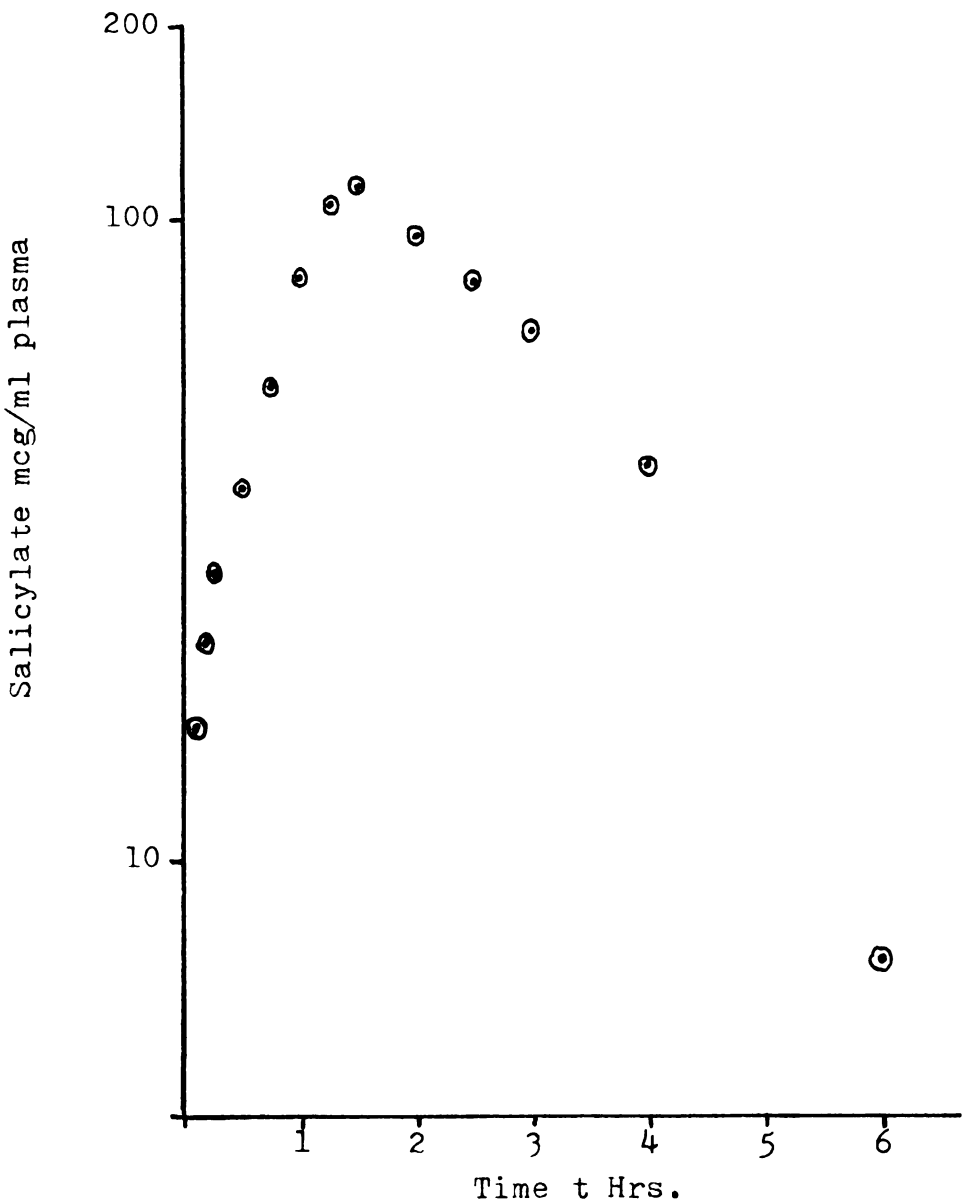


Fig. B-5.--Sample Semilogarithmic Plot of Plasma Concentration of Salicylic Acid Versus Time.

Simultaneous Instillation of Salicylic Acid (42 mg/kg), Antipyrine (14 mg/kg), ^{14}C Antipyrine (22.93 μc) and levo-Amphetamine HCl (1.0 mg/kg) into the Stomach of Monkey A.

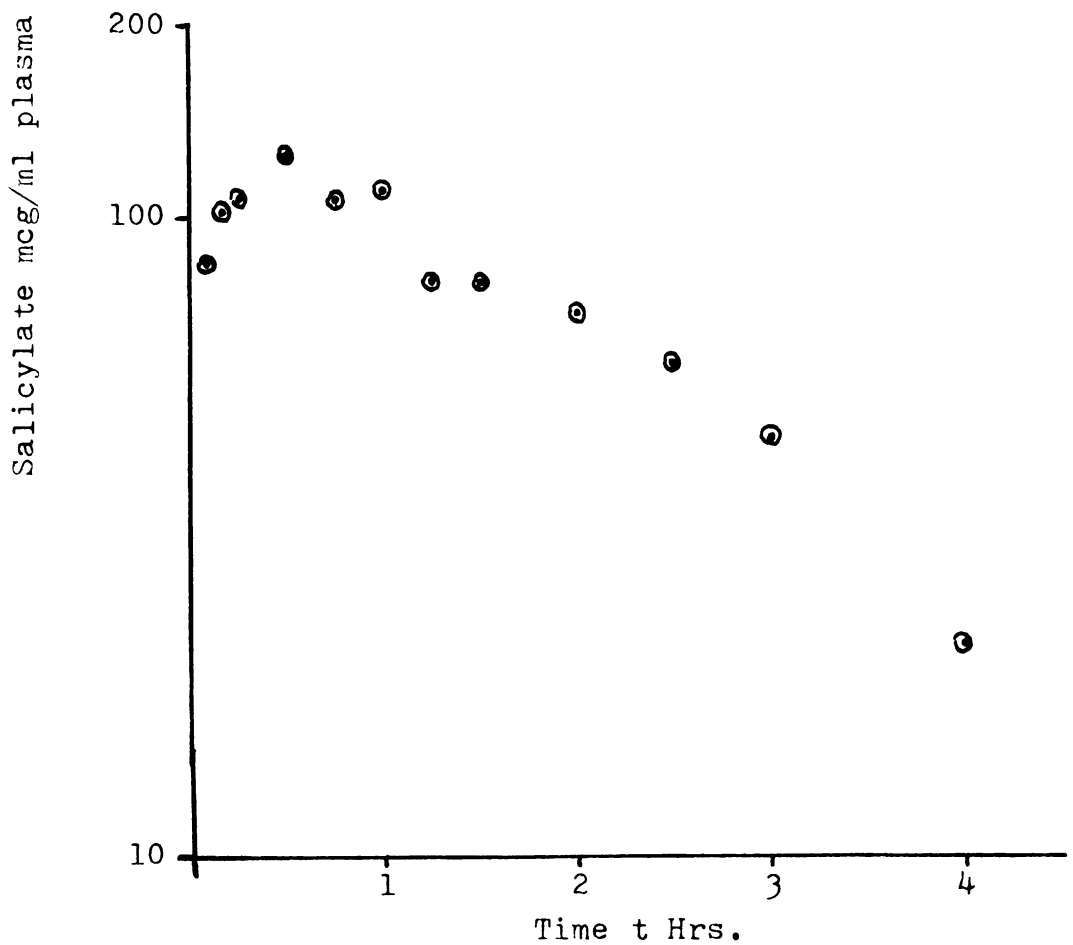


Fig. B-6.--Sample Semilogarithmic Plot of Plasma Concentration of Salicylic Acid Versus Time.

Simultaneous Instillation of Salicylic Acid (42 mg/kg), Antipyrine (14 mg/kg), ^{14}C Antipyrine (22.93μc) and levo-Amphetamine HCl (0.3 mg/kg) into the Duodenum of Monkey A.

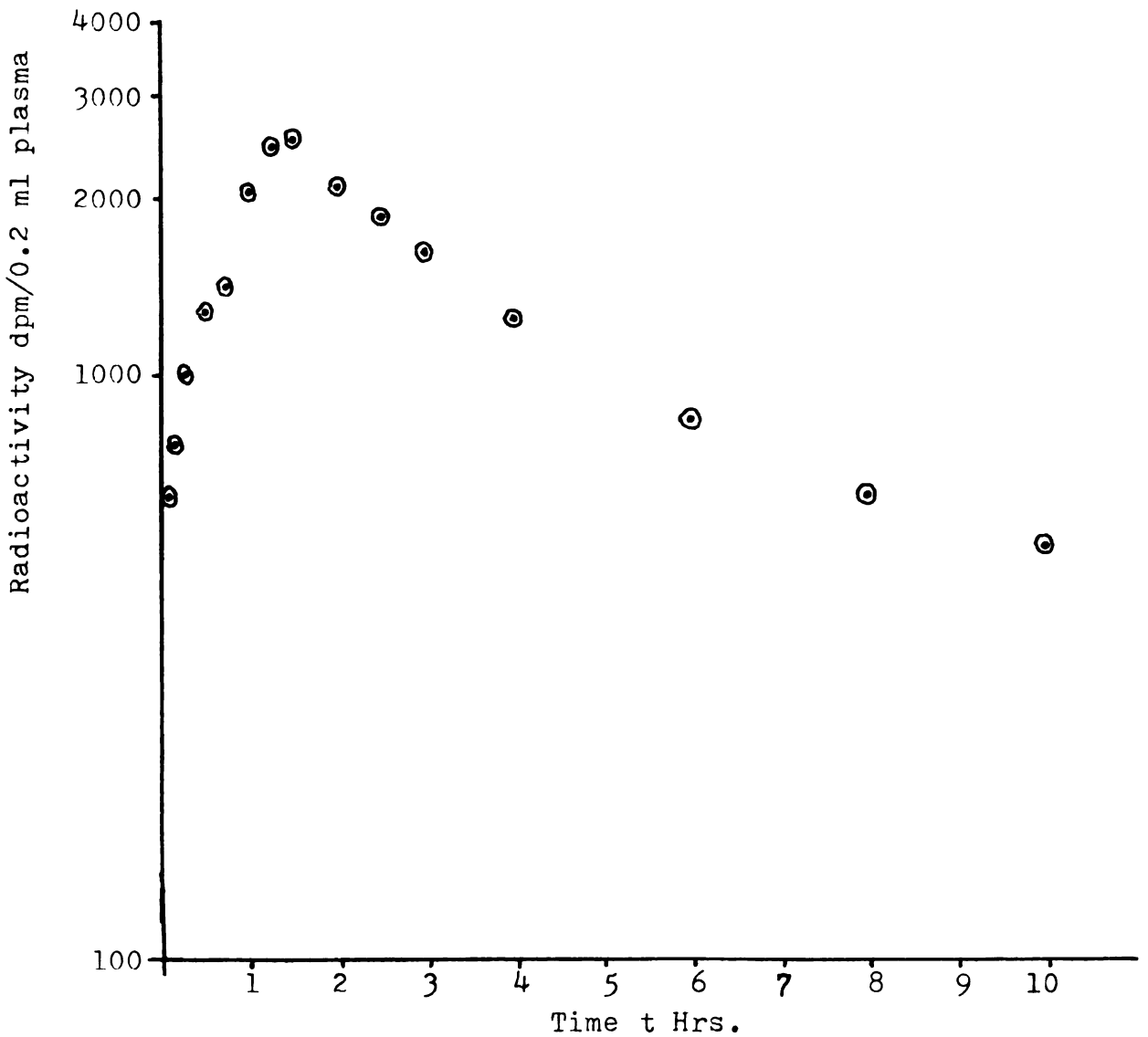


Fig. B-7.-- Sample Semilogarithmic Plot of Plasma Radioactivity due to Antipyrine and its Metabolites Versus Time.

Simultaneous Instillation of Salicylic Acid (42 mg/kg), Antipyrine (14 mg/kg), ^{14}C Antipyrine (22.93μc) and levo-Amphetamine HCl (1.0 mg/kg) into the Stomach of Monkey **A**.

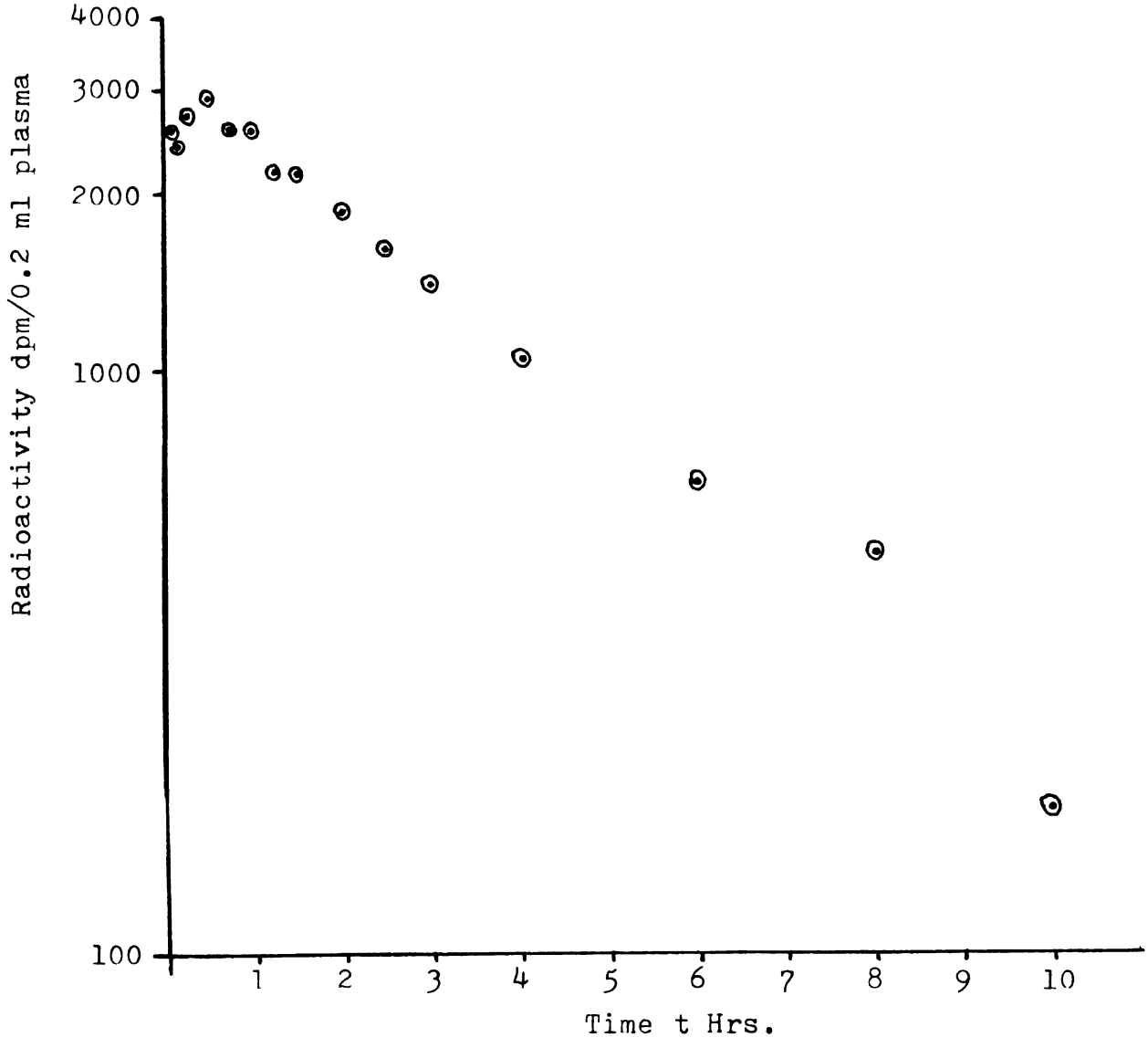


Fig. B-8.--Sample Semilogarithmic Plot of Plasma Radioactivity due to Antipyrine and its Metabolites Versus Time.

Simultaneous Instillation of Salicylic Acid (42 mg/kg), Antipyrine (14 mg/kg), ^{14}C Antipyrine (22.93μc) and levo-Amphetamine HCl (0.3 mg/kg) into the Duodenum of Monkey A.

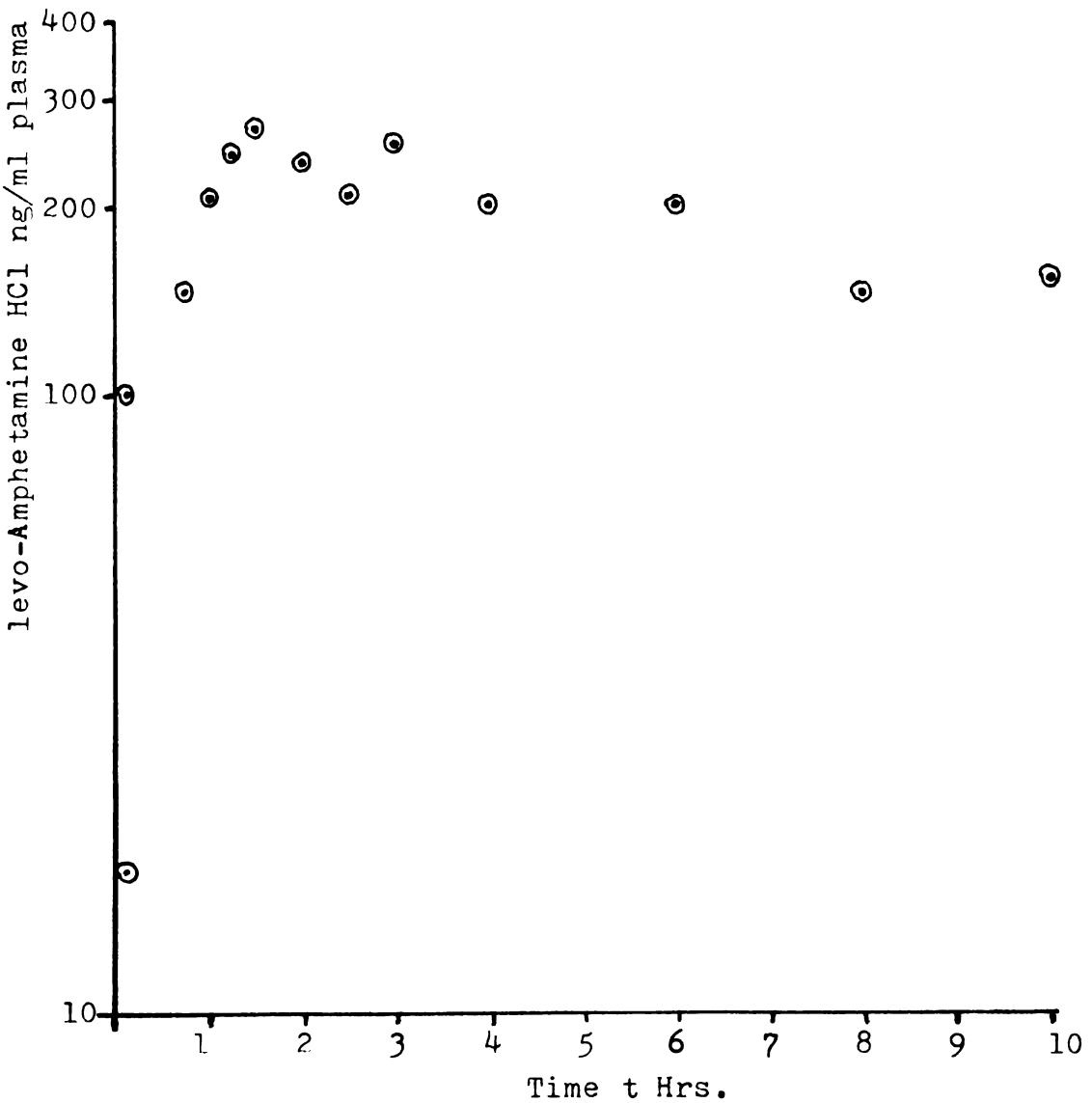


Fig. B-9.--Sample Semilogarithmic Plot of Plasma Concentration of levo-Amphetamine HCl Versus Time.

Simultaneous Instillation of Salicylic Acid (42 mg/kg), Antipyrine (14 mg/kg), ^{14}C Antipyrine (22.93 μc) and levo-Amphetamine HCl (1.0 mg/kg) into the Stomach of Monkey A.

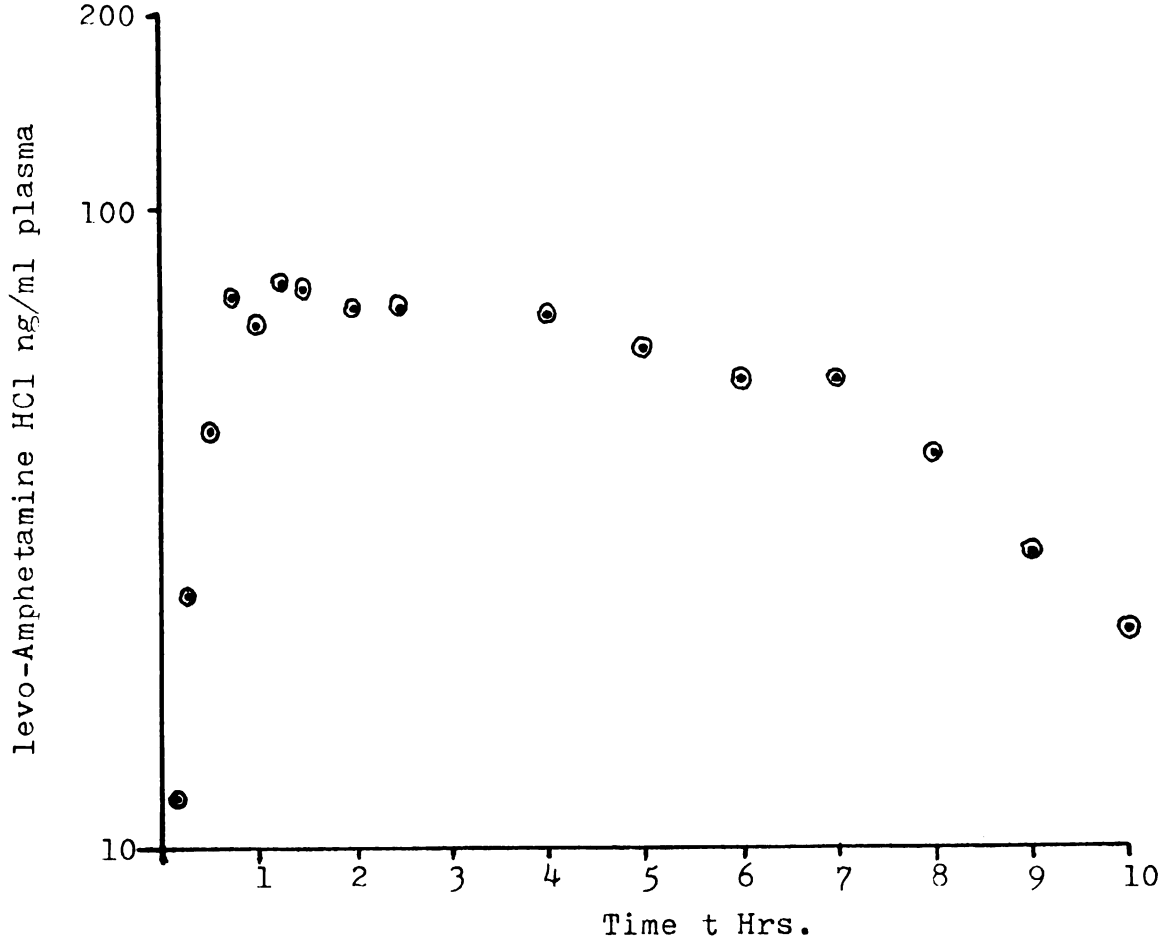


Fig. B-10.--Sample Semilogarithmic Plot of Plasma Concentration of levo-Amphetamine HCl Versus Time.

Simultaneous Instillation of Salicylic Acid (42 mg/kg), Antipyrine (14 mg/kg), ^{14}C Antipyrine (22.93μc) and levo-Amphetamine HCl (0.3 mg/kg) into the Duodenum of Monkey A.

REFERENCES

1. "Guidelines for Biopharmaceutical Studies in Man",American Pharmaceutical Association Academy of Pharmaceutical Sciences, Washington, D. C., 1972.

2. Gibaldi, M., Chapter 11 in "The Theory and Practice of Industrial Pharmacy" Edited by L. Lachman, H. Lieberman and J. L. Kanig, Lea and Febiger, Philadelphia, 1970.

3. Levy, G., Chapter 2 in "Prescription Pharmacy" 2nd edition, Edited by J. B. Sprowls, Lippincott, Philadelphia, 1970.

4. Levy, G., Proc. Int. Pharmacol. Meet., 3rd, 7, 33 (1966).

5. Barr, W. H., Drug Info. Bull., 3, 27 (1969).

6. Riegelman, S., ibid., 3, 59 (1969).

7. Benet, L. Z., Chapter 1 in "Drug Design" Volume IV, Edited by E. J. Ariens, Academic Press, New York, 1973.

8. Hunt, J. N., and Spurell, W. R., J. Physiol., 113, 157 (1951).

9. Hunt, J. N., Physiol. Rev., 39, 491 (1959).

10. Hunt, J. N., and Pathak, J. D., J. Physiol., 154, 254 (1960).

11. Hunt, J. N., ibid., 154, 270 (1960).

12. Hunt, J. N., Amer. J. Dig. Dis., 8, 885 (1963).

13. Hunt, J. N., and Knox, M. T., Alim. Canal, 4, 1917 (1968).

14. Ibid., J. Physiol., 201, 161 (1969).

15. Thomas, J. E., Physiol. Rev., 37, 453 (1957).

16. Thomas, J. E., and Baldwin, M. V., Alim Canal, 4, 1937 (1968).

17. Wagner, J. G., Drug Intelligence, 2, 244 (1968).

18. Hunt, J. N., and MacDonald, I., J. Physiol., 126, 459 (1954).

19. Davenport, H. W., Pg. 50, 149, in "Physiology of the Digestive Tract", Year Book Publisher, Chicago, 1961.

20. Bachrach, W. H., Ciba Clin. Sympos., 11, 3 (1959).

21. Levy, G., and Jusko, W., J. Pharm. Sci., 54, 219 (1965).

22. Lagerlof, H. O., Rudewald, M. B., and Perman, G., Acta Med. Scand., 168, 269 (1960).

23. Lewis, R. A., and Said, D., Int. Pharmacol. Meet., 1st, Stockholm, Sweden, August 1961.

24. Weikel, J. H., Jr., and Lish, P. M., Arch. Int. Pharmacodyn., 119, 398 (1959).

25. Varga, F., Arch. Int. Pharmacodyn., 163, 38 (1966).

26. Cummins, A. J., and Almy, T. P., Gastroenterology, 23, 179 (1953).

27. Uyeyama, K., Giansiracusa, J. E., Althaesen, T. L., and Harper, H. A., Gastroenterology, 23, 143 (1953).

28. Levy, G., and Jusko, W., J. Pharm. Sci., 55, 285 (1966).

29. Reynell, P. C., and Spray, G. H., Brit. J. Pharmacol., 12, 104 (1957).

30. Uleri, G., and Reggerini, R., Ann. Anesthesiol., 9, 135 (1968).

31. Farrar, J. T., and Zfass, A. M., Gastroenterology, 52, 1019 (1967).

32. Jaffe, J. M., Collaizzi, J. L., and Barry, H., III, J. Pharm. Sci., 60, 1646 (1971).

33. Wood, J. H., Lancet, 2, 212 (1967).

34. Hirsch, H. A., and Finland, M., Amer. J. Med. Sci., 237, 693 (1959).

35. Peterson, O. L., and Finland, M., ibid., 204, 581 (1942).

36. Kirby, W. M. M., Roberts, C. E., and Burdich, R. E., Antimicrob. Ag. Chemotherap., 1, 286 (1961).

37. Rosenblatt, J. E., Barett, J. E., Brodie, J. L., and Kirby, W. M. M., ibid., 6, 134 (1966).

38. Klein, J. O., and Finland, M., New Engl. J. Med., 269, 1019 (1963).

39. Jusko, W., and Levy, G., J. Pharm. Sci., 56, 58 (1967).

40. Crounse, R. R., J. Invest. Dermatol., 37, 529 (1961).

41. Kabasakalian, P., Katz, M., Rosenkrantz, B., and Townley, E., J. Pharm. Sci., 59, 595 (1970).

42. Giannina, T., Steneitz, B. G., and Meli, A., Proc. Soc. Exptl. Biol. Med., 121, 1175 (1966).

43. Reininger, E. J., and Sapirstein, L. A., Science, 126, 1176 (1957).

44. Herrick, J. F., Essex, H. E., Mann, F. C., and Baldes, E. J., Am. J. Physiol., 108, 621 (1934).

45. Brandt, J. L., Castleman, L., Ruskin, H. D., Greenwald, J., and Kelly, J. J., J. Clin. Invest., 34, 1017 (1955).

46. Doluisio, J. T., Tan, G. H., Billups, N. F., and Diamond, L., J. Pharm. Sci., 58, 1200 (1969).

47. Ochsenfahrt, H., and Winne, D., Life Sciences, 7, 493, (1968).

48. Winne, D., and Remischovsky, J., J. Pharm. Pharmacol., 22, 640 (1970).

49. Diamond, L., Doluisio, J. T., and Crouthamel, W. G., Eur. J. Pharmacol., 11, 109 (1970).

50. Reuning, R. H., and Levy, G., J. Pharm. Sci., 56, 843 (1967).

51. Levine, R. R., and Pelikan, E. W., J. Pharmacol. Exp. Ther., 131, 319 (1961).

52. Bates, T. R., Gibaldi, M., and Kanig, J. L., Nature, 210, 1331 (1966).

53. Faloon, W. W., Paes, I. C., Woolfolk, D., Nankin, H., Wallace, K., and Haro, E. N., Ann. N. Y. Acad. Sci., 132, 879 (1966).

54. Shore, P. A., Brodie, B. B., and Hogben, C. A. M., J. Pharmacol. Exp. Ther., 119, 361 (1957).

55. Schanker, L. S., Shore, P. A., Brodie, B. B., and Hogben, C. A. M., J. Pharmacol. Exp. Ther., 120, 528 (1957).

56. Schanker, L. S., Tocco, D. J., Brodie, B. B., and Hogben, C. A. M., J. Pharmacol. Exp. Ther., 123, 81 (1958).

57. Hogben, C. A. M., Schanker, L. S., Tocco, D. J., and Brodie, B. B., J. Pharmacol. Exp. Ther., 120, 540 (1957).

58. Hogben, C. A. M., Tocco, D. J., Brodie, B. B., and Schanker, L. S., J. Pharmacol. Exp. Ther., 125, 275 (1959).

59. Hogben, C. A. M., Fed. Proc., 19, 864 (1960).

60. Schanker, L. S., J. Med. Pharm. Chem., 2, 343 (1960).

61. Wagner, J. G., Drug Intelligence, 2, 244 (1968).

62. Turner, R. H., Mehta, C. S., and Benet, L. Z., J. Pharm. Sci., 59, 590 (1970).

63. Kakemi, K., Arita, T., Hori, R., Konishi, R., and Kenji, N., Chem. Pharm. Bull., 17, 248 (1969).

64. Kakemi, K., Arita, T., Hori, R., Konishi, R., Nishimura, K., Matsui, H., and Nishimura, T., Chem. Pharm. Bull., 17, 255 (1969).

65. Wilson, T. H., and Wiseman, G., J. Physiol., 123, 116 (1954).

66. Hoffman, H., Radke, E. U., and Ankermann, H., Acta Biol. Med. Ger., 26, 833 (1971).

67. Schanker, L. S., and Jeffrey, J. J., Nature, 190, 727 (1961).

68. Dikstein, S., and Sulman, F. G., Biochem. Pharmacol., 14, 355 (1965).

69. Crane, R. K., and Wilson, T. H., J. Appl. Physiol., 12, 145 (1958).

70. Feldman, S., Salvino, M., and Gibaldi, M., J. Pharm. Sci., 59, 705 (1970).

71. Benet, L. Z., Orr, J. M., Turner, R. H., and Webb, H. S., J. Pharm. Sci., 60, 234 (1971).

72. Nayak, R. K., and Benet, L. Z., J. Pharm. Sci., 60, 1508 (1971).

73. Wiseman, G., J. Physiol., 120, 63 (1953).

74. Darlington, W. A., and Quastel, J. H., Arch. Biochem. Biophy., 43, 194 (1953).

75. Wilson, T. H., J. Appl. Physiol., 9, 137 (1956).

76. Acland, J. D., Biochem. J., 73, 23 (1959).

77. Smyth, D. H., and Taylor, C. B., J. Physiol., 136, 632 (1957).

78. Misra, A. L., Hunger, A., and Keberle, H., J. Pharm. Pharmacol., 18, 246 (1966).

79. Koizumi, T., Arita, T., and Kakemi, K., Chem. Pharm. Bull., 12, 421 (1964).

80. Barr, W. H., and Riegelman, S., J. Pharm. Sci., 59, 154 (1970).

81. Kakemi, K., Sezaki, H., Muranishi, S., and Tsujimura, Y., Chem. Pharm. Bull., 17, 1641 (1969).

82. Doluisio, J. T., Billups, N. F., Dittert, L. W., Sugita, E. T., and Swintosky, J. V., J. Pharm. Sci., 58, 1196 (1969).

83. Doluisio, J. T., Tan, G. H., Billups, N. F., and Diamond, L., J. Pharm. Sci., 58, 1200 (1969).

84. Crouthamel, W. G., Tan, G. H., Dittert, L. W., and Doluisio, J. T., J. Pharm. Sci., 60, 1160 (1971).

85. Lanman, R. C., Stremsterfer, C. E., and Schanker, L. S., Xenobiotica, 1, 613 (1971).

86. Dowset, E. E., and Wingate, D. L., J. Physiol., 210, 1P (1970).

87. Schedl, H. P., and Clifton, J. A., J. Clin Invest., 40, 1079 (1961).

88. Holdsworth, C. D., and Dawson, A. M., Clin. Sci., 27, 371 (1964).

89. Sladen, G. E., and Dawson, A. M., Gut, 11, 947 (1970).

90. Pyorala, K., Jussila, J., Mustala, O., and Siurala, M., Scand. J. Gastroent. (Suppl.), 9, 95 (1970).

91. Kekki, M., Pyorala, K., Mustala, O., Salmi, H., Jussila, J., and Siurala, M., Int. J. Clin. Pharmacol., 5, 209 (1971).

92. Siurala, M., Mustala, O., Pyorala, K., Kekki, M., Airaksinen, M., Jussila, J., Salmi, H., and Jalkunen, R., Acta Hepato-Gastroenterol., 19, 190 (1972).

93. Beermann, B., Hellstrom, K., and Rosen, A., Circulation, 48, 852 (1971).

94. Beermann, B., Hellstrom, K., and Rosen, A., Acta Pharmacol. Toxicol., 29, (Suppl. 3), 98 (1971).

95. Hellstrom, K., Rosen, A., and Soderlund, K., Scand. J. Gastroent., 5, 585 (1970).

96. Beermann, B., Hellstrom, K., and Rosen, A., Eur. J. Clin. Pharmacol., 4, 46 (1971).

97. Beermann, B., Hellstrom, K., and Rosen, A., Clinical Science, 40, 95 (1971).

98. Beermann, B., Hellstrom, K., and Rosen, A., Eur. J. Clin, Pharmacol., 3, 93 (1971).

98a. Gibaldi, M., and Kanig, J. L., Arch. Intern. Pharmacodyn. Therapie, 161(2), 343 (1966).

99. Kramar, J., and Simay-Kramar, M., Endocrinology, 52, 453 (1953).

100. Kramar, J., Peetz, D. J., and McCarthy, H. H., Psychosomat. Med., 16, 393 (1954).

101. Kramar, J., Science, 119, 790 (1954).

102. Kramar, J., Am. J. Physiol., 175, 69 (1953).

103. Zeman, W. V., Lab. Anim. Care, 16, 386 (1966).

104. Zeman, W. V., Surgery, 59, 387 (1966).

105. Zeman, W. V., and Symchowicz, S., Am. J. Vet. Res., 31, 1293 (1970).

106. Zeman, W. V., Am. J. Vet. Res., 31, 1287 (1970).

107. Kennedy, G. Y., Comp. Biochem. Physiol., 36, 415 (1970).

108. Saslaw, S., and Carlisle, H. N., Ann. N. Y. Acad. Sci., 162, 646 (1969).

109. Coulston, F., and Serrone, D. M., Ann. N. Y. Acad. Sci., 162, 681 (1969).

110. Dowling, R. H., Mack, E., Picott, J., Berger, J., and Small, D. M., J. Lab. Clin., Med., 72, 169 (1968).

111. Campbell, C. B., Cowley, D. J., and Dowling, R. H., Eur. J. Clin. Invest., 2, 332 (1972).

112. Boass, A., and Wilson, T. H., Proc. Soc. Exp. Biol. Med., 112, 654 (1963).

113. Fausnaugh, C. L., DeVito, R. V., Chapman, N. D., Nyhus, L. M., and Harkins, H. N., Surgery, 48, 682 (1960).

114. Lanciault, G., Merritt, A., Rosato, E., Bonoma, C., and Brooks, F. P., Am. J. Dig. Dis., 17, 523 (1972).

115. Winship, D. H., Poindexter, R. E., Thayer, W. R., Jr., and Spiro, H. M., Gastroenterology, 48, 231 (1965).

116. Lien, W. M., McCormick, J. R., Davies, M. W., and Egdahl, R. H., Endocrinology, 87, 206 (1970).

117. Forsyth, R. P., Hoffbrand, B. I., and Melmon, K. L. Circulation, 44, 119 (1971).

118. Forsyth, R. P., Psychosom. Med., 31, 300 (1969).

119. Polish, E., Brady, J. V., Mason, J. W., Thach, J. S., and Niemeck, W., Gastroenterology, 43, 193 (1962).

120. Sweet, A. Y., Levitt, M. F., Hodes, H. L., Haber, H., and Kurzman, B., Am. J. Physiol., 201, 975 (1961).

121. Mann, G. V., J. Nutr., 47, 213 (1952).

122. Overman, R. R., and Feldman, H. A., Am. J. Physiol., 148, 455 (1947).

123. Krise, G. M., and Wald, N., J. Appl. Physiol., 12, 482 (1958).

124. Forsyth, R. P., Nies, A. S., Wyler, F., Neutze, J., and Melmon, K. L., J. Appl. Physiol., 25, 736 (1968).

125. Forsyth, R. P., and Hoffbrand, I., Am. J. Physiol., 218, 214 (1970).

126. de los Santos, Bucaille, M., Delgado, J. M. R., and Spiro, H. M., Gastroenterology, 42, 595 (1962).

127. Brooks, F. P., Ridley, P., Attinger, F., Bjovedt, G., and Neff, K., Am. J. Physiol., 205, 1096 (1963).

128. Brooks, F. P., Ridley, P., Attinger, F., and Neff, K., Am. J. Physiol., 205, 1093 (1963).

129. Brodie, B. B., and Axelrod, J., J. Pharmacol. Exp. Ther., 98, 97 (1950).

130. Yoshimura, H., Shimono, H., and Tsukamoto, H., Biochem. Pharmacol., 17, 1511 (1968).

131. Reid, J. T., Balch, C. C., and Glascock, R. F., Brit. J. Nutr., 12, 43 (1958).

132. Mendelsohn, D., and Levin, N. W., J. Lab. Clin. Med., 54, 311 (1959).

133. Welch, R. M., Harrison, Y. E., and Burns, J. J., Toxicol. Appl. Pharmacol., 10, 340 (1967).

134. Davies, D. S., and Thorgeirsson, S. S., Ann. N. Y. Acad. Sci., 179, 411 (1971).

135. Hucker, H. B., Stauffer, S. C., and White, S. E., J. Pharm. Sci., 61, 1490 (1972).

136. Woodbury, D. M., Chapter 17 in "The Pharmacological Basis of Therapeutics" 3rd Edition, Edited by Goodman, L. S. and Gilman, A., The MacMillan Company, New York, 1965.

137. Kucera, J. L., and Bullock, F. J. J. Pharm. Pharmacol., 21, 293 (1969).

138. McArthur, J. N., Dawkins, P. D., and Smith, J. H. M., J. Pharm. Pharmacol., 23, 32 (1971).

139. Brzezinski, J., Acta Pol. Pharm., 25, 91 (1968).

140. Alles, G. A., and Wisegarver, B. B., Toxicol. Appl. Pharmacol., 3, 678 (1961).

141. Beckett, A. H., Van Dyke, J. M., Chissick, H. M., and Gorrod, J. W., J. Pharm. Pharmacol., 23, 560 (1971).

142. Dring, L. G., Smith, R. L., and Williams, R. T., Biochem. J., 109, 10P (1968).

143. Davis, J. M., Koping, I. J., Lemberger, L., and Axelrod, J., Ann. N. Y. Acad. Sci., 179, 493 (1971)

144. Beckett, A. H., Rowland, M., and Turner, P., Lancet, 1, (No. 7380), 303 (1965).

145. Werdegar, D., Johnson, D. G., Mason, J. W., J. Appl. Physiol., 19, 519 (1964).

146. Forsyth, R. P., and Rosenblum, M. A., J. Exp. Anal. Behav., 7, 367 (1964).

147. Forsyth, R. P., and Baireuther, R., Am. J. Physiol., 212, 1461 (1967).

148. Rowland, M., and Riegelman, S., J. Pharm. Sci., 56, 717 (1967).

149. Rowland, M., unpublished report.

150. Levy, G., J. Pharm. Sci., 54, 496 (1965).

151. Levy, G., J. Pharm. Sci., 54, 959 (1965).

152. Cummings, A. J., Martin, B. K., and Renton, R., Brit. J. Pharmacol. Chemother., 26, 461 (1966).

153. Cummings, A. J., and Martin, B. K., J. Pharm. Sci., 57, 891 (1968).

154. Barry, R. J. C., Jackson, M. J., and Smyth, D. H., J. Physiol., 182, 150 (1966).

155. Benet, L. Z., Intestinal pH and Its Effect on Drug Absorption I. An Alternative to the Virtual pH Hypothesis, Presented to the AphA Academy of Pharmaceutical Sciences, Las Vegas Meeting, 1967.