ABSORPTION AND BIOAVAILABILITY OF PREDNISOLONE IN THE DOG

Robert Paul Weatherby, B.Pharm., M.Sc.

A thesis submitted to The University of Sydney to fulfil the requirements for the degree of Doctor of Philosophy.

March, 1980

ACKNOWLEDGEMENTS

I wish to thank Professor T.R.Watson, Professor of Pharmaceutical Chemistry, for allowing me to be a candidate within the Department of Pharmacy, and Professor R.H.Thorp, Professor of Pharmacology, for offering and allowing me to use the facilities of his Department without which this work could not have been undertaken. I am grateful for the receipt of financial support in the form of a Commonwealth Post-Graduate Studentship from 1971 to 1975.

I also wish to express my gratitude to various members of the Department of Pharmacology for assistance during the progress of the experimental work, namely Associate Professor G.A.Starmer, Drs R.Einstein, J.A.Angus and H.M.Franks and Mr D.Richardson. Also to Dr C.R.Bellenger of the Department of Veterinary Surgery, Dr G.Benness of the Department of Surgery and Dr L.Arnold of the Department of Pathology.

During the revision of this thesis, many fruitful hours of discussion were spent with Dr Garry G.Graham, Department of Physiology and Pharmacology, University of New South Wales who was appointed Associate Supervisor in 1978, for which I am very appreciative. Thanks must also be recorded to Associate Professor R.A.Anderson, Supervisor since 1978, for his editorial attention.

I am deeply indebted to my Mother for the assistance she provided for the maintenance of the dogs, for much needed encouragement at some critical moments and for financial support from 1975 to 1976 and during periods from 1978 to 1980 whilst the thesis was revised, also my sister, Noelene for her help in the care of the dogs. I am also indebted to Mr P.W.Holley for assistance in preparing the original figures and diagrams some of which have been re-used. Finally I must mention my Mother and Sister again, for their assistance in proof reading of my typing of this revised manuscript and in the preparation of new figures.

R.P.W. 1980.

TABLE OF CONTENTS

Table of Contents (continued) Page

Table of Contents (continued) Page

Table of Contents (continued) Page

CHAPTER FOUR —— DISCUSSION

CHAPTER ONE

INTRODUCTION

1.1 General

"INSOLUBLE TABLOIDS

To the editors of THE LANCET

Sirs, - A fact has just come under my notice which I think should be brought before the profession, and I should like to ask whether other practitioners have observed any similar instances. A typhoid fever patient has been taking tabloids of quinine - two grains in each - three times a day at first and lately four times. Three days ago I discovered in one stool two tabloids - whole, but slightly enlarged - and I have found one and pieces of others each day since. This is the seventeenth day, as far as the date of invasion can be fixed, and there has been no diarrhoea, the bowels having acted once each day after an enema. In view of the number of tabloids of various kinds that are used now it appears to me a very serious matter if they are liable to pass through the stomach and intestines unaltered. - I am, Sirs, yours truly, WILLIAM G. GROVES.

Woodford-green, Feb. 15th, 1893. "

From The Lancet, page 507, March 4, 1893.

Incomplete gastrointestinal absorption of a drug from a formulation has long been recognised, as the 19th century quotation above shows. Only in the past 10 to 15 years has attention been directed to studying this problem and determining the factors necessary for efficient absorption from the gastrointestinal tract and at devising methods for the study and routine testing of indiv-

idual batches of formulations intended for oral administration.

The methods that have been used to measure the gastrointestinal absorption of drugs have suffered from various disadvantages. The rate of absorption *in vitro* is very much lower than *in vivo* whilst *in vivo* methods performed with anaesthetised animals may give altered absorption due to the surgical procedure and the anaesthetic. Other *in vivo* methods suffer from complications arising from simultaneous metabolism, distribution and elimination. In the present studies, the absorption of prednisolone was determined from the measurement of portal vein - arterial concentration differences. This method allows the amount of drug absorbed to be measured in a direct fashion. The studies required the development of a method of chronic catheterisation of the portal vein of the dog. In the present work the term "*intestinal absorption*" is defined as the net transfer of a substance from the lumen of the gastrointestinal tract to the mesenteric or portal blood.

Prednisolone is a potent anti-inflammatory corticosteroid used in a wide variety of diseases including, congenital adrenal hyperplasia, severe asthma and certain haematological, rheumatic, gastrointestinal and malignant conditions (Pickup, 1979). A closely related corticosteroid, prednisone, was first reported to be incompletely absorbed in 1963 (Campagna, Cureton, Mirigian & Nelson, 1963). Since then concern has been expressed about the bioavailability of both prednisone and prednisolone and there have been reports of therapeutic ineffectiveness of prednisolone tablets (Schneller, 1970; Engel, 1972). However, *in vivo* studies of prednisolone absorption have not provided any insight into the nature of the problem. Different prednisolone formulations all appear to produce adequate plasma concentrations despite very different rates of dissolution *in vitro*.

1.2 Methods of Studying Gastrointestinal Absorption

1.2.1 Introduction

Methods used to investigate gastrointestinal absorption vary considerably and therefore it is necessary to consider the various aspects of absorption that each method measures. A diagrammatic representation of the routes of gastrointestinal absorption is shown in Figure 1.1.

Drug absorption can be studied both *in vitro* and *in vivo*. In *in vitro* experiments, intestinal segments are isolated and removed from the animal. The tissue does not have an intact blood supply. In such experiments the substance being studied is usually added to the fluid bathing the mucosa and the rate of transport to the fluid bathing the serosa is measured.

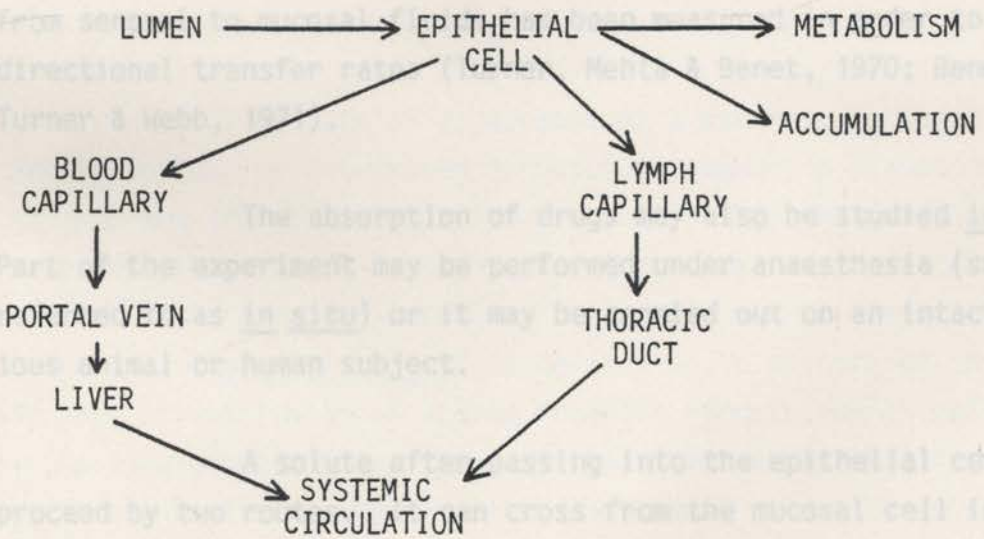


Figure 1.1. Diagrammatic representation of the routes of absorption from the lumen of the intestine.

In the process of absorption, a solute must first leave the lumen of the intestine (or stomach) and enter the mucosal (epithelial) cells of the intestinal wall. All in vitro methods measure the disappearance of drug from the mucosal fluid. The rate of appearance in the serosal fluid is also measured in most in vitro experiments. Metabolites formed in intestinal tissue, which may be either accumulated within the tissue or transferred to serosal fluid, can also be measured. Because of metabolism and/or storage in tissue, disappearance from the lumen and appearance in serosal medium are not necessarily equal. However, the pathway of absorption of drugs in in vitro experiments differs from the mode of absorption in vivo. To reach the serosal solution, the substance must cross the intestinal musculature, which is a significant barrier in vitro (Wolfe, Forland & Benet, 1973). In vivo, the drug is removed by the blood circulating through the intestine.

An important aspect of the mucosal cell is that the transfer of a substance across a cell wall is not uni-directional but bi-directional. In most studies, the drug is added to the mucosal fluid and the net transfer from mucosal to serosal fluids is measured. However, drugs have been added to the serosal fluids and net transfer from serosal to mucosal fluids has been measured in order to determine directional transfer rates (Turner, Mehta & Benet, 1970; Benet, Orr, Turner & Webb, 1971).

The absorption of drugs may also be studied in vivo. Part of the experiment may be performed under anaesthesia (sometimes referred to as in situ) or it may be carried out on an intact conscious animal or human subject.

A solute after passing into the epithelial cell, can proceed by two routes. It can cross from the mucosal cell into the blood capillaries which richly supply the mucosal layer of the intestinal wall and can then be transported (in normal subjects) by the mesenteric veins and the portal vein to the liver and the systemic circulation. In patients with portal hypertension or cirrhosis of the liver, the presence of portal-vena caval anastomoses may allow

drugs to be absorbed directly into the systemic system. Alternatively, the substance may cross into the lymphatics and gain access to the systemic circulation *via* the thoracic duct, thus avoiding passage through the liver although absorption of drugs *via* the lymphatic system has not been shown to be significant (Sieber, Cohn & Wynn, 1974). However the portal system is the major route by which drugs gain entry to the systemic circulation.

Disappearance of material from the lumen can be measured directly, using techniques such as perfusion of the intestinal segment. In intact man intubation or recovery of a solid dosage form from the faeces can be used, and in animals a variety of more invasive methods, such as fistulas, can be applied. It is not possible to measure the appearance of materials in the capillary blood directly, but it can be measured by collecting the blood from an intestinal segment after it has flowed into a mesenteric vein. Alternatively the appearance can be monitored by sampling either a mesenteric vein from a segment of intestine or the portal vein which represents the complete intestinal blood flow. Vascular perfusion also is an attempt to measure appearance directly from the capillaries but suffers serious disadvantages with regard to the integrity of the intestinal tissue.

The rate of appearance of substances in the systemic circulation can be determined directly by measuring plasma concentrations and indirectly by urinary excretion and pharmacological response. This is the basis of the widely applied pharmacokinetic approach. The rate and extent of absorption can be estimated from such data. "Bioavailability" is defined as, a measure of the extent and rate of absorption of a drug from its formulation as reflected by the time-concentration curve of the administered drug in the systemic circulation (Commonwealth Department of Health, NDF 4, 1976). Usually the bioavailability of a test preparation is measured relative to that from some standard preparation or dosage form of the same drug. The extent of absorption can be estimated by comparing the area under the time course of plasma concentrations after administration of the oral dosage form compared with the correspond-

ing area after intravenous dosage. Bioavailability is affected by the dissolution of the dosage form in the lumen, metabolism of the drug in the gut and liver, as well as the absorption into the mesenteric veins.

It should be noted that considerable metabolism may take place before an absorbed drug reaches the systemic circulation. Metabolism may occur during transit through the gut wall and/or during its first pass through the liver. This is known as the "first-pass effect". To distinguish between metabolism in the gut wall and in the liver during the absorption process, it is necessary to employ a method which measures the concentrations of the drug in mesenteric or portal blood. In vitro studies may also yield information on the metabolism of the drug in the gut wall. In order to measure accumulation in the gut wall in vivo, methods that measure disappearance and appearance must be used in combination.

When using in vivo methods involving measurement of concentrations in the vascular system, it is necessary to consider whether the measurement should be of blood or plasma. For total absorption, blood is required. However, for technical reasons the easier measurement in plasma is made. To obtain total absorption using plasma concentrations, the red blood cell-plasma partition ratio and the haematocrit are required and the assumption must be made that the red blood cell-plasma equilibrium is rapid. In very few studies of total absorption has either the measurement of blood concentration or the correction of plasma concentrations been carried out.

It is thus apparent that no single method of measuring absorption will give an understanding of the complete process and that information from several sources must be collated. The choice of method is influenced by which aspect of the absorption process is to be studied or whether the complete process is to be investigated.

1.2.2 *In vitro* methods

Early *in vitro* experiments using isolated intestinal segments were carried out in the 19th century, and have been reviewed by Parsons (1968). These early experiments used both everted and non-everted segments and were crude and uncontrolled. Most studies involved the absorption of dyes. However, the experiments of Waymouth Reid (1892, 1901b) are notable. He devised an apparatus for holding a living membrane in a vertical position between two compartments containing identical solutions. The transfer of fluid across the membrane was measured by the movements of the menisci in horizontal tubes connected to each compartment. With a membrane of rabbit intestine, a marked transfer of fluid occurred from the saline in the mucosal side to the serosal side. Waymouth Reid (1901b) also performed experiments using everted sacs of cat intestine, and measured the rise in the level of fluid in an observation tube tied into the sac.

(i) Non-everted preparations: Circulation methods

Fisher & Parsons (1949) described a preparation using isolated rat small intestine. This was the first effective *in vitro* intestinal preparation and is important because it recognised the need for the mucosal cells to receive an oxygen supply to remain viable. The original Fisher-Parsons method is quite elaborate but briefly the method is as follows:
The complete small intestine of the rat is suspended and immersed in oxygenated serosal fluid (the fluid bathing the serosal side of the intestine) and oxygenated mucosal fluid is circulated through it. Circulation of fluid is achieved by gas bubbles. Samples of both mucosal and serosal fluid can be taken at any convenient time, and at the conclusion of the experiment, the intestinal tissue can be homogenised and assayed for any substance which may have accumulated within it. Such preparations can transfer substances from the mucosal fluid to the serosal fluid. The method was modified by Wiseman in 1953 who used only segments of intestine instead of the

whole small intestine.

The original Fisher-Parsons method has not been widely used as it was quickly supplanted by more convenient methods. Curry, D'Mello & Mould (1970, 1971) used the original method to study chlorpromazine absorption. Glucose transfer (shown to be an active process) was taken as evidence of structural and functional integrity of the mucosa; histological examination revealed no damage to mucosa or villi. It was found that chlorpromazine, which was circulated in the mucosal solution, was rapidly absorbed by the tissue. Some of the chlorpromazine was converted to products which, together with unchanged drug, were partly retained in the intestinal wall and partly transferred to the serosal side of the tissue. Observations made using three different concentrations of chlorpromazine supported the hypothesis that transfer of unchanged drug occurred by passive diffusion. Nogami & Matsuzawa (1961) used the Wiseman apparatus with minor modification to study the kinetics of salicylic acid transfer. These techniques have not been employed as widely as everted sac methods although some experiments have been conducted using the everted intestine with perfusion and oxygenation of both mucosal and serosal sides.

(ii) Everted preparations

(a) Everted sacs

Early experiments using everted sacs were carried out by Waymouth Reid in 1901, but the technique was unsatisfactory because the relationship between the measured parameter (rate of fluid rise in a tube) and the rate of absorption was not simple (Bell & Parsons, 1976). However, the everted intestinal sac method, introduced by Wilson & Wiseman in 1954, has gained wide acceptance.

Briefly the technique (Figure 1.2) is as follows: A small segment of rat intestine is everted over a rod introduced into the lumen. The everted segment is placed in glucose-saline and tied at its ends into a sac, the sac being filled with a physiological

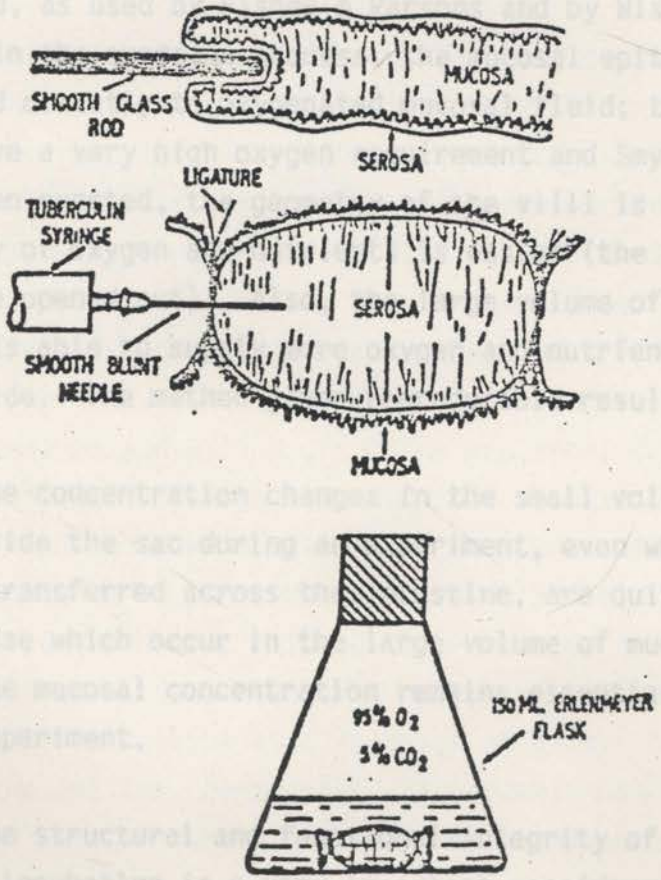


Figure 1.2. Technique for the preparation of everted sacs (Wilson & Wiseman, 1954).

buffer (serosal fluid). The sac is then immersed in a flask containing a larger volume of buffer solution in which the test substance is dissolved. The flask and its contents are in equilibrium with an appropriate gas mixture and incubated at 37^{o} for a specified period of time, after which the amount transferred is measured.

The everted sac technique has been a success largely because it is simple and convenient. More elaborate apparatus for circulating fluid, as used by Fisher & Parsons and by Wiseman, is not necessary. In the eversion process, the mucosal epithelial cells are exposed directly to oxygenated mucosal fluid; the epithelial cells have a very high oxygen requirement and Smyth (1974) suggests that when everted, the geometry of the villi is such that the accessibility of oxygen and nutrients is easier (the spaces between villi are opened out). Also, the large volume of fluid outside the sac is able to supply more oxygen and nutrients than the small volume inside. The method gives reproducible results.

The concentration changes in the small volume of serosal fluid inside the sac during an experiment, even when only a small amount is transferred across the intestine, are quite large compared with those which occur in the large volume of mucosal fluid. In many cases, the mucosal concentration remains essentially constant throughout the experiment.

The structural and functional integrity of everted intestine during incubation is a very important consideration. Everted intestine is "viable" for several hours after its removal from an intact blood supply with respect to certain metabolic processes and to active transport mechanisms. Bamford (1966) found that the rate of oxygen consumption by isolated ileal and jejunal segments of 18-day old rats was essentially constant over 3 hours. Similar findings were reported by Jordana & Ponz (1969) using intestinal segments from adult rats. These workers also observed substantial respiratory activity for up to 7 hours after excision of the intestine. Robinson & Felber (1966) showed that the active uptake of L-methionine and L-phenylalanine by rat intestine at 37^{o} was maintain-

ed for 2 to 3 hours after isolation. Benet, Orr, Turner & Webb (1971) have found that Krebs bicarbonate pH 7.4 buffer provided the best environment for maintenance of the in vitro membrane in a uniformly viable condition. Despite the strong case which can be made for the "viability" of the everted gut preparation, its usefulness in drug absorption studies (where passive mechanisms appear to predominate) is probably more dependent on structural than on metabolic integrity (Gibaldi & Grundhofer, 1972a).

Until recently, no evidence of the structural integrity of the rat everted sac preparation under the conditions of its use had been reported. In 1970, Levine, McNary, Kornguth & Leblanc observed that intestinal sacs of rats were morphologically intact after eversion but progressively lost structural integrity. Changes in the epithelial tissue from animals sacrificed by decapitation were apparent 5 minutes after incubation at 37^{o} in oxygenated buffer; at 30 minutes, 50 to 75 per cent of the normal epithelium had disappeared and at 1 hour, there was total disruption of the epithelial border. Damage occurred more slowly in tissues of animals sacrificed under anaesthesia and even after 60 minutes of incubation only 10 to 15 per cent destruction and disruption of the epithelial cells was noted. Gibaldi & Grundhofer (1972a) determined the mucosal-to-serosal flux of a number of solutes across the rat everted small intestine as a function of time. Certain compounds including aniline, benzocaine, salicylamide and antipyrine, which demonstrated initial clearance values of > 1 ml per hour showed little change in permeability over the entire 2-hour period of study under the experimental conditions. On the other hand, the transfer rates of compounds which were initially cleared at substantially lower rates, such as pralidoxime, riboflavine, methyl orange, eosin blue and bromothymol blue increased markedly and continually during the course of the experiment. Determination of drug transfer rates after the intestine was incubated in buffer revealed that the marked increase in the clearance of these polar compounds with time is due, in part, to a loss of functional integrity of the preparation.

An ultrastructural examination of everted rat jejunum (Lepper & Mailman, 1977) has shown an altered geometry. Intercellular "lakes" were found in the epithelia and it was suggested that eversion produced a mechanical blockage of normally free flowing capillary and lymph channels forcing transported solutes and water to form the "lakes" instead of being "washed out" into the lamina propria.

Changes in functional integrity of everted intestine with respect to mucosal-to-serosal transfer of riboflavine and methyl orange appeared to parallel the changes in structural integrity of the mucosal epithelium of isolated intestine as reported by Levine & others (1970). The loss of structural integrity appears to have no effect on the transfer rate of nonpolar, lipid-soluble compounds across the everted intestine, indicating that the epithelial border is not the rate-limiting barrier to transfer of these compounds across the rat everted intestine (Benet & others, 1971).

Parsons (1968) reviewed the objections to the use of everted sacs of small intestine. With rat intestine, the addition of glucose to the mucosal fluid stimulated vigorous fluid movements which were dependent on the initial degree of distension of the sac. Fluid transport in an actively functioning sac might cause considerable distension over the experimental period. The possibility of leaks and differing degrees of distension producing differing degrees of unfolding of the villous surface must also be considered. Wilson (1956) found that when hydrostatic pressure on the serosal surface of an everted segment of intestine exceeded about 4 cm of water, filtration of fluid from the interior of the sac tended to occur. Parsons (1968) suggested that the increase in hydrostatic pressure caused the mucosal cells to separate upward from their bases and to be attached only at the terminal bar region. This resulted in leakage between the cells. This disadvantage of closed sacs could be overcome by the use of a cannulated sac which is described later. The effective oxygenation of the serosal surface is doubtful, although some workers inject a bubble of oxygen with the fluid into the interior of the sac to improve this. A further criticism of everted sac techniques has been the influence of the change of the pH in the mucosal solution

(Chowhan & Amaro, 1977; Graham, 1979).

A major practical disadvantage which limits the use of the closed everted sac for drug absorption studies is the inability to follow a transfer with respect to time because samples cannot be removed during the course of the experiment.

However, the method continues to be used for drug absorption studies. An investigation of the transport mechanisms of β-lactam antibiotics was carried out by Penzotti & Poole (1974). They tested active transfer of penicillins and cephalosporins by measuring the ratios of drug concentrations between serosal and mucosal solutions. Equal concentrations of drug or glucose were established in the serosal and mucosal solutions and a serosal-to-mucosal concentration ratio greater than one was used as an indication of active transport. Experiments with glucose, known to be actively transported in everted sacs, resulted in a positive ratio. None of the β-lactam antibiotics tested demonstrated active transport. A linear relationship between rate of transfer and concentration provided further evidence for passive transport. The mechanism of transfer of pralidoxime has also been studied with the method (Hurwitz & Gutman, 1979; Hurwitz, 1979).

(b) Crane and Wilson modification of the everted sac technique

The Crane & Wilson (1958) modification (Figure 1.3) involves tying a cannula into the end of the sac, thereby creating an open-ended sac, from which samples can be withdrawn at any time.

With the sampling problems of closed sacs overcome, "Crane-Wilson sacs" have become the most widely used in vitro method for intestinal drug absorption studies. Disadvantages common to most in vitro methods remain, however. For example, since the intestine is removed from the animal and from its normal blood supply, both the functional and structural integrity of the preparation are suspect and the drug must traverse the entire thickness

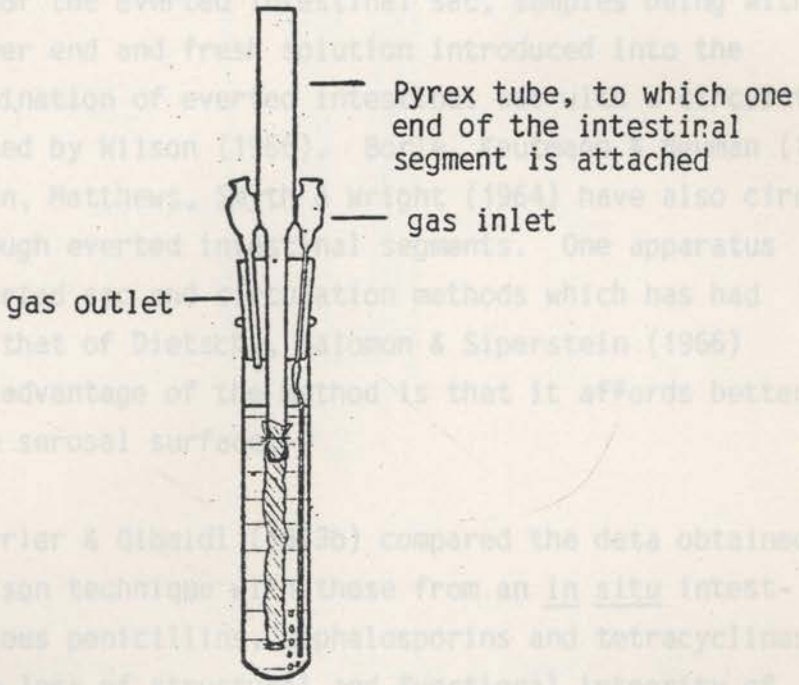


Figure 1.3. Apparatus for the Crane-Wilson Modification of the Everted Sac Technique. It is partially immersed in a constant temperature bath maintained at 37^{o} (after Crane & Wilson, 1958).

of the intestinal wall. This is in marked contrast to the intact animal, where the vessels supplying blood to the small intestine are interspersed between epithelial cells and the muscularis. Consequently, the transfer of a drug in an everted sac is quite different from that in vivo. Wolfe, Forland & Benet (1973) have attempted to overcome this problem by using everted sacs from which the muscularis has been removed (see page 19).

Variations and modifications to everted-intestine techniques have been made. Jorgensen, Landau & Wilson (1961) cannulated both ends of the everted intestinal sac, samples being withdrawn from the lower end and fresh solution introduced into the upper end. A combination of everted intestinal sac with a circulation method has been used by Wilson (1956). Borle, Keutmann & Neuman (1963) and Barry, Dikstein, Matthews, Smyth & Wright (1964) have also circulated fluids through everted intestinal segments. One apparatus combining both everted sac and circulation methods which has had subsequent use is that of Dietschy, Salomon & Siperstein (1966) (Figure 1.4). An advantage of the method is that it affords better oxygenation of the serosal surface.

Perrier & Gibaldi (1973b) compared the data obtained from the Crane-Wilson technique with those from an in situ intestinal loop for various penicillins, cephalosporins and tetracyclines. To account for the loss of structural and functional integrity of everted gut reported by Levine & others (1970) and Gibaldi & Grundhofer (1972b), experiments were conducted where the everted intestinal preparation was incubated in drug-free buffer solution for up to 90 minutes. During the incubation of these preparations, "sham" samples of serosal solution were taken at 30 minute intervals and discarded. When the 90 minute incubation period had expired, the preparations were immediately transferred to a mucosal solution and the serosal compartment sampled. From this Perrier & Gibaldi (1973b) calculated clearances for the first 30 minute period as a standard, assuming negligible loss of integrity (Levine & others, 1970). For all the antibiotics studied, the first 30-minute period clearances were less than the steady-state clearances but a rank-order agree-

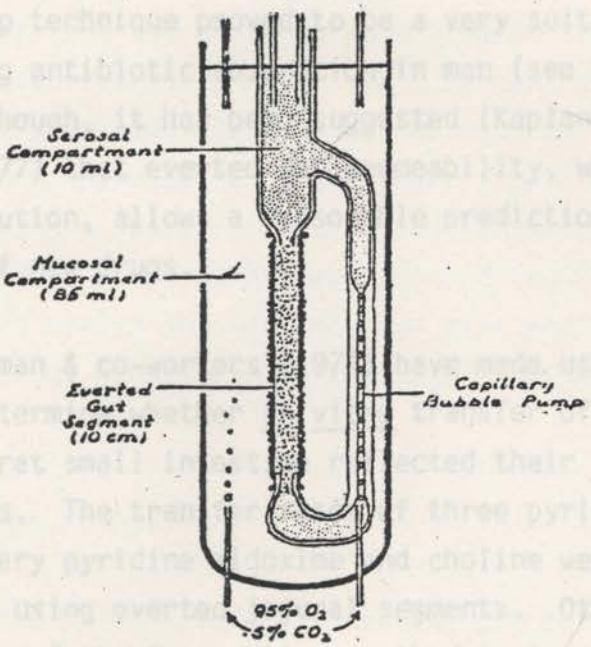


Figure 1.4. Perfusion apparatus of Dietschy & others (1966). A 10 cm segment of small intestine is everted on a glass rod, then mounted on the central glass carrier. This carrier contains the serosal perfusion solution (10 ml volume) which is constantly circulated through the gut segment by means of a capillary bubble pump. The outer vessel contains the mucosal perfusion solution (85 ml volume) which is constantly mixed by bubbling 95 per cent oxygen:5 per cent carbon dioxide through it. The entire apparatus is placed in a constantly stirred water bath at 37^{o}.

ment was found. When compared with data from the in situ intestinal loop preparation, a rank-order agreement was found for the various penicillins and the tetracyclines tested but not for the cephalosporins. When data obtained from the in vitro sacs and the in situ loops were compared with those from humans (obtained using the urinary excretion method), Perrier & Gibaldi concluded that in vitro sac methods were unsuitable as a model for human drug absorption, but the in situ loop technique proved to be a very suitable animal model for predicting antibiotic absorption in man (see page 35). On the other hand though, it has been suggested (Kaplan & Cotler, 1972; Chowhan & Amaro, 1977) that everted sac permeability, when coupled with data on dissolution, allows a reasonable prediction of problems in the absorption of new drugs.

Feldman & co-workers (1974) have made use of the Crane-Wilson method to determine whether in vitro transfer of drug complexes across the everted rat small intestine reflected their in vivo absorption characteristics. The transfer rates of three pyridinium aldoximes, a non-quaternary pyridine aldoxime and choline were measured by Crone & Keen (1969) using everted jejunal segments. Other examples of the extensive use of the Crane-Wilson method to investigate drug absorption phenomena are-: (a) the effect of various cations on passive transfer (Mayersohn & Gibaldi, 1970; Mayersohn, Gibaldi & Grundhofer, 1971) and (b) the effect on transfer of pH and other substances, such as gelatin, methylcellulose, skim milk, albumin and sodium lauryl sulphate (Lovering & Black, 1974).

The everted intestinal segment perfusion apparatus of Dietschy & others (1966) has been used by Turner, Mehta & Benet (1970) in their study of apparent directional permeability coefficients of drug ions, and by Benet & co-workers (1971) to follow the absorption kinetics of salicylate ion and acetanilide.

(iii) Transport cells using an intestinal segment as a membrane

The first use of a flat segment of intestine as a membrane separating two compartments in a cell was by Waymouth Reid in

1892. Ussing (1949) devised a more refined version but little use has been made of these preparations. Mayersohn & Suryasaputra (1973) have designed a transport cell, which they claim obviates eversion. A section of flat intestine is placed between the two halves of a cell and the drug in buffer solution introduced into the mucosal side and buffer solution into the serosal side. The compartments are stirred and oxygenated and sampling carried out via a pipette. A disadvantage of this method is that the small surface area of the membrane available for diffusion results in only small concentrations being found in the serosal side, and limits assay methods to those that are very sensitive. Since Mayersohn & Suryasaputra (1973) everted the intestine before taking a section for use, the claimed advantage of non-eversion seems to be lost. Considerable handling is inevitable when the intestine is placed in position over pins and the possibility of stretching the tissue or of introducing leaks must also be considered.

(iv) Intestinal rings

Everted or non-everted intestine can be cut into small rings and the measurement of the rate of accumulation or uptake of a substance from the solution in which these rings are bathed can be made (Agar, Hird & Sidhu, 1954). The ring technique can provide useful information on accumulation and metabolism in the intestinal wall and also for comparison of the rate and extent of active transport under various conditions. It is simple and highly reproducible (Crane & Mandelstam, 1960). The retraction of the circular and longitudinal muscle coats, which occurs after the rings have been prepared, causes a separation and spreading of the villi in a manner which aids the access of oxygen and substrates. Boass & Wilson (1962) studied the absorption mechanisms of vitamin B_{12} using uptake by small rings of hamster ileum.

(v) Isolated fractions of the intestinal wall

A problem in the use of isolated intestinal preparations *in vitro* is that transfer of a compound from the mucosal to the serosal side involves passage through the muscularis. In the intact animal, transport occurs *via* small blood vessels lying between the muscularis and the epithelium. Consequently, the rate of transfer of a compound in an *in vitro* situation is often slower than *in vivo*. In attempts to overcome this difficulty, some investigators have used fractions of the intestinal wall.

Hakim, Lester & Lifson (1963) stripped away enough of the muscularis from dog small intestine for a diaphragm of mucosal tissue to be set up between two compartments. These consisted only of epithelium and lamina propria and were found to have similar transport characteristics to whole rat small intestine *in vitro*: dependence of water transport from isotonic Ringer fluid on the presence of glucose in the medium, water absorption against an osmotic activity difference, sensitivity of water transport to small excess hydrostatic pressure on the serosal side and uphill glucose movement. This preparation was considered suitable for absorption studies. Unstripped rat jejunum and tissues from which the serosa and muscularis had been separated were used by Barnett, Hui & Benet (1978) in a transport cell to study salicylate transport. The flux values for the stripped preparations were found to be approximately double those of intact intestine.

Parsons & Paterson (1965) removed the muscle layers from a segment of rat colon leaving mucosa, supported by a thin layer of muscularis and prepared an everted sac which was used to investigate fluid and sugar transport. The absorption kinetics of salicylate ion through intestinal membranes from which the serosa and both the longitudinal and transverse layers of the muscularis externa were surgically removed were followed by Wolfe & others (1973). These sections consisted of epithelium, underlying lamina propria, muscularis mucosa and submucosa. Transfer rates were measured using

everted intestine in a perfusion apparatus (Dietschy & others, 1966) and a Crane-Wilson sac. The rates of salicylate transport through the stripped intestinal segments were found to be 1.5 to 1.8 times greater than those through intact segments.

A different line of approach was taken by Nayak & Benet (1971) who separated the columnar epithelium from rat intestine by treatment with ice-cold edetic acid solution. This preparation of denuded intestinal musculature was used in a perfusion apparatus (Dietschy & others, 1966) to monitor the kinetics of salicylate ion and acetanilide. Differences in directional transfer rates, which occur with untreated intestinal membrane, were absent and transfer rates through the muscle layer were found to be at least double those in untreated membrane. However oedema occurred in the muscularis mucosa after the edetic acid treatment but could be eliminated by rinsing with magnesium chloride or calcium chloride solutions. Results obtained with this preparation were at variance with those of Mayersohn, Gibaldi & Grundhofer (1971), possibly because of edetic acid induced damage.

Perrier & Gibaldi (1973b) separated the epithelial layer from the mucosal surface of rat intestine and made Crane-Wilson everted sacs. Little change was found in the mucosal-to-serosal clearance of cephalosporins compared with whole intestinal wall and they suggested that the epithelial layer was not the primary barrier *in vitro* and that the muscle layer was equally important. A similar finding was reported for prednisolone and its amide complex by Feldman & co-workers (1974).

1.2.3 *In vivo* methods

(i) Difference methods

Difference methods are relatively simple techniques involving oral administration of the drug and measurement of residual material. Absorption is determined as the difference between the

dose and the amount recovered. Many of the early studies have been carried out using these techniques (Parsons, 1968), but a recent example of this type of experiment is a study by Grundy, Mcainsh & Taylor (1974). They investigated the effect of food on the release of propranolol from a PVC matrix tablet. Dogs were given tablets, either one hour or 24 hours after feeding, which were subsequently recovered intact from the faeces and assayed for propranolol. They found that feeding decreased the transit time from 44 to 25 hours and the amount of propranolol absorbed from 90 per cent to 47 per cent.

An alternative type of difference method is represented by the experiment carried out by Cori in 1925. He administered sugar solutions by gavage to 48-hour fasted rats. After a predetermined time the animals were killed and the gastrointestinal tracts were removed and the contents assayed. Variation of this method includes homogenisation of the gut (Wright & Crowne, 1972; Skellern, Stenlake & Williams, 1973) and incorporation of a dye marker (Reynell & Spray, 1956a,b; Feldman, Wynn & Gibaldi, 1968) to monitor gastric emptying or intestinal transit time. The Cori method has been extensively used for investigating physiological substances, but since difference methods treat the intestinal tract as a single entity, they do not allow for detailed analysis of absorption data.

(ii) Pharmacokinetic methods

(a) Blood levels

Drug absorption can be monitored by following concentration changes in systemic blood. It is relatively easy to obtain several blood samples by venipuncture, although in some experiments, chronically implanted catheters have been used. The relationship between blood concentrations and the rate of absorption is not simple. Absorption experiments in which blood levels of carbohydrates, amino acids etc. are measured for clinical tests are

sometimes termed tolerance curve studies, the tolerance curve being a plot of blood concentration against time.

Barlow & Climenko (1941) used time course of blood concentrations to study the absorption profiles of sulphapyridine and sulphathiazole in the monkey and man, and in the 1950's increasing interest was shown in using blood concentration studies to follow drug absorption (Truitt, McKusick & Krontz, 1950; Smith, 1951; Swintosky & co-workers, 1957, 1958). A large amount of work has since followed.

Analysis of the time course of plasma concentrations of a drug yield both qualitative and quantitative data of the rate and extent of absorption. Since the blood concentrations of a drug are influenced not only by the rate of absorption, but also by distribution into the tissues, metabolism and by the rate of excretion, it is necessary to postulate a mathematical model of the processes involved. The first detailed mathematical treatment of the kinetics of absorbed substances in the bloodstream was given by Teorell in 1937.

The simplest model that can be assumed to represent the administration of a drug is a one-compartment model. In this, the body is represented by the single compartment from which drug is eliminated (metabolism or renal excretion) by a first-order process. Dominguez & Pomerene (1945) assumed first-order absorption and elimination and derived an equation for the calculation of the rate constant of absorption based on the one-compartment model:

$$\frac{dA}{dt} = V\left(\frac{dC}{dt} + K.C\right) \qquad \text{equation (1.1)}$$

where $\frac{dA}{dt}$ is the rate of absorption, V is the apparent volume of distribution, C is the blood level in concentration units at time t, $\frac{dC}{dt}$ is the slope of the blood level-time curve at time t and K is the first-order rate constant for loss of drug from the volume of distribution. To use the equation it is usually necessary to estimate the apparent volume of distribution of the drug in the compartment. The integrated equation for a one-compartment open

model with first-order absorption used for single dose studies is

$$C = \frac{F.D}{V} \cdot \left(\frac{k}{k-K}\right)\left(e^{-Kt} - e^{-kt}\right) \quad \text{equation (1.2)}$$

where D is the dose of drug administered, F is the fraction of the dose which is absorbed and k is the first-order rate constant for absorption. The values of K and k can be estimated by standard feathering methods (Notari, 1975). Subsequently, better estimates of the pharmacokinetic constants can be determined by computerised iterative methods (Notari, 1975). Wagner & Nelson (1963) introduced a method which plots cumulative per cent absorbed against time and which does not assume that absorption is first-order.

Loo & Riegelman (1968) tested the assumption that the body is a single compartment by infusing drugs intravenously at a known rate and found that the mathematical methods based on the single-compartment model did not result in acceptable estimates of the rate constants of absorption. They then presented an equation based on a two-compartment model which gave accurate estimates of the known infusion rates. The model assumes that the drug distributes between a central and a peripheral (tissue) compartment with elimination occurring only from the central compartment. In order to calculate the rate of absorption, the drug must be administered on two occasions, orally on one occasion and intravenously on another.

The amount absorbed is related to the area under the blood concentration-time curve. Tidwell (1950) computed areas under curves to study fat absorption and similar methods are currently used to determine bioavailability. The area under the plasma concentration curve (AUC) following intravenous administration represents complete absorption. The fraction of an oral dose effectively absorbed into the systemic circulation is given by

$$F = \frac{AUC_{0-\infty(oral)}}{AUC_{0-\infty(intravenous)}} \quad \text{equation (1.3)}$$

This fraction may be less than one (1) because of incomplete absorp-

tion from the gastrointestinal tract but also because of metabolism of the drug in the intestinal wall or in the liver. A disadvantage of blood concentration studies is that the measurement is removed from the actual absorption site in the gastrointestinal tract. A drug may be well absorbed into the mesenteric blood, but only a small amount may gain access to the systemic circulation because of this first-pass metabolism in the liver. An example is propranolol, which after infusion into the portal vein of a rat, gives an area under the curve much smaller than that obtained after an infusion into a peripheral vein (Suzuki & co-workers, 1974). Clearly therefore the use of systemic blood levels to follow absorption after the oral administration of propranolol, can underestimate the amount absorbed from the gastrointestinal tract.

The advantages of blood concentration studies are many. The experimental procedures are simple to perform, although they must be very carefully controlled. Such studies can be carried out in human subjects without undue risk. Using human subjects avoids possible species differences in response which can arise with use of other animals; this is important when the absorption behaviour of a dosage form is required. Methods involving the analysis of plasma concentrations or urinary excretion data (see next section) are the only routine techniques available for studying the rate of absorption of drugs in man.

(b) Urinary excretion

The urinary excretion methods are useful when only comparative measures of total absorption are needed; the determinations are obviously less direct than blood levels, being a step further away from the absorption process. Advantages of this approach are that it is non-invasive in nature and that many drugs are concentrated in the urine and are therefore more easily assayed.

In 1867, Bence Jones & Dupre identified quinine in the urine of human subjects 10 to 20 minutes after they had taken the drug by mouth. McCance & Madders (1930) used a urinary excretion

method to study sugar absorption. Hevesy & Hofer (1934) found that deuterium oxide appeared in the urine of humans within 30 minutes of drinking. In 1941, Barlow & Climenko used urinary excretion in their study of the rate of absorption of sulphapyridine and sulphathiazole. Melnick, Hochberg & Oser (1945) devised a method using urinary excretion for determining the physiological availability of vitamins. They compared the oral absorption of tablets with that of a solution. The same method was used by Chapman, Crisafio & Campbell (1954, 1956) for determining the relative availability of sugar-coated tablets of riboflavine and sodium p-aminosalicylate.

The urinary excretion method does have disadvantages. The limited number of samples that can be obtained, even when a subject is water loaded, hinders the analysis of data. Water loading itself may be undesirable, since it may alter the rate of absorption of the drug concerned. The determination of total absorption requires collection of urine over many half-lives. In bioavailability studies it is suggested that urine specimens be collected for seven half-lives or more (Commonwealth Department of Health, NDF 4, 1976). The concentrations of drug and/or metabolites in the later urinary collections may be very low and difficult to assay. Alternatively, excretion can be extrapolated to infinite time by the method of Beckett & Rowland (1965) from urinary collections over 2 to 3 half-lives. Another problem concerned with the remoteness of urinary excretion from the absorption process is when in addition to absorption, a drug is simultaneously lost to an extravascular compartment via a parallel process (Perrier & Gibaldi, 1973a). The possible presence of a lag time between changes in blood concentrations and changes in the rate of excretion makes studies on the rate of absorption of drugs very difficult using this method (Wagner & Northam, 1968).

For valid conclusions about absorption to be drawn from urinary excretion data, similar mathematical treatments to those used for blood concentration data is required. The methods available have been extensively reviewed by Wagner (1971, 1975), Gibaldi & Perrier (1975) and Notari (1975).

(c) Pharmacological response

The onset, duration and intensity of a pharmacological response to a drug may be used as a measure of its absorption, provided the response can be accurately measured without subjective bias, and can be related to the amount of drug in the body.

However, the use (where possible) of a pharmacological response to measure availability has a number of advantages over urinary excretion and blood sampling methods. No further assay is required, since the response is the assay, and time is thus saved. Blood sampling is an invasive process, while measurement of response may not be. Also, blood and urine samples are taken at discrete time intervals, whereas pharmacological response can often be continuously measured.

Portmann, Minatoya & Lands (1965) calculated the absorption rate of isoprenaline from the changes in heart rate of unanaesthetised dogs, and Dittert & co-workers (1968) have shown that a relationship exists in mice between the oral LD_{50} and the rate of absorption of paracetamol. The mydriatic response has been used to estimate the quantity of a drug in the body after oral administration (Irwin & co-workers, 1969; Smolen & Schoenwald, 1971; Smolen, 1971). The use of pharmacological response data in absorption analysis has been reviewed by Smolen & Weigand (1973) and Smolen (1978).

In most cases the pharmacological effect is very difficult to determine accurately and absorption studies involving the measurement of pharmacological effects are rare.

(iii) Intubation methods

In intubation techniques, gastric or intestinal fluid is removed for analysis via a tube which is passed into a specified section of the gastrointestinal tract. Test solutions can be administered by the same tube and disappearance of material from the lumen

of the intestine is measured.

The first intubation technique allowing removal of samples was introduced by Miller & Abbott in 1934. It involved the use of a double-lumen rubber tube of relatively small calibre, to the distal end of which was attached a collapsible rubber balloon which could be distended at will. The balloon when distended in the bowel, stimulated sufficient peristalsis to propel the apparatus through the length of the small intestine in three to four hours. During this time, it formed an obstruction to the flow of intestinal contents so that they could easily be aspirated from above the balloon through suitably located holes in the other lumen of the tube. However, the samples obtained through the tube were contaminated with biliary and pancreatic secretions, and quantitative studies could not be carried out.

A modified apparatus was devised (Abbott & Miller, 1936) containing three lumens (Figure 1.5). Two of the lumens were used for the control of collapsible balloons, one at the distal end of the tube and the other at a variable distance from it. An isolated segment of small intestine is produced between the balloons when they are inflated. The third lumen of the tube, through

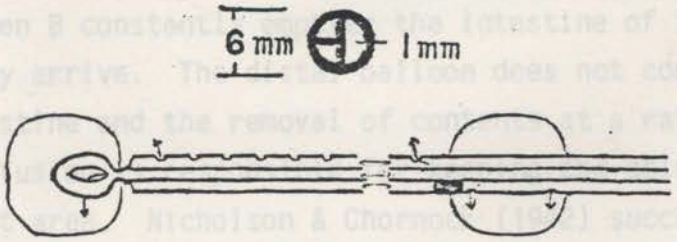


Figure 1.5. Three lumen intubation apparatus of Abbott & Miller (1936).

perforations in the outer wall between the balloons, communicates with the interior of the isolated segment, allowing the contents to be aspirated at any time. Considerable use has been made of the double and triple lumen tubes in studying absorption in humans. Glucose absorption was studied by Abbott, Karr & Miller (1938), and Miller (1944) reviewed the use of the intubation methods up to that time.

However the use of the triple-lumen tube with balloons isolating an intestinal segment was considered to have disadvantages in absorption studies (Nicholson & Chornock, 1942; Miller, 1944). The damming back of large volumes of the test solution above the distal delimiting balloon often leads to an escape of some of the material beyond the test area. Conditions do not duplicate those that occur during normal digestion of food and any mechanical obstruction, such as a balloon above the area under study, might interfere with the normal progress of peristaltic waves. Nicholson & Chornock (1942) introduced an improved technique to overcome these problems. The method involves the maintenance of a continuous perfusion of a solution downward through the lumen of a selected segment of small intestine. The apparatus (Figure 1.6) consists of a double-lumened rubber tube with a single-lumen extension. The end of the single-lumen extension has multiple suction holes in the distal 10 cm and a rubber balloon tied to it which is controlled by a separate rubber tube. When the apparatus is in place, a solution flowing through lumen A enters the mid-duodenum while suction applied to lumen B constantly empties the intestine of its contents as fast as they arrive. The distal balloon does not completely block the intestine and the removal of contents at a rate faster than their perfusion is responsible for keeping the solution entirely within the test area. Nicholson & Chornock (1942) successfully tested this apparatus measuring ascorbic acid absorption. It was claimed that the functions of the small intestine were not disturbed by the procedure itself, however the presence of the tube must influence the intestinal tone.

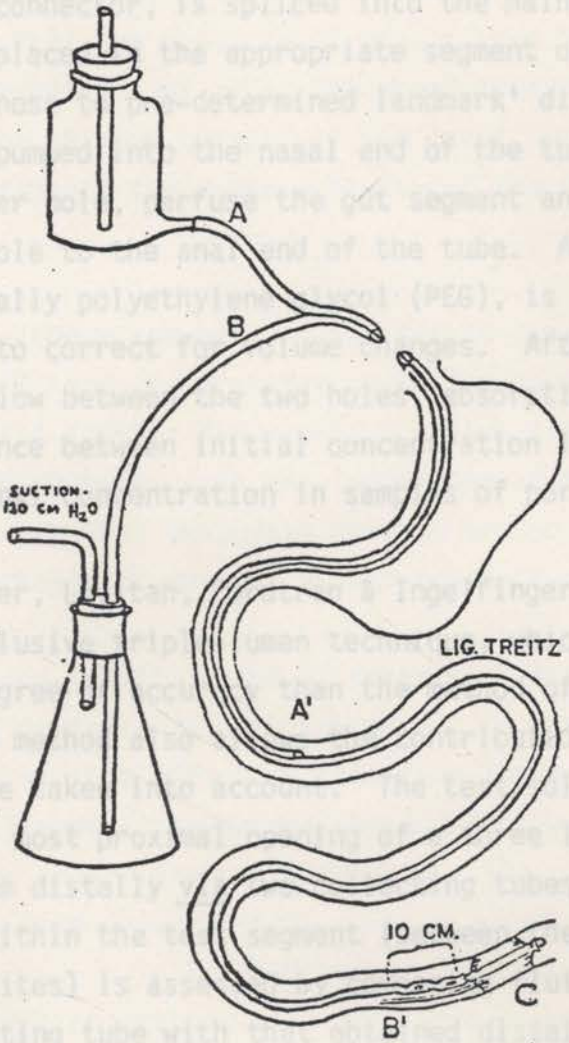


Figure 1.6. Arrangement of the apparatus used by Nicholson & Chornock (1942).

Blankenhorn, Hirsch & Ahrens (1955) introduced an intubation method without the use of balloons. First, a single-lumen tube is passed from the nares to the anus. Then, a section, made up of two pieces of tubing attached to a solid connector with holes for aspiration (15 to 30 cm apart) cut in the tubing on either side of the connector, is spliced into the main tube. The spliced section is placed in the appropriate segment of gut for study by means of 'nose to pre-determined landmark' distances. Test solutions are pumped into the nasal end of the tube, enter the gut through the upper hole, perfuse the gut segment and are sampled through the lower hole to the anal end of the tube. A nonabsorbable indicator, usually polyethylene glycol (PEG), is included in the test solutions to correct for volume changes. After achieving a steady-state of flow between the two holes, absorption is measured by the difference between initial concentration in the solution perfused and the final concentration in samples of perfusates.

Cooper, Levitan, Fordtran & Ingelfinger (1966) developed a non-occlusive triple-lumen technique, which is claimed to give a higher degree of accuracy than the method of Blankenhorn & others (1955). The method also allows the contribution of endogenous substances to be taken into account. The test solution is infused through the most proximal opening of a three lumen tube and samples 10 and 40 cm distally via two collecting tubes. Net absorption or secretion within the test segment (between the proximal and distal collecting sites) is assessed by comparing fluid obtained from the proximal collecting tube with that obtained distally. The fluid from the proximal collecting tube is a mixture of infused solution plus endogenous solute and water. The test solutions contain a non-absorbable marker as in other intubation methods. However practical disadvantages of this method are the long equilibration period and the need for simultaneous sampling at two levels.

A four-lumen proximal occlusive balloon method was introduced by Phillips & Summerskill (1966). One lumen controls the inflation of the balloon, another serves to aspirate the gut above the inflated balloon. Test solution is infused immediately

below the balloon through the third lumen and perfusate is aspirated at the end of the test segment through the fourth lumen which opened 25 cm below the tip of the third lumen. Because of its simplicity, this proximal occlusive balloon method has been widely used (Modigliani, Rambaud & Bernier, 1978). However criticism of the method centres on the possibility, held in common with all methods involving balloons, that the presence of an inflated balloon at the entry of the test segment might alter the absorptive process in the segment. This can be through changes in the local mucosal blood flow or of modification in the motor activity of the underlying segment.

In a comparison between the triple-lumen technique of Cooper & others (1966) and the proximal occlusive balloon method of Phillips & Summerskill (1966), Modigliani & others (1978) demonstrated identical absorption rates with the two methods for water and ions, concluding that the proximal occlusive balloon method was valid for these substances although it may not be for nonionic compounds.

Shields (1972) looked at intestinal tubes in position when some intubated patients required surgery. In some of these patients, a concertina-like effect of intestine around the tube was found. This would increase the mucosal area and give rise to varying absorption rates in different experiments. The significance of this effect has not been investigated.

Intubation methods have been widely used by physiologists and clinicians in absorption studies, with various modifications to the techniques for specific purposes. The methods have also been adapted for use in animals (Bucher, Anderson & Robinson, 1950). Schedl & Clifton (1963) studied the absorption of hydrocortisone in man using the technique of Blankenhorn & others (1955). The simultaneous measurement of the rate of disappearance from the small intestine of hydrocortisone and its appearance in the peripheral blood (Schedl, Clifton & Nokes, 1964), showed that during perfusion the initial rise in plasma hydrocortisone was rapid and tended to plateau at 2 to 3 hours. The concentration of plasma hydrocortisone

paralleled the disappearance rate. When the intestinal disappearance rate of hydrocortisone was 59 per cent (from constant rate perfusion at steady state), the plasma concentrations rose more rapidly to higher levels with less tendency to reach a plateau than when the disappearance rate was 32 per cent. Lower disappearance rates (27 to 40 per cent) resulted in no differences in the blood levels, suggesting that rate of inactivation of hydrocortisone in passing through the liver appears to be more important than the rate of disappearance (absorption) from the small intestine in determining its physiological availability. Hogben, Schanker, Tocco & Brodie (1957) studied the absorption of drugs from the intubated stomach of man. A stomach tube was passed while the subject rested in the left supine position. This position is used since it prevents the loss of infused solution from the stomach and reflux of intestinal contents including bile into the stomach. Phenol red was included in the drug solutions introduced into the stomach to allow correction for the change in drug concentration due to dilution by gastric secretion and swallowed saliva. The absorption of aspirin and salicylic acid has been similarly investigated (Overholt & Pollard, 1968; Ivey, Morrison & Gray, 1972).

Although intubation methods have been widely used for measuring absorption in physiological studies, little direct comparison with other methods has been made to ascertain the relative accuracy of the various techniques. No comparisons have been made concerned with drug absorption. The methods yield direct measurements of the rate of absorption, but it is considered that the experimental conditions differ from the normal situation existing in the intestine (Schedl, 1965). Schedl (1965) admits the conditions he used exaggerate the absorption of slowly absorbed compounds. The methods also involve considerable discomfort to the subjects especially when occlusive balloons are used. Subjects are clearly apprehensive of the intubation methods and their invasive nature limits use with conscious subjects although patients requiring intubation as a clinical procedure can be used experimentally. Intubation methods have a place in investigations of drug absorption since they are the only practical way of determining in humans the

rate of absorption of drugs at different sites in the gastrointestinal tract and the disappearance of a drug from the lumen.

Nonabsorbable indicators or markers are routinely included in the test solutions instilled *via* intubation. This allows for correction of the concentration of test compound from changes which may occur as a result of water absorption or secretion during the test period. The validity of these nonabsorbed indicators was tested by Schedl, Miller & White (1966), using the method of Blankenhorn & others (1955) and by French, Brown, Good & McLeod (1968) using a modified Nicholson-Chornock tube. Both groups found that polyethylene glycol and phenol red were suitable indicators in limited segments of intestine, although polyethylene glycol was preferred because it is absorbed to a lesser extent and any absorption is not influenced by the pH of the environment. $^{51}CrCl_3$ is also a good nonabsorbable marker (Ivey & Schedl, 1970) and is probably the best indicator because it is more easily estimated and gives reproducible results. It does not need immediate assay and the presence of bile, blood, mucus etc. does not effect the counting efficiency. Schedl & others (1966) found discrepancies between the indicators in patients with malabsorption, which could not be explained solely by some absorption of phenol red or by interference with phenol red analysis by colour in the samples. A general characteristic of indicators is that recovery of indicator is initially low. This low recovery could be due to saturation of a surface adsorption process or adsorption to contents of the stomach such as mucus or other suspended material (Ivey & Schedl, 1970).

(iv) Catheterisation of lymphatics

The first interest in gastrointestinal absorption resulted from the discovery of lacteals in the 17th century. Following this discovery many experiments were undertaken to trace the origin and fate of the chyle after the administration of food. These early experiments are described in the review by Parsons (1968). *In situ* experiments involving lymph collection are

usually difficult because inhibition of flow occurs for many reasons. In 1948, Bollman, Cain & Grindlay introduced methods for cannulating the lymphatic vessels from the liver and intestine and the thoracic duct in rats. With the animals held in a restraining cage, lymph was collected from the conscious rats for periods of up to 10 days. Grindlay, Cain, Bollman & Mann (1950) described techniques for the continuous collection of lymph through flexible cannulas inserted into various lymphatic trunks including those draining the intestine of unanaesthetised dogs. They reported that the lymph flow could be maintained for up to 8 days from the thoracic duct and for 2 to 3 days from intestinal lymph vessels. Gralla, Cappiello & Jonas (1973) devised a technique for chronic cannulation of the thoracic duct in dogs. Samples of lymph were collected from the side arm of the cannula which passed through the dorsal thoracic wall. A small infusion line was attached which conveyed a constant, low velocity stream of anticoagulant solution to avoid clotting of the lymph. Samples of lymph were obtained for up to three weeks. Lymphatic fistulas in human subjects have been used and the thoracic duct has been cannulated for lymph collection (Bierman & co-workers, 1953).

DeMarco & Levine (1969) used the method of Bollman & others (1948) to cannulate the thoracic duct or the mesenteric lymph vessel in order to study the role of the lymphatic system in the absorption of drugs. They found 1.5 per cent of the absorbed dose of both p-aminosalicylic acid and benzomethamine in the thoracic duct. With tetracycline, only 0.26 per cent of the absorbed dose was found in the thoracic duct, but evidence for direct absorption of tetracycline into the lymph was found. Fluorescence in the central lacteals was noted within one minute of administration.

If a drug is absorbed via the lymphatics it avoids a "first-pass" of the liver but the rate of transport of lymph away from the small intestine is minimal compared with the rate of blood flow. The role of the lymphatic system in the absorption of drugs from the intestine appears to be minor (DeMarco & Levine, 1969; Sieber, Cohn & Wynn, 1974), although the lymph route is a very important pathway for the absorption of triglycerides and

cholesterol. For significant lymphatic absorption of a foreign compound to occur, it must be highly lipid soluble and is dependent upon the concomitant absorption of lipids. For example, DDT is carried in the lipid core of chylomicrons (Sieber & others, 1974). Except in studies on the absorption of very lipid soluble drugs, the measurement of drug concentrations in lacteals is not considered because of the small percentage of drugs absorbed _via_ the route and also because of the technical difficulties in collecting lymph. Furthermore, the presence of drugs in lymphatics does not indicate that drugs are directly transported from cells lining the gastro-intestinal tract to lacteals. Drugs could diffuse from blood to lacteals as part of the general distribution of the drug in body fluids.

(v) The use of closed or perfused segments with or without mesenteric blood collection

(a) Closed segments

With closed segments the abdomen is opened under anaesthesia and a segment of intestine is isolated, rinsed and filled with a known amount of the test solution. The isolated segment is then returned to the abdomen. After a predetermined time, the contents of the segment are aspirated and absorption is determined by difference. Early experiments of this type were carried out by Waymouth Reid (1900, 1901a). Dogs, which had been previously fasted for 24 hours were anaesthetised and two adjacent segments of intestine were isolated, one of which served as a control. Extreme care was taken not to impede the mesenteric circulation of the isolated segment. After 15 minutes, the fluid remaining was analysed. Using this technique the absorption of water, peptone, glucose and maltose was investigated.

Sollmann, Hanzlik & Pilcher (1910) studied the absorption of phenol using _in situ_ gut preparations in cats and dogs. Two types of procedures were followed. In one, animals were killed at various intervals after administration of the drug; in

the other, the drug was divided among several loops in the same animal, the loops being excised successively. They noted very rapid absorption of phenol in the first 5 minutes, a steady decrease to 30 minutes and almost no absorption after one hour. Total absorption averaged 44 per cent. Identical behaviour was found in cats and dogs and in both stomach and intestine. The effect of other substances on the absorption of phenol was also studied. It was found that "local changes in the intestinal circulation have the most conspicuous effect on the absorption". Local vasoconstriction diminished the absorption of phenol whilst vasodilation increased it. Hanzlik & Collins (1913) investigated the absorption of ethanol in the same manner and also looked at the distribution of ethanol between the lumen and the wall of the intestine. Closed intestinal segments have been used extensively by physiologists but it was not until the 1950's that they were employed to measure drug absorption.

In 1955, Levine, Blair & Clark described the preparation of an intestinal loop in the rat which they used to study the factors influencing the absorption of benzomethamine. In the preparation, the abdominal cavity of fasted rats is opened under light ether anaesthesia. A single loop of intestine is isolated and filled with a quantity of drug solution which does not distend the loop. The incision is closed and anaesthetic removed allowing the animal to regain consciousness within 10 to 15 minutes. After a predetermined time interval, the animal is again lightly anaesthetised and the isolated section of gut, including its contents is removed from the animal, homogenised and assayed. A similar method was used by Schanker, Shore, Brodie & Hogben (1957) to study the absorption of drugs from the stomach. The stomach is isolated under pentobarbitone anaesthesia and the absorption is allowed to proceed with the animal remaining under anaesthesia.

Levine & Pelikan (1961) modified the method of Levine & others (1955) so that multiple loops could be used in the same animal, and found that the rate of absorption of benzomethamine was greatest in the intestinal segments closest to the pylorus. Feldman, Salvino & Gibaldi (1970) used two loops per animal to investigate

the effect of sodium deoxycholate on phenol red absorption. They found that the bile salt markedly enhanced the absorption of phenol red by altering the permeability of the intestinal membranes and in the intact rat, these effects appeared to be reversible. Nightingale, Axelson & Gibaldi (1971) used single loops to study the effect of bile flow on sulphadiazine absorption. The loop contents were collected to estimate volume and determine pH. Four experimental conditions were used namely control, bile duct ligation, sham bile duct ligation and sodium dehydrocholate-stimulated bile flow.

"Sham-operated" rats absorbed sulphadiazine to the same extent as control animals and it was evident that the prior surgical procedure had no influence on the rate or total absorption of sulphadiazine. Enhanced bile flow increased the absorption of the drug about 50 per cent, apparently by increasing the solubility and dissolution rate. Bile duct ligated rats had significantly reduced absorption compared to controls. The results suggested that bile plays an important role in the absorption of sulphadiazine under these experimental conditions. Again using isolated loops, Walsh & Levine (1974) examined the effect of antibiotic pretreatment on ileal absorption and found that antibiotic regimens which alter the enteric bacterial population apparently do not produce significant changes in the absorption process of the drugs studied.

Koizumi, Arita & Kakemi (1964a) have used the technique of Schanker & others (1957) to study the absorption of a series of 17 sulphonamides from rat stomach. Kakemi & co-workers (1970) adapted the technique to use a closed intestinal loop, consisting of the whole small intestine from the pylorus to the ileo-caecal junction. They studied the mechanism of enhancement of absorption of sulphaguanidine by the bile salts sodium taurocholate and sodium glycocholate. It was found that the enhancement was caused by the direct action of bile salts to the structure of the absorptive surface. A similar method was used by Hwang & Schanker (1973) when they studied the absorption of organic arsenical compounds.

An important disadvantage of the closed loop techniques of Levine & others (1955) and Schanker & others (1957) is that it is not possible to withdraw samples during the course of an experiment. Only at the termination can a measurement be made, resulting in only one datum point per animal. However the method developed by Doluisio, Billups, Dittert, Sugita & Swintosky (1969) overcomes this problem and enables frequent samples to be taken during an experiment. The technique is as follows :
The small intestine of fasted, anaesthetised rats is exposed and two L-shaped glass cannulas are inserted at the duodenal and ileal ends as shown in Figure 1.7. The intestine is returned to the abdominal cavity and a syringe, fitted with a three-way stopcock and containing perfusion fluid, is attached to the duodenal cannula (Figure 1.7). The ileal cannula is connected to another syringe and the operator is able to pump the drug solution in the lumen into either the ileal or the duodenal syringe, remove an aliquot and return the remaining solution. The procedure was similarly applied to the stomach. The technique is simple, gives reproducible results and yields absorption rates which are realistic in terms of the known absorption behaviour of drugs in humans and intact animals. The technique of Doliusio & others (1969) has been extensively used for the investigation of drug absorption. Its great advantage over the single perfusion or recirculation methods (see page 42) is that, in general, closed loops give better reproducibility and faster absorption rates. For example, barbitone was found by Kakemi & coworkers (1967), using a single perfusion technique, to have an absorption half-life of 41 minutes, whilst Doluisio & others (1969) found an absorption half-life of 19 minutes.

Amongst some of the many examples of use of the method is the study on the effect of a series of dialkylpropionamides on the absorption of prednisone and prednisolone by Hayton & Levy (1972a) and work by Houston & Levy (1975) which showed that some alcohols increase net water flux from the small intestine.

Crouthamel, Doluisio, Johnson & Diamond (1970) have adapted the method of Doluisio & others (1969) for use in dogs.

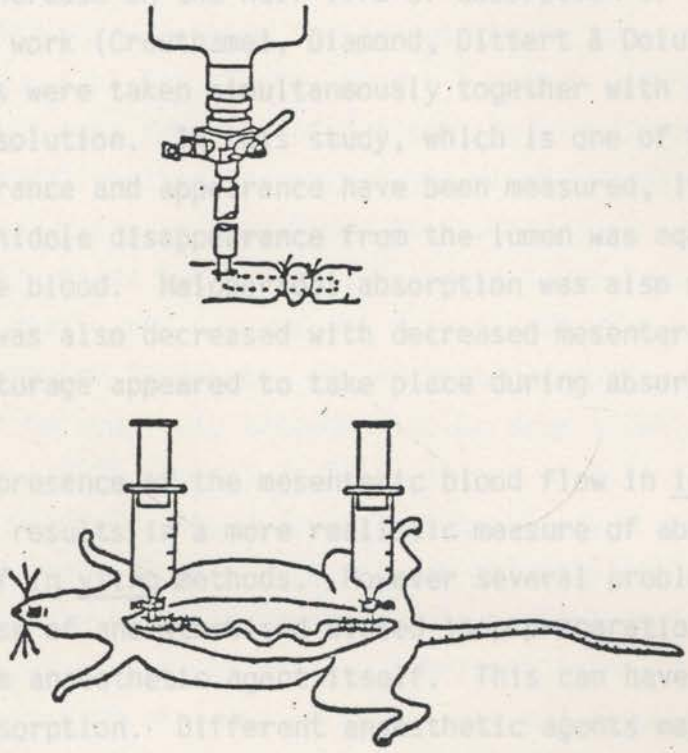


Figure 1.7. Arrangement of Doluisio & others (1969) technique.

This was done so that the effect of mesenteric blood flow on intestinal drug absorption could be measured, the dog being a more convenient animal to use for experiments involving blood flow measurements due to the larger size of the blood vessels. In addition to measuring the absorption from a segment of jejunum, the blood flow in the mesenteric artery was measured using an electromagnetic flowmeter. The intestinal blood flow was altered by means of an hydraulic occluder located immediately distal to the flow probe. They found that a 40 to 60 per cent reduction in mesenteric blood flow resulted in a considerable increase in the half-life of absorption of sulphaethidole. In later work (Crouthamel, Diamond, Dittert & Doluisio, 1975), blood samples were taken simultaneously together with samples of intestinal drug solution. In this study, which is one of the few where both disappearance and appearance have been measured, it was found that sulphaethidole disappearance from the lumen was equivalent to appearance in the blood. Haloperidol absorption was also measured and its absorption was also decreased with decreased mesenteric blood flow but membrane storage appeared to take place during absorption.

The presence of the mesenteric blood flow in <u>in situ</u> closed loop methods results in a more realistic measure of absorption than with the use of <u>in vitro</u> methods. However several problems are apparent with the use of anaesthetised closed loop preparations. One is the effect of the anaesthetic agent itself. This can have a marked effect on absorption. Different anaesthetic agents may vary in their effect on the absorption of a single drug species. In the rat, pentobarbitone anaesthesia has been found to increase liver blood flow by 40 per cent whereas urethane anaesthesia has almost no effect (Hiley, Yates & Back, 1978). However, more importantly it has been found that when a surgical procedure involving the vascular system is performed, namely portal vein catheterisation, rats anaesthetised with pentobarbitone showed no haemodynamic changes whereas in animals anaesthetised with urethane there was an 18 per cent decrease in liver blood flow. Therefore, since splanchnic blood flow is an important factor in intestinal absorption, the choice of anaesthetic agent is extremely important. The minimal use of anaesthetic in the method of Levine & others (1955), was an attempt

to overcome the problem. Another solution to the presence of an anaesthetic agent would be to use decerebrate animals. One would expect then, that the method of Schanker & others (1957) would be more affected by problems with anaesthetic agents than that of Levine & others (1955). The effects of surgical trauma on the intestinal blood flow has also been of concern to experimenters since the time of Waymouth Reid (1900, 1901a).

Water loss through absorption is common to all *in situ* methods; Levine's group did not take it into account whereas Schanker & others (1957) did by using the nonabsorbable indicators phenol red or inulin. Schanker, Tocco, Brodie & Hogben (1958) found 3 per cent water loss from perfused intestine over 30 minutes. Doluisio & others (1969) checked for water absorption by adding phenol red to drug solutions and concluded that water loss was insignificant. However, Bates & Gibaldi (1970) have found, using the same technique, that water loss became significant when an experiment extended beyond 30 minutes and recommended that a nonabsorbable indicator be routinely included in the drug solution. Pelzmann & Havemeyer (1972) also found 30 to 40 per cent (4 ml) net water absorption per hour in closed loops of rat intestine. As discussed previously (page 33), phenol red and polyethylene glycol have been considered to be suitable nonabsorbable markers in intubation studies. However a study by Admans, Cobcroft, Finnin, Nolan & Reed (1976) found that 20 per cent of phenol red was absorbed from closed segments of rat small intestine. The absorption was increased markedly in the presence of dioctyl sodium sulphosuccinate. From this study it appears that considerably more phenol red can be absorbed from the *in situ* small intestine of the rat than the earlier studies have indicated and phenol red may not be suitable for use as a nonabsorbable indicator in *in situ* studies.

Kojima, Smith, Crouthamel & Doluisio (1972), using the method of Doluisio & others (1969), found that the lumenal solution tended to change to a higher tonicity value. Water loss from isotonic solutions ranged between 7 to 17 per cent in 3 hours. It is of note that the tonicity of the medium alters the rate of absorp-

tion of sulphaethidole (Kojima & others, 1972). The tonicity of the perfusing solution and water transport should be considered in the analysis of data on the absorption of drugs from loops of small intestine.

Other problems of closed loop preparations are the difficulty in the complete removal of the intestinal contents and the usual absence of bile. This is important because the intestinal contents are usually rinsed prior to experimentation and therefore the experimental conditions may not closely resemble physiological conditions with respect to the presence of bile.

(b) Perfused segments

These methods are similar to closed segments, except that fluid is circulated through the lumen of the segment. Schanker & co-workers in 1958 developed methods for the perfusion of intestinal segments in rats which led them to formulate the pH-partition theory of gastrointestinal absorption. Two types of methods were developed. One was based on a "single perfusion" of fluid through the segment with the outflow fluid being collected; the other used a "recirculation" of the outflow fluid back through the segment with samples being withdrawn from a reservoir. These methods have the advantage over the closed loop methods of Levine & others (1955) and Schanker & others (1957) in that it is possible to take samples during an experimental run.

The methods of Schanker & others (1958) are as follows:

Fasted rats are anaesthetised with pentobarbitone, and the small intestine exposed by a midline abdominal incision. The intestine is cannulated at the duodenal and ileal ends with polyethylene cannulas and the stomach and caecum closed off by ligatures. The intestine is replaced in the abdomen, the incision closed and perfusion from pylorus to ileo-caecal junction started immediately. For single perfusion, the small intestine is first cleared of part-

iculate matter by perfusion with drug-free solution for 30 minutes. The solution containing drug is then perfused for 30 minutes to displace the previous wash after which four 10-minute samples are collected from the ileal outflow. The concentration of drug is measured in each of the collected samples and the relative rate of absorption is calculated from the difference in the concentration entering and leaving the intestine. The need for perfusing the intestine with drug solution for 30 minutes before measuring drug absorption was demonstrated by observing that the outflowing concentration of inulin-^{14}C, a substance which was not absorbed, did not become constant until 25 minutes after perfusion had commenced. At steady-state the concentration of inulin was 1.03 ± 0.02 times the concentration of the inflowing solution. This volume change, due to absorption of water, was determined by direct measurement of the rates of inflow and outflow and found to agree with the inulin concentration ratio of 1.03.

The single perfusion technique is not suitable for monitoring slowly absorbed drugs because of very small differences in drug concentrations in perfusate flowing into and out of the intestine. Recirculation of the perfusate increases changes in drug concentration by increasing the time for effective contact of drug with absorbing surfaces. With recirculation, the intestine is first perfused for 30 minutes with drug solution containing either inulin-^{14}C or phenol red. The tubing attached to the inflow and outflow cannulas are then transferred to a flask containing 30 ml of drug solution. This volume was then continuously circulated through the small intestine for three hours. The relative rate of absorption is calculated from the decrease in drug concentration, corrected for the volume change.

The perfusion methods avoid the potential problems of overfilling the segment. The difficulty of removing only the contents of a closed segment is also avoided. The greatest disadvantage of the perfused loop technique is the slower absorption rates which are obtained because of less effective contact of drug with the absorbing surface. A large number of experiments utilising

perfused segments have been carried out by workers interested in drug absorption. Again the use of the anaesthetic is a disadvantage, as it is with all *in situ* experiments.

The single perfusion technique was used by Hogben, Tocco, Brodie & Schanker (1959) in experiments that supported and verified the hypothesis that the intestinal mucosa preferentially allows absorption of the unionised form of a drug. It was also used by Schanker (1959) to study the absorption of drugs from the rat colon.

Investigation of the recovery of nonabsorbable indicators in studies on perfused segments was carried out by Miller & Schedl (1970). They considered that the loss of indicator was significant and that absorption data should be corrected for this loss. Miller & Schedl (1970) tried to recover the total amount of indicator from serum and urine, but could not explain the 2 to 3 per cent loss. Possibly, it was irreversibly adsorbed to the absorbing surfaces. A small loss of indicator also occurs in intubation methods (page 33) but the large amount of phenol red absorbed in a closed loop method (Admans & others, 1976) may occur because the closed loop allows for greater contact of indicator with the absorbing surface than the perfused loop.

Methods involving the use of recirculation of fluid through the intestine have been rarely used in studies on drug absorption. Examples of the use of this method include studies on the absorption of sulphonamides (Koizumi, Arita & Kakemi, 1964b; Kakemi, Arita & Muranishi, 1965), prednisolone (Hayton & Levy, 1972b) and the effects of blood glucose and plasma osmolality on absorption (Kitazawa & Johno, 1977).

(c) Closed or perfused segments with complete mesenteric blood collection

A great disadvantage of the closed loop and perfused loop preparations is that only the loss of material from the lumen

is measured. Accumulation or metabolism in the gut wall is not detected and as a result the absorption may appear to be more rapid and more complete than would otherwise be indicated. Crouthamel & others (1975) while using closed loops in dogs, obtained systemic venous samples concurrently. However this method enables only concentration changes to be monitored instead of measurement of the total amount of drug absorbed. The total drug absorbed can be measured by collecting the total mesenteric venous blood flow from a segment of intestine, which has been isolated as either a closed or an open perfused loop. In this way, both the disappearance of drug from the lumen and appearance in the blood after crossing the intestinal wall can be simultaneously measured. Collecting the venous blood draining an isolated segment means that the total amount of drug absorbed is collected and complications resulting from its gaining access to the systemic circulation are avoided. It is thus possible to calculate an intestinal transfer rate for a drug and, since it is a closed system, to carry out kinetic analysis. The volume of blood collected per unit time is a measure of the blood flow through the isolated segment and it is therefore possible to study the effect of blood flow on absorption. In the rat, the blood flow through the intestinal loop can be altered by adjusting the blood pressure. In larger animals, the superior mesenteric artery can be constricted.

Matthews & Smyth (1954) were the first workers to collect the complete mesenteric venous blood flow from an intestinal segment. They studied the absorption of amino acids in cats, but the intestinal lumen contents were not sampled. Atkinson, Parsons & Smyth (1957) used a closed loop of dog intestine, from which the complete mesenteric blood flow was collected, to investigate the absorption of glucose. The contents of the lumen were sampled at the conclusion of the experimental period. Pindell, Cull, Doran & Dickison (1959) used a similar technique to investigate tetracycline absorption in the dog. Diczfalusy, Franksson & Martinsen (1961) used patients undergoing surgery to study oestrogen conjugation. During, surgery, oestriol was injected into a jejunal loop which had been isolated with clamps and blood was collected by catheterisation of the transected vein draining the loop. It was found that both the

effluent venous blood and the intestinal wall contained high concentrations of conjugated oestriol.

Barr & Riegelman (1970) introduced an excellent version of the method using rabbits (Figure 1.8). Rabbits were chosen because of their convenient size. Cannulation of the mesenteric vein in smaller animals is difficult, while larger animals, such as dogs, require the replacement of large amounts of blood to maintain blood flow. A midileal portion of the intestine was selected because its accessibility and suitable vasculature facilitated cannulation. The mesenteric arcades to adjacent portions were carefully tied off. The intestine was cut and cannulated with Tygon tubing for either perfusion (Figure 1.8A) or a closed loop (Figure 1.8B). The mesenteric vein was cannulated and the mean-flow rate determined directly by recording the quantity of blood collected in successive intervals, usually 10 minutes. The blood loss from the mesenteric vein was continuously replaced with an equal volume of heparinised blood-saline mixture or whole blood previously collected from donor animals.

Barr & Riegelman compared their *in situ* method with the *in vitro* cannulated everted sac method using intestinal segments from the same region. The effects of metabolism, tissue accumulation and blood flow on the transport of salicylamide were studied. A lag time of 4 minutes was found for the appearance of free salicylamide into mesenteric blood *in situ*, whereas the appearance of free drug into serosal fluid *in vitro* had a lag time of about 10 minutes. Lag times in everted sac preparations are considered to be due to drug crossing unphysiological barriers such as the muscularis. However the lag time in the *in situ* preparation was found to be a real effect attributable to the intestinal tissue and not to either delayed release of dosage forms or diffusion in the lumen fluid as has been suggested for lag times *in vivo*. The steady-state rate of appearance of free drug into the plasma (*in situ*) was five to ten times greater than the rate of appearance of free drug into the serosal fluid (*in vitro*) at similar mucosal concentrations.

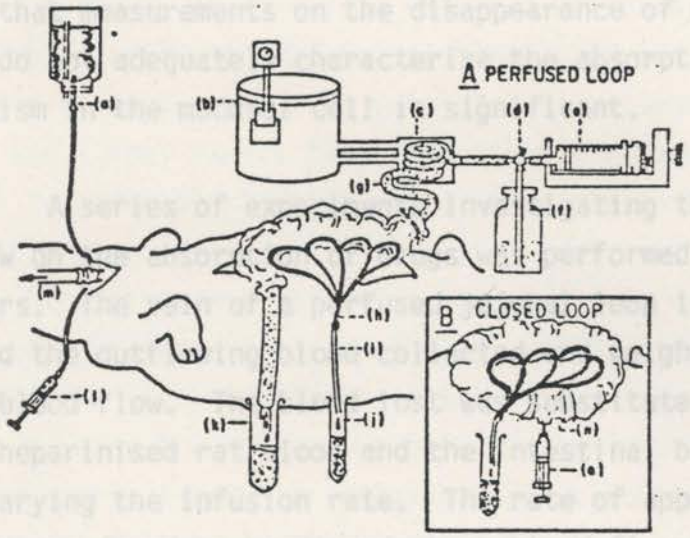


Figure 1.8. Diagram illustrating the in situ intestinal preparation, with complete venous collection, of Barr & Riegelman (1970).
A. Perfused intestine; B. Closed intestinal loop.
Key: (a) blood (or blood-saline mixture) infused into jugular vein; (b) constant temperature water bath; (c) heat exchange coil for perfusate; (d) three-way stopcock to refill infusion pump (e) with perfusate (f); (g) "Tygon" tubing connecting the perfusion system to the isolated intestinal loop; (h) mesenteric vein draining ileal segment; (i) polyethylene cannula draining mesenteric venous blood; (j) calibrated centrifuge tube; (k) centrifuge tube to collect perfusion effluent; (l) carotid cannulation for arterial sample; (m) attachment to animal respirator; (n) "Tygon" tubing attaching both ends of intestine to form closed loop; (o) syringe used to collect samples of lumen fluid and also periodically to mix lumen fluid.

Barr & Riegelman found that the in situ intestinal loop with complete venous collection has many advantages in studying physiological factors of intestinal drug absorption. Very importantly they showed that measurements on the disappearance of drug from the lumen alone do not adequately characterise the absorption process when metabolism in the mucosal cell is significant.

A series of experiments investigating the influence of blood flow on the absorption of drugs was performed by Winne and his co-workers. The vein of a perfused jejunal loop in a rat was punctured and the outflowing blood collected and weighed to give an estimate of blood flow. The blood lost was substituted by an infusion of heparinised rat blood and the intestinal blood flow was changed by varying the infusion rate. The rate of appearance of most drugs studied was found to be dependent on blood flow (Ochsenfahrt & Winne, 1968, 1969; Winne & Remischovsky, 1971), although the absorption of ribitol was shown to be independent of blood flow (Winne & Remischovsky, 1970).

The effect of arteriovenous anastomoses present in the gastrointestinal circulation on mesenteric collection preparations has been investigated (Barr & Riegelman, 1970). Salicylamide solution was placed in the lumen of a closed loop and the venous blood collected and assayed for free drug and glucuronide. During the absorption process, the carotid artery was sampled periodically. The amount of drug reaching the systemic circulation was found to be negligible.

Despite the great advantage of knowing what is happening on both sides of the intestinal membrane (lymph being neglected), very little use has been made of methods employing whole mesenteric blood collection in drug absorption studies. It is a technically complex method because of the perfusion pumps and other equipment required and the considerable volume of blood which needs to be collected and stored.

(d) Vascular perfusion

This involves perfusion of the vascular bed of the intestine with a fluid, which may be either blood or a blood substitute. The blood flow is arbitrarily fixed, and the outflow is collected either from a mesenteric vein draining a segment or from the complete intestinal venous flow from the portal vein. It thus represents an "artificial circulation" of the intestinal area.

The first experiment of this type was carried out by Salvioli in 1880. Parsons & Prichard (1965, 1968) studied sugar absorption from the small intestine of the frog. The vascular perfusion fluid was a bicarbonate-Ringer solution containing either dextran, bovine serum albumin or polyvinylpyrrolidone. They also perfused the lumen of the intestine with oxygenated bicarbonate-Ringer solution, since with an artificial circulation, no oxygen can gain access to the serosal side of the intestinal wall. The apparatus necessary to perform a vascular perfusion experiment is elaborate and there have been few examples of its use. Ochsenfahrt (1974, 1979) has measured drug absorption from vascularly perfused jejunal loops of rats. No morphological alterations were found in blood-perfused segments and metabolic viability was preserved. However, the method remains difficult to use.

(e) Animals with fistulas

Most methods of measuring absorption using fistulas are referred to collectively as "Thiry-Vella loops". In these methods a loop of intestine is surgically separated from the rest of the intestinal tract and the ends of the loop brought to the skin surface. The isolated intestinal loop retains a complete blood supply and its nervous connections. The experimental animals used are almost invariably dogs, but recently the pig (Fox, Care & Swaminathan, 1978) and guinea-pig (Fabri, Scott & Nelson, 1977) have been used. Drug solutions are instilled into the loop and after a period of time are recovered and the loss of test material calculated.

The experiments are carried out while the animal is conscious. There are various modifications to the basic method.

The method was first described by Thiry in 1864. He prepared intestinal segments which were closed at one end and had a fistulous opening at the other end. The filling and emptying of these segments was difficult and the technique was modified by Vella in 1880 and 1888, to have openings at both ends of the isolated intestinal segment, thereby creating a loop (see Figure 1.9).

Some criticisms of the technique have been discussed by Berger, Kanzaki, Homer & Steele (1959). They found, over a 5-year period, that there was no evidence of physiological or histological deterioration of loops although there is still some doubt as to whether the isolated loop maintains its normal function (Smyth, 1974). A modified method whereby the Thiry-Vella loop retains its continuity with the gastrointestinal tract has been described by Phillips, Webb & Fontenot (1978). The surgical techniques required to produce Thiry-Vella loops have been fully detailed by Markowitz (1964), and the techniques for perfusing the loops were described in detail by Vaughan Williams (1951) who also designed plastic cannulas which eliminated leaks from the ends of the fistulas.

Sample, Rossi & Packman (1968) evaluated the Thiry-Vella dog as a model for intestinal drug absorption. Their findings are interesting, although it is unfortunate that they used the loops only for administering drug solutions, relying on blood levels for the measurement of absorption. One problem they encountered was that continuous perfusion of the loops caused swelling and distension of tissue. Oedema did not occur after introduction of relatively small fixed volumes into the segment and it was concluded that the physiological integrity could best be preserved in this way. Sample & others (1968) found that the rate of absorption of paracetamol was more constant after placement into the Thiry-Vella loops than after oral administration to dogs without fistulas. They concluded that the "Thiry-Vella dog" was a stable quantitative and reproducible

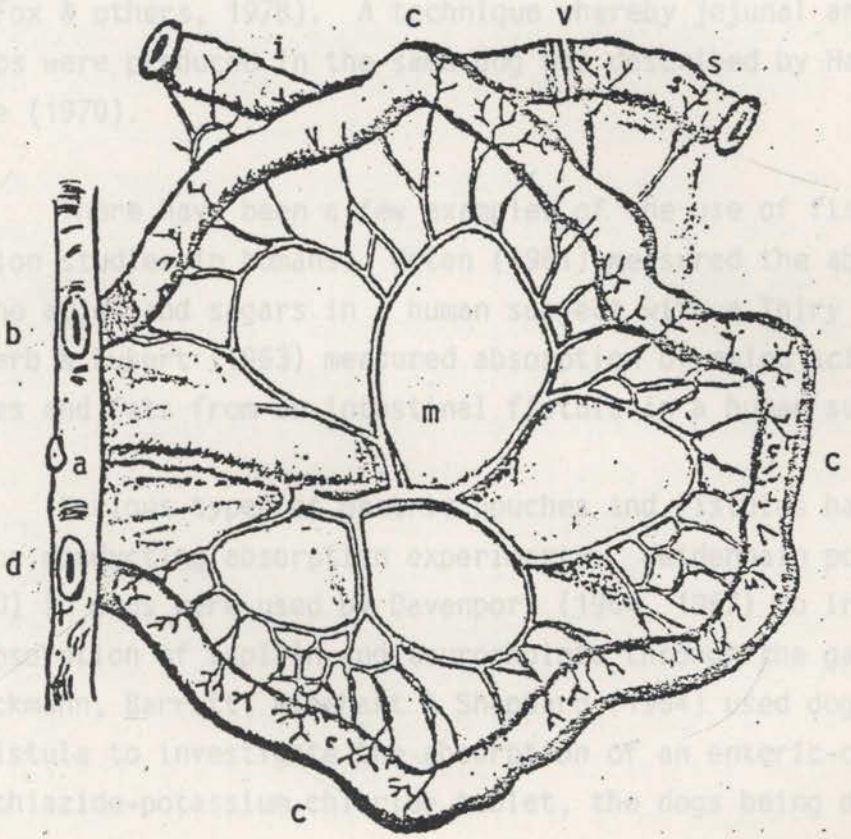


Figure 1.9. Typical Thiry-Vella loop, c. b and d are the openings in the abdominal wall, a; the segment being removed from continuity with the intestinal tract, i-s; at the point, v. m is the intact blood supply continuing to supply the segment (from Vella, 1888).

system for evaluating intestinal drug absorption especially since the chronic nature enables multiple experiments on the same animal. The only disadvantage is that major abdominal surgery is required. In consequence, Thiry-Vella loops have only had limited use so far in absorption studies and those performed have been concerned with nutrients (Fox & others, 1978). A technique whereby jejunal and colonic loops were produced in the same dog was described by Hauge & Krippaehne (1970).

There have been a few examples of the use of fistulas for absorption studies in humans. Orten (1961) measured the absorption of amino acids and sugars in a human subject with a Thiry fistula. Schloerb & Lukert (1963) measured absorption of amino acids, carbohydrates and fats from an intestinal fistula in a human subject.

Various types of gastric pouches and fistulas have been used for conducting absorption experiments. Heidenhain pouches (Figure 1.10) in dogs were used by Davenport (1964, 1967) to investigate the absorption of aspirin and taurocholate through the gastric mucosa. Lackmann, Barrett, Rinehart & Sheppard (1964) used dogs with a gastric fistula to investigate the absorption of an enteric-coated hydrochlorothiazide-potassium chloride tablet, the dogs being dosed

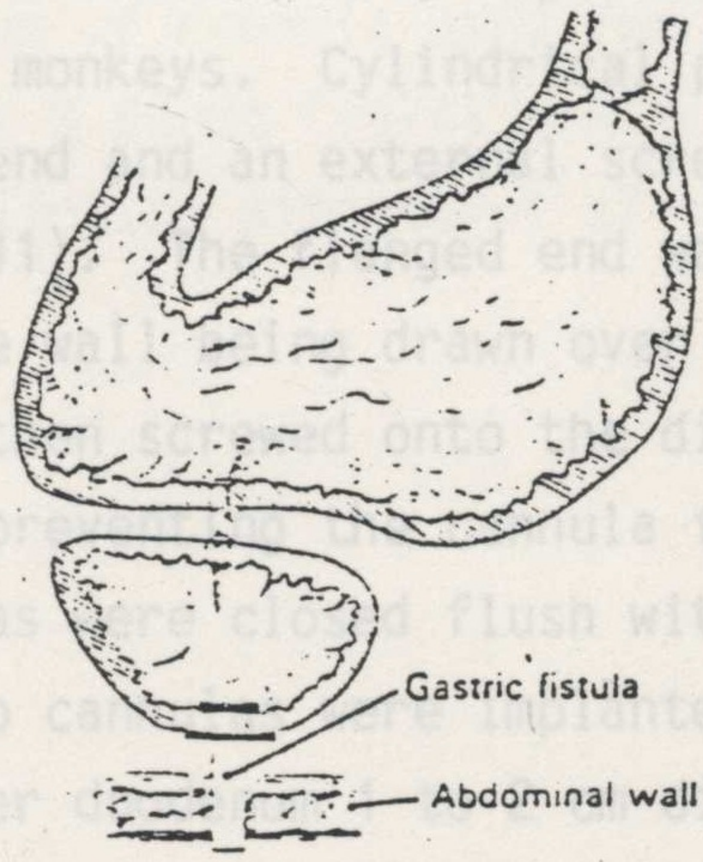


Figure 1.10. Cross section of Heidenhain pouch (from Stedman's Medical Dictionary, Twenty-third edition, 1976).

orally and gastric fluid collected through the fistula for analysis.

Recently, another approach to gain access to intestinal segments has been to implant cannulas between the intestinal lumen and the surface of the skin. In effect, it is an artificial fistula but allows the gastrointestinal tract to function normally when the cannulas are occluded. Zeman & Symchowicz (1970) developed a method in dogs in which cannulas were implanted at specific locations in the duodenum, jejunum and ileum which were used to deliver accurately known amounts of compounds to specific absorption sites. They did not use the cannulas to remove luminal samples however, and absorption was monitored by analysis of blood samples. This same method was used by Symchowicz, Zeman, Williams & Tabachnick (1965) to study the absorption of betamethasone. Wangsness & McGilliard (1972) implanted duodenal and ileal cannulas in calves and used a London-type cannula (page 68) to sample portal blood. Solutions were infused into the duodenal cannula and the amount of material not absorbed was determined by recovery through the ileal cannula. At the same time, the concentrations in portal and arterial blood were measured together with the portal vein blood flow (page 70) permitting determination of the total amount effectively absorbed through the gastrointestinal tract. The difference allowed the effect of "first-pass" metabolism arising from either the intestine or the liver to be studied.

Nayak & Benet (1974) implanted cannulas in the duodenum and stomach of monkeys. Cylindrical plastic cannula tubes with flanges at one end and an external screw thread at the other were used (Figure 1.11). The flanged end was inserted into the stomach or intestine, the wall being drawn over the flange and secured. Star wing nuts were then screwed onto the distal end until they rested against the skin preventing the cannula from being drawn into the abdomen. The cannulas were closed flush with the flange using a screw-in insert. Two cannulas were implanted, one in the stomach, the other in the upper duodenum 1 to 2 cm distal to the pylorus. A suture was passed through the lumen between the two cannulas and its ends tied to the tips of the screw-in inserts of the cannulas. This

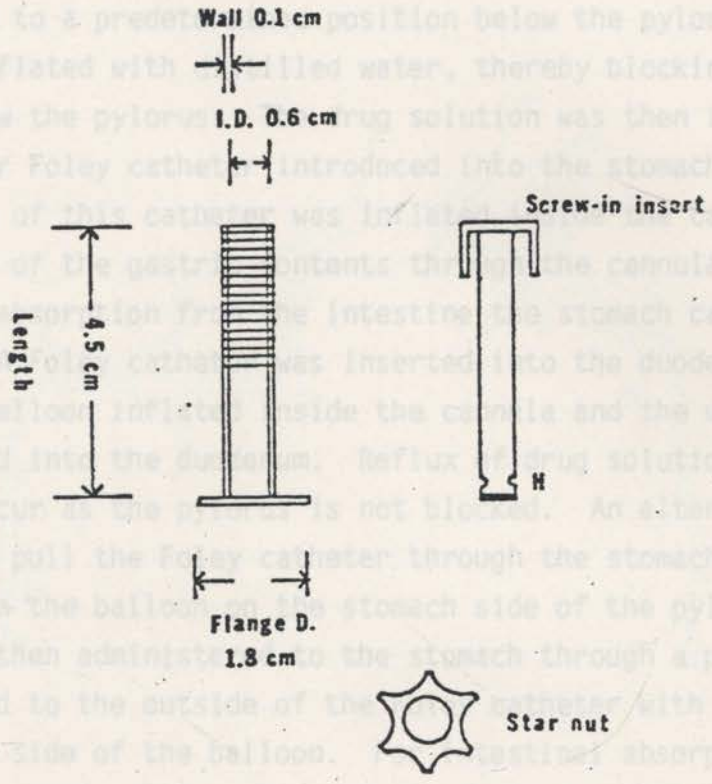


Figure 1.11. Diagram of cannula used for stomach and duodenal implantation by Nayak & Benet (1974). H, indicates the hole drilled through the insert allowing attachment of the surgical nylon thread (suture).

allowed the investigator to pull a Foley catheter through either cannula when occlusion of the pylorus or duodenum was required. When studying the absorption of drugs from the stomach, a double lumen Bard-Foley catheter with a 5 ml balloon was inserted into the duodenal cannula and pulled by the suture attached to the stomach cannula insert to a predetermined position below the pylorus. The balloon was inflated with distilled water, thereby blocking the duodenum just below the pylorus. The drug solution was then instilled through another Foley catheter introduced into the stomach cannula. The balloon of this catheter was inflated inside the cannula to prevent leakage of the gastric contents through the cannula. When studying the absorption from the intestine the stomach cannula remained closed. A Foley catheter was inserted into the duodenal cannula with its balloon inflated inside the cannula and the drug solution introduced into the duodenum. Reflux of drug solution into the stomach may occur as the pylorus is not blocked. An alternative method used was to pull the Foley catheter through the stomach cannula and to inflate the balloon on the stomach side of the pylorus. Drug solution was then administered to the stomach 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, 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 1.12 schematically shows the arrangement for the alternate method for stomach absorption. During experiments, the monkeys remained in restraining chairs and catheters were placed for simultaneous sampling of arterial and venous blood.

Nayak & Benet used the monkeys for simultaneous measurement of disappearance of drug from the lumen and appearance of drug in the systemic blood. The size of the cannulas allowed the administration of tablets or capsules directly, obviating the need to disguise the drug in the monkey's food or physically to force the monkey to swallow a tablet. The animals remained healthy and pharmacokinetic measurements of salicylic acid were reproducible over a 9-month period. This model can be used to study any oral

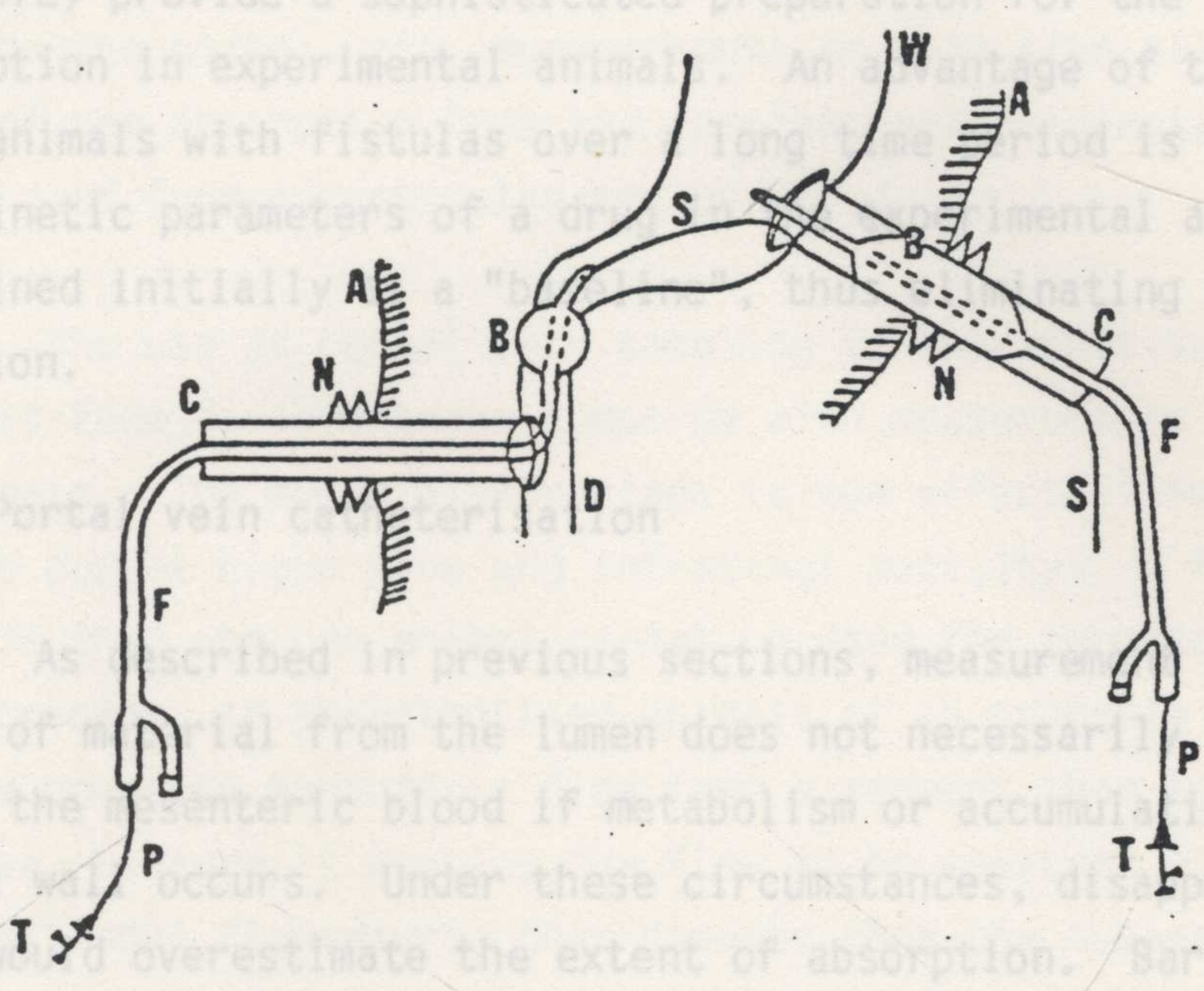


Figure 1.12. Schematic representation of the positioning of Foley catheters by the alternate method for stomach absorption study. Key: A - abdominal skin; B - balloon of Foley catheter; C - cannula; D - duodenum; F - Foley catheter; N - star nuts; P - polyethylene tubing; S - nylon suture attached to cannula inserts and used to position Foley catheters; T - three-way stopcock; W - stomach wall (from Nayak & Benet, 1974).

dosage form. The influence of variables such as food or starvation, which could modify absorption, can also be evaluated. It is theoretically possible to introduce other cannulas, for example, at the ileo-caecal junction, to study the effect of residence time. These gastrointestinal cannulation methods, especially when combined with portal vein sampling and flow measurement (as used by Wangsness & McGilliard, 1972) provide a sophisticated preparation for the study of drug absorption in experimental animals. An advantage of the use of conscious animals with fistulas over a long time period is that the pharmacokinetic parameters of a drug in the experimental animal can be determined initially as a "baseline", thus eliminating inter-animal variation.

(vi) Portal vein catheterisation

As described in previous sections, measurement of the disappearance of material from the lumen does not necessarily equal appearance in the mesenteric blood if metabolism or accumulation in the intestinal wall occurs. Under these circumstances, disappearance measurements would overestimate the extent of absorption. Barr & Riegelman (1970) showed that at low initial concentrations in the lumen, over 60 per cent of salicylamide was conjugated with glucuronic acid in the mesenteric blood of anaesthetised rabbits; Diczfalusy & others (1961) found that large amounts of conjugated oestriol were present in the mesenteric blood of anaesthetised humans who were administered oestriol into isolated intestinal loops. Estimates of absorption using blood-level or urinary-excretion measurements are made difficult by the fact that, in a dynamic system, distribution, metabolism and elimination also occur. In particular, since the liver is the major site of metabolism for most drugs, it follows that blood level measurements are liable to be markedly influenced by the extent of metabolism which has occurred. For example, propranolol is extensively metabolised in the liver and Suzuki & others (1974) showed that after a direct infusion into the portal vein of an anaesthetised rat, considerably less propranolol entered the systemic circulation than when the same dose was infused directly into a peripheral vein. Nogami & co-workers (1970) found that 80 per cent of

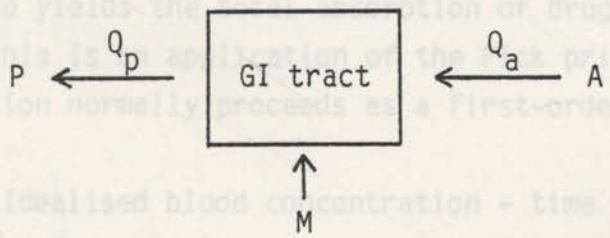
orally-administered riboflavine was metabolised on its first pass through the liver. Thus, to avoid complications of liver metabolism in absorption measurements it is highly desirable that measurements should be made prior to the entry of the drug into the liver. There is thus considerable advantage to be gained from the use of the portal vein for the measurement of intestinal absorption. In addition, since nearly all the blood from the intestinal capillary bed drains into the portal vein, measurements at this site allow the quantitation of the absorption from the complete gastrointestinal tract and not just from a particular region.

The use of portal vein sampling in anaesthetised animals suffers from disadvantages compared with measurements in conscious animals. The major disadvantage is the effect of the anaesthetic on portal blood flow and intestinal motility. A further disadvantage is that the one animal cannot be used for repeated experiments. Moreover, in anaesthetised animals it is probably easier to collect the complete mesenteric flow from a segment than to sample the portal vein, because it enables the flow to be measured without the aid of additional flowmeters; this assumes that the segment is representative of the gastrointestinal tract as a whole. However, useful data have been obtained from preparations involving sampling from the portal vein in situ. In experiments in an intact conscious animal, simultaneous measurements of drug concentrations in arterial and portal vein blood are the most direct way in which the influence on the absorption process of entero-hepatic cycling and first-pass metabolism in the gut wall and liver can be investigated. It cannot be used in man, except in special circumstances, because of the severe invasive nature of the technique, but other mammalian species can be used. The study of portal vein - arterial (P - A) concentration differences in conscious animals permits the direct quantitation of gastrointestinal absorption of drugs, avoiding complicating factors such as distribution, metabolism and elimination which must be considered in the analysis of the time course of concentration of drug in arterial or peripheral venous blood (page 21).

Portal vein sampling by itself has the disadvantage that concentration changes only can be monitored and therefore the total amount of drug absorbed is not known. For this to be known, the portal vein blood flow needs to be measured simultaneously. In addition it is also necessary to take into account the effect of drug that passes through the liver and into the systemic circulation. This drug is present in the arterial blood arriving in the intestinal capillaries. After this drug-containing blood reaches the intestinal area, the portal blood then contains newly absorbed drug plus systemic drug and the portal vein blood levels remain higher than the systemic blood levels until absorption ceases.

(a) Theoretical considerations

The process of absorption can be considered diagrammatically as follows.



where P = the concentration of drug in portal venous blood
A = " " " " " arterial blood
M = amount of drug in the lumen of the intestine
$\dot{Q}_a$ = arterial blood flow
$\dot{Q}_p$ = portal vein blood flow

Now, over any time interval, dt,

$$\dot{Q}_p \times P = \dot{Q}_a \times A + \frac{dM}{dt} \qquad \text{equation (1.4)}$$

where $\frac{dM}{dt}$ is the net rate of transport of drug into the portal vein.

By integration,

$$M_{o-t} = \int_o^t (\dot{Q}_p \, . \, P - \dot{Q}_a \, . \, A) \, dt \qquad \text{equation (1.5)}$$

where M_{o-t} is the amount of drug absorbed during the time period from 0 to t.
If $\dot{Q}_p = \dot{Q}_a$, ie at any instant the arterial blood flow in, equals the portal vein blood flow out, which is usually assumed, then

$$M_{o-t} = \int_o^t \dot{Q}_p \, (P - A) \, dt \qquad \text{equation (1.6)}$$

therefore the AUC_{o-t} of a plot of $\dot{Q}_p.(P - A)$ versus time yields the amount of drug absorbed to time t. To use equation (1.6), continuous monitoring of the portal blood flow during an experiment is necessary.

Now, if $\dot{Q}_p$ is constant, then

$$M_{o-t} = \dot{Q}_p \int_o^t (P - A) \, dt \qquad \text{equation (1.7)}$$

Thus, the area under the plot of P - A versus t multiplied by the portal flow rate yields the total absorption of drug up to any time t. Basically this is an application of the Fick principle (Fick, 1870). Absorption normally proceeds as a first-order process.

Idealised blood concentration - time curves that would be expected from simultaneous sampling from the portal vein and an artery are shown in Figure 1.13, and the resulting P - A difference curve assuming constant portal blood flow shown in Figure 1.14. In both figures, the hatched areas are equivalent and represent input of drug into the bloodstream. A very detailed treatment of the theory of the use of arterial - venous concentration differences in general has been given by Zierler (1961).

The portal vein catheterisation method is useful for studying the first-pass effect and for identifying where the metabolism of drugs occurs. For metabolism taking place primarily in the liver, the amount absorbed is determined first and the areas under the (P - A) difference curve and the arterial concentration curve compared. If

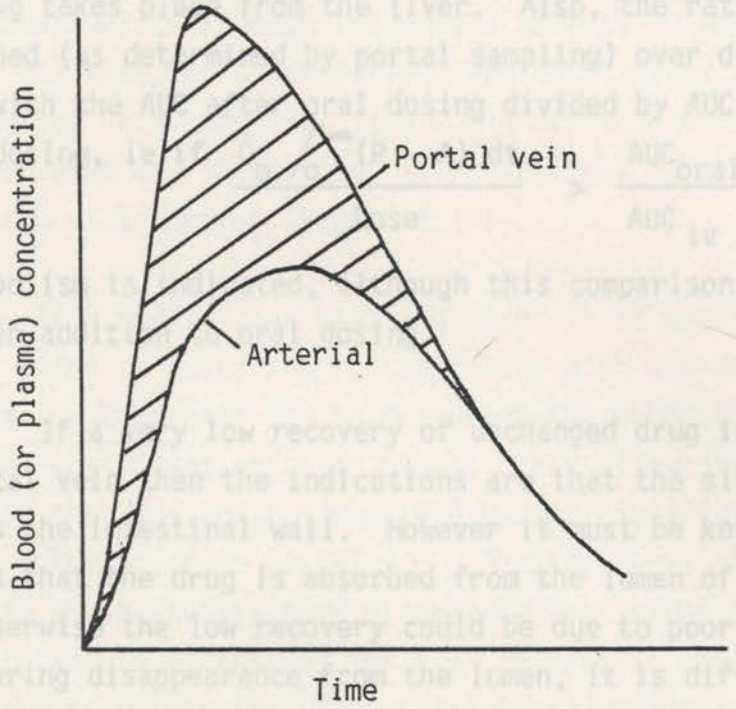


Figure 1.13. Typical concentration versus time curves obtained from portal vein sampling, assuming constant portal blood flow (Q).

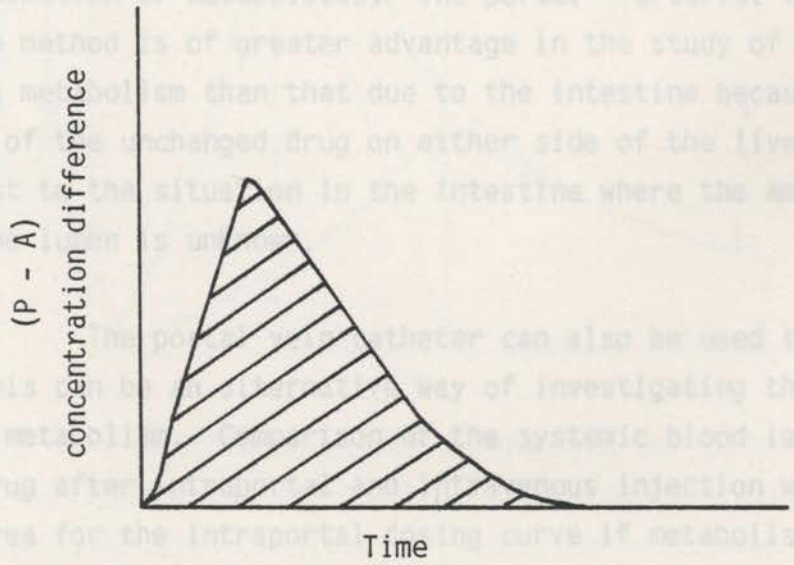


Figure 1.14. The resulting P - A difference curve from Figure 1.13.

$AUC_{(P-A)} >> AUC_A$, then significant hepatic metabolism has occurred. This requires only a single oral dose to indicate that loss of unchanged drug takes place from the liver. Also, the ratio of the amount absorbed (as determined by portal sampling) over dose can be compared with the AUC after oral dosing divided by AUC after intravenous dosing, ie if $\frac{\dot{Q}_p \int_0^\infty (P - A)\, dt}{Dose} > \frac{AUC_{oral}}{AUC_{iv}}$ then hepatic metabolism is indicated, although this comparison requires intravenous in addition to oral dosing.

If a very low recovery of unchanged drug is made from the portal vein then the indications are that the site of metabolism is the intestinal wall. However it must be known from other sources that the drug is absorbed from the lumen of the intestine otherwise the low recovery could be due to poor absorption. Without measuring disappearance from the lumen, it is difficult to identify positively the intestine as a site of metabolism. However intestinal metabolism can be identified if metabolites are quantitated. P - A difference curves for the metabolite(s) are constructed and the areas combined with that of the curve for unchanged drug to give total absorption. Radioactive-labelled compounds can simplify the determination of metabolites. The portal - arterial concentration difference method is of greater advantage in the study of hepatic first-pass metabolism than that due to the intestine because the behaviour of the unchanged drug on either side of the liver is known in contrast to the situation in the intestine where the amount of drug in the lumen is unknown.

The portal vein catheter can also be used to administer drugs. This can be an alternative way of investigating the liver as a site of metabolism. Comparison of the systemic blood level curves for the drug after intraportal and intravenous injection will show a smaller area for the intraportal dosing curve if metabolism has occurred in the liver. Some use of intraportal dosing in anaesthetised animals has already occurred (Iwamoto & Klaassen, 1977), although the use of conscious animals is preferable because of the lack of

anaesthetic agent. Comparison of P - A difference curves after oral and intraportal dosing can also be used to detect first-pass metabolism in the intestinal wall, although incomplete absorption should be considered too. The time for distribution into the tissues can be investigated with portal vein sampling. The P - A difference curve shows the true absorption peak and the lag until the arterial curve shows a peak is due to tissue distribution (Figure 1.15).

If P < A for any sampling pair, it is possible that absorption of water is occurring concomitantly with the drug absorption. This can be corrected for by measuring the haemoglobin concentrations of the portal and arterial blood. This also corrects for any water secretion that may occur. Moodie & Walker (1963) used haemoglobin ratios to demonstrate positive P - A differences in sheep; however the few other studies have disregarded net water change as it probably is negligible because of the high splanchnic blood flow. An alternative possibility for P < A, is loss of systemic drug through

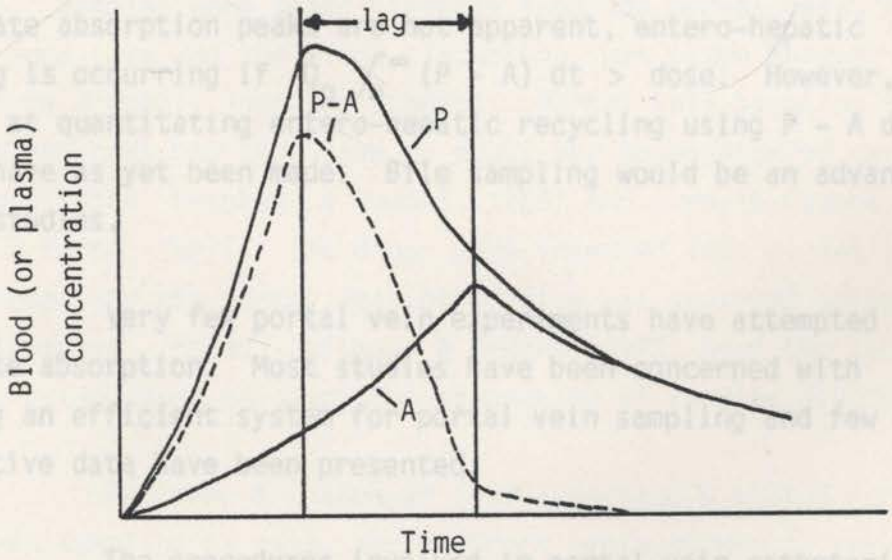


Figure 1.15. P - A difference, portal and arterial concentration curves showing the lag in the arterial concentration peak from the true absorption peak (P - A), due to tissue distribution.

splanchnic metabolism and/or secretion into the intestine. A negative P - A difference would only be observed where absorption was either very small or not occurring. The AUC of the P - A difference and the systemic arterial concentration will not show intestinal secretion of a drug if the drug is re-absorbed since the negative loss and positive gain will tend to cancel each other out.

Some compounds undergo entero-hepatic recycling and this can be shown with portal - arterial difference curves. P - A concentration differences after normal absorption has ceased indicate re-absorption after biliary excretion. It is necessary to establish that normal absorption is complete, because incomplete and erratic absorption may exhibit positive P - A differences after zero values that follow the first absorption peak. If after intraportal or intravenous dosing, there are no P - A concentration differences, then entero-hepatic recycling can be excluded. An absorption peak after intraportal dosing indicates biliary excretion and the amount of drug absorbed equals the amount undergoing entero-hepatic cycling. If separate absorption peaks are not apparent, entero-hepatic recycling is occurring if $\dot{Q}_p \int_0^\infty (P - A)\, dt >$ dose. However, no attempts at quantitating entero-hepatic recycling using P - A differences have as yet been made. Bile sampling would be an advantage in such studies.

Very few portal vein experiments have attempted to quantitate absorption. Most studies have been concerned with achieving an efficient system for portal vein sampling and few quantitative data have been presented.

The procedures involved in portal vein catheterisation are technically difficult because of poor access and because of the rapidity with which catheters become blocked. Consequently, only a small number of experiments has been attempted and each one has aimed to produce a usable reproducible method in some original way.

(b) Portal vein sampling in anaesthetised animals

Historically, the idea of portal vein sampling to trace the fate of absorbed substances is very old. Prior to the 1870's many experiments were carried out in which animals were fed various meals, allowed time for digestion to occur, killed, and as quickly as possible after death, portal vein blood was taken and analysed. Von Mering made a great advance in 1877 when he sampled the portal vein blood of anaesthetised dogs (page 67). Many other experiments using anaesthetised animals were carried out during the following years in investigations of the absorption of physiological materials. For example, Magee & Reid in 1931, investigated the absorption of glucose in cats and rabbits by sampling from the portal vein after glucose solutions had been injected into a closed intestinal loop. Only recently has any use been made of in situ portal vein sampling for drug absorption studies.

Hartiala, Leikkola & Savola (1958) studied intestinal glucuronide synthesis, using dogs anaesthetised with sodium pentobarbitone. Samples were taken directly from the portal vein, hepatic vein and vena cava and the drug, cinchophen, was given through a gastric tube directly into the stomach. Portal vein sampling in anaesthetised cats has been used to study the absorption of cardiac glycosides (Forth, Furukawa & Rummel, 1969) and phenylbutazone (Seebald & Forth, 1977). The drugs were injected into closed intestinal loops and the portal vein blood flow was measured using a bubble flowmeter. Haass, Lullmann & Peters (1972) used the same method in guinea-pigs to study the absorption of cardiac glycosides.

Pelzmann & Havemeyer (1971) devised a method for portal vein sampling in rats, where direct venipuncture is very difficult because of the fragility of the portal vein. A shunt of polyethylene tubing cannulated in opposite directions in the portal vein (Figure 1.16) was used and the bile duct was also cannulated to prevent entero-hepatic circulation. Absorption was measured from intestinal loops. Lumen samples were taken by inserting a 27-gauge needle through the intestinal wall. The surgery involved in this

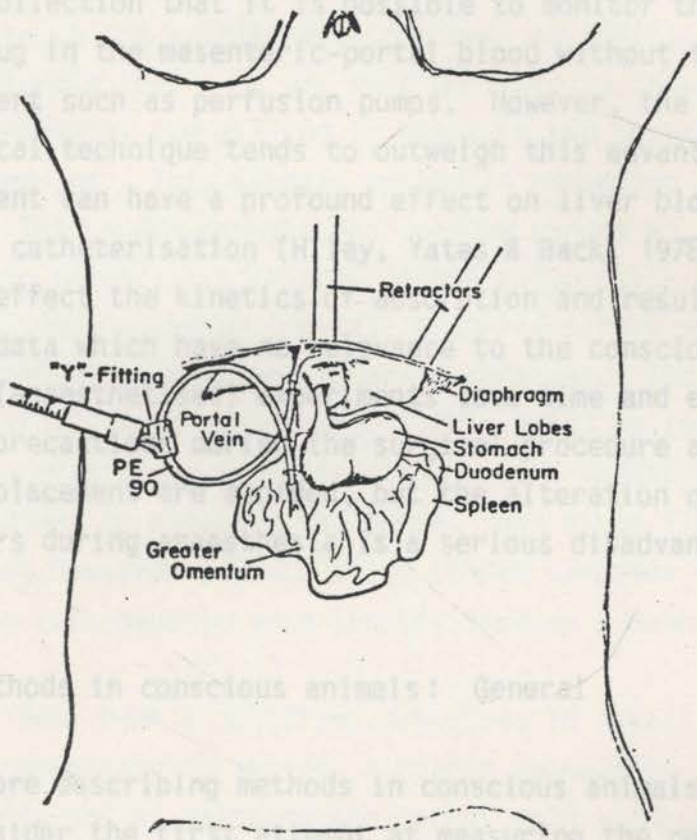


Figure 1.16. Portal vein cannulation method of Pelzmann & Havemeyer (1971).

technique is delicate, requiring speed and accuracy to prevent excessive engorgement of the intestinal veins and acute loss of blood to the liver. The technique was used to study the absorption of chlormadinone acetate and norethisterone (Pelzmann, 1973).

In situ portal vein sampling has the advantage over mesenteric blood collection that it is possible to monitor the appearance of a drug in the mesenteric-portal blood without the need for complex equipment such as perfusion pumps. However, the difficulty of the surgical technique tends to outweigh this advantage. The anaesthetic agent can have a profound effect on liver blood flow during portal vein catheterisation (Hiley, Yates & Back, 1978). This can markedly effect the kinetics of absorption and result in the collection of data which have no relevance to the conscious situation. Acute (anaesthetised) experiments save time and effort because sterility precautions during the surgical procedure and maintenance after placement are avoided, but the alteration of physiological parameters during anaesthesia is a serious disadvantage of such experiments.

(c) Methods in conscious animals: General

Before describing methods in conscious animals, it is interesting to consider the first attempt at measuring the concentration of a substance in the portal vein of a living animal. This was done by von Mering in 1877. He used anaesthetised dogs in which a metal T-piece was inserted into the splenic vein close to the spleen and the portal vein was ligatured between the junction of the portal and splenic veins and the liver. The portal vein flow was then diverted to the splenic vein and was collected through the metal T-piece. Average portal vein flows of 240 ml per minute were found. With this arrangement, it was necessary to feed the dogs prior to administration of anaesthetic so that absorption of food was well advanced when the portal blood was collected. Von Mering measured the concentrations of glucose in the portal blood and compared this with the concentration in carotid arterial samples making very significant observations on the nature of gastrointestinal absorption.

(d) London cannula

The first method used to sample portal vein blood in conscious animals was introduced by London in 1928. At that time, flexible tubing was not available and the only material from which cannulas could be made was metal or glass. Such cannulas could not be permanently introduced into a remote inaccessible vein, so London's solution to the problem was to create an artificial fistula from the portal vein wall. It was then possible to insert a long needle down the metal cannula and to puncture the portal vein and remove blood samples. This classic method is now known as the "London cannula". The London cannula was used by London & Kotschneff (1934) to investigate changes in the polypeptide content of serum during absorption and by Crandall & Cherry (1936) and Cherry & Crandall (1939) to examine glucose absorption. The London cannula was successfully used by Schambye (1951, 1955) in sheep to study the influence of glucose administration on the volatile acids in the portal blood and to estimate the portal vein blood flow. The liver blood flow was measured in dogs using London cannulas to the portal vein and the left hepatic vein (Pratt, Burdick & Holmes, 1952).

In 1949, Dent & Schilling described in detail the surgical manipulations necessary to implant a London cannula in a dog. The first stage consisted of painting the portal vein with iodine solution and immobilising it by sewing it to the side of the adjacent part of the inferior vena cava. Two weeks later the cannula was sutured to the painted area on the portal vein and then wrapped around with an elongated mass of excised free omentum (see Figure 1.17). A problem encountered by Dent & Schilling (1949) was that frequently samples were found to have been withdrawn from the vena cava or the bile duct rather than the portal vein. Other problems included adhesions around the cannula causing both the gallbladder and the bile duct to be distended. On some occasions the cannula end slipped away from the portal vein. The method cannot be considered to be really successful; Dent & Schilling (1949) had many failures. Even when a successful operation was performed the useful life of the preparation was short.

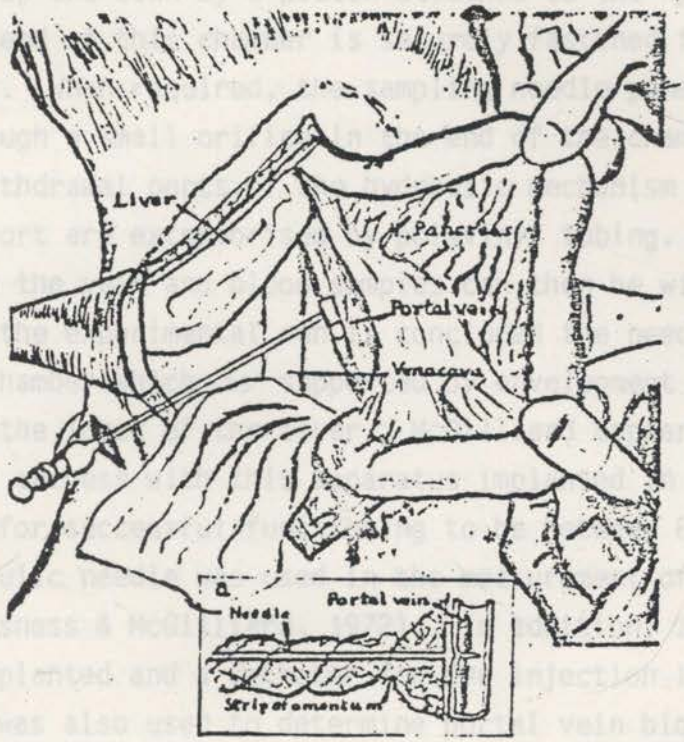


Figure 1.17. Drawing showing a London cannula sutured in its final position to the portal vein. A strip of omentum is wrapped round the cannula as shown in the inset before the abdomen is fully closed. To withdraw portal blood the trocar is removed and a long needle is inserted through the cannula until the wall of the vein is pierced. From Dent & Schilling (1949).

A modification of the "London cannula" was devised by Amirov (1966), who used a split polyethylene T-tube placed around a section of the portal vein. McGilliard (1971) has designed what may be described as an "automatic London cannula". It consists of an hydraulically operated needle which is permanently implanted. The hydraulic needle consists of a chamber in which the sampling needle is moved up and down by a piston attached to the hydraulic mechanism. The end of this chamber is securely fastened to the portal vein wall. When required, the sampling needle penetrates the portal vein through a small orifice in the end of the chamber. The injection and withdrawal ports of the hydraulic mechanism and the blood sampling port are exteriorised by polyvinyl tubing. The needle is inserted into the vein and blood samples can then be withdrawn as required. When the experimental run is concluded the needle is withdrawn into the chamber which is supported by envelopment in the fissure between the lobes of the liver. McGilliard appears to have had considerable success with this apparatus implanted in calves and found the range for successful functioning to be between 84 and 148 days. The hydraulic needle was used in the measurement of glucose absorption (Wangsness & McGilliard, 1972). In addition, intestinal cannulas were implanted and a catheter for dye injection into the mesenteric vein was also used to determine portal vein blood flow using an indicator dilution method.

(e) Direct catheterisation

The advent of flexible plastic tubing enabled indwelling catheters to be used to obtain portal vein blood samples. In 1953, Denton, Gershoff & Elvehjem used "Genflex" plastic tubing coated with silicone to catheterise the portal vein of dogs directly. Earlier in situ investigations had shown that portal vein blood must be collected anterior to any vein entering it, that is anterior to the junction with the gastroduodenal vein. A needle containing the tubing was inserted into the portal vein approximately one inch from the liver and advanced almost to the liver. After the needle was removed, saline was immediately infused through the tubing to prevent clotting. The tubing was looped in the form of a knot and

sutured firmly to the cleared connective tissue surrounding the point of insertion. The other end of the tubing was passed subcutaneously to a point behind the shoulder and brought to the surface. A canvas harness was placed on the dog to protect the cannula. Denton & others (1953) were able to keep the cannula patent for a period of 6 to 8 weeks. After that period a clot formed on the end of the tubing and although the dog was in a healthy condition and saline could be infused through the tubing it was impossible to withdraw blood, apparently because of the valve-like action of the clot. They suggested that the use of heparin instead of saline might alleviate the clotting problem. Their dogs were left for two weeks to recover and were then used to study the absorption of amino acids.

Other investigators have followed this direct approach. Jackson, Radeleff & Buck (1960) used highly flexible polyethylene tubing catheters in sheep. The end of the catheter was bevelled to 45 degrees to allow for easier penetration. A small puncture was made in the portal vein wall near the liver and the bevelled end of the catheter was passed through the opening until approximately 2.5 cm of catheter lay within the vein. A previously glued on suture was then used to anchor the catheter to the capsule of the liver. Jackson & others obtained about 7 days use from their catheters before clots formed. A similar method was used by Bensadoun & Reid (1962), also in sheep. A purse-string suture was first made in the wall of the portal vein. The blood flow in the vein was then occluded and an opening in the vein wall made and polyethylene tubing with a small flange at its tip introduced. The suture was completed and the tubing pulled slowly until the flange rested against the vein wall. These catheters were flushed every 6 hours which allowed blood to be withdrawn for up to 5 weeks.

A special split-needle forceps and catheter was devised by Waldern, Frost, Harsch & Blosser (1963). A 70-mm, 10-gauge needle was split longitudinally and each half was soldered to the jaws of a curved Carmalt forceps leaving about 25 mm from the tips free. The catheter consisted of polyethylene tubing

through which was threaded a stainless steel piano wire with a stainless steel plug at its tip. To anchor the catheters firmly to tissue adjoining the catheterised vein, two 1-inch square plastic tabs were cemented to the catheters and used as a base for suturing. The catheter and split-needle forceps were used as follows: The sealed catheter containing heparin in saline solution was clamped and held in the needle of the split-needle forceps when the jaws were closed with the sealed tip of the catheter just behind the bevel of the needle. When the portal vein was freed of connective tissue, the needle was inserted into the vessel, the forceps opened very slightly and the catheter threaded into the vessel. The needle was then retracted and removed from around the catheter. When the catheter was fixed in position, the wire was pushed about 1 cm into the vessel and the catheter flushed with heparin-saline solution. A blood sample was withdrawn, the tube reflushed, the wire pulled tight to close the catheter and a clamp used to hold the wire in position. The catheters, implanted in dairy cattle, remained patent for 5 to 40 days with daily flushing with heparin-saline solution. Final blockage of the indwelling catheters was due primarily to formation of thrombi at their tips.

Martin, Loriaux & Farnham (1965) placed catheters directly into the portal vein of dogs together with a cannula in the bile duct to study the entero-hepatic cycling of testosterone. Katz & Bergman (1969) used polyvinyl tubing with attached "cuffs" in catheterising the portal vein in sheep. The catheter was inserted directly into the portal vein approximately 2.5 cm from its point of entry into the liver. Purse-string sutures were tied between the cuffs on the catheter to secure the catheter in the vein. Katz & Bergman found that their catheters remained patent for approximately 5 weeks. It was found that a wire guide could be used to push the catheter tips away from the vessel walls or to dislodge clots acting as one-way valves. McGilliard & Thorp (1971) used a unique method to introduce silicone rubber catheters into the portal vein of calves. A sharpened point of bone was fitted into the tip. This point was forced through the portal vein wall, the elasticity of the wall preventing blood loss. The catheter was then fixed in place by suturing a collar, fixed on the catheter, to adjacent connective tissue. It

was then flushed with saline to dislodge the sharpened bone. The catheter remained functional for between 12 and 88 days and thrombus formation occurred at the catheter tip. The use of sharpened points of bone was considered by McGilliard & Thorp to be highly successful because intimal damage to the vessel was kept to a minimum and extravascular bleeding was eliminated. No undesirable effects were observed from dislodging points into the circulation, all of which were located at autopsy in the liver.

Resection of the twelfth rib has been used to approach the portal vein for direct insertion of a catheter (Hoover, Wolfrom & Paddleford, 1976). Good exposure was achieved, allowing an excellent working area around the portal vein. A needle was inserted into the vein and "Silastic" tubing was threaded through it. The needle was then withdrawn. This catheter had a life of 19 days; thrombus was found surrounding the catheter and partly occluding the portal vein.

A slightly different way of directly introducing a catheter into the portal vein was used by Annison, Hill & Lewis (1955) in sheep. The catheter was passed through the substance of the caudate lobe of the liver and then via the portal branch of that lobe into the portal vein. It was anchored into position with a plastic sponge. The life of these catheters was 7 to 14 days, when they became plugged by clots. Lewis, Hill & Annison (1957) used this method to study the absorption of ammonia from the rumen of sheep.

The two main problems associated with direct catheterisation of the portal vein are the difficult access to the vein and the effective anchorage of the catheter in the vein. These difficulties have led to other approaches being used.

(f) Splenic vein approach

In 1959, Shoemaker, Walker, Van Itallie & Moore described a technique for inserting a catheter into the portal vein

of a dog *via* the splenic vein. The catheter, after insertion into the splenic vein, was guided upward until its tip projected several centimetres into the portal vein. Splenectomy was then carried out. The catheter was flushed with heparin solution at 8 to 12 hour intervals. The major difficulty encountered by Shoemaker & co-workers was intravascular clotting. Shoemaker, Yanof, Turk & Wilson (1963) used such dogs to measure the intestinal absorption of glucose and fructose. The same basic approach as that of Shoemaker & others (1959) was used by Harris & Riegelman (1969). However the catheter was inserted into a branch of the splenic vein and then guided upward until its tip lay within the portal vein. The branch of the splenic vein was ligated at the point of entry of the catheter. Harris & Riegelman used these catheters only to infuse solutions of drugs. Hauge & Krippaehne (1970) also placed a catheter into the portal vein of dogs *via* the splenic vein. These dogs in addition were prepared with jejunal and colonic Thiry-Vella loops.

(g) Mesenteric vein approach

Another approach was attempted by Conner & Fries in 1960, who used one of the small mesenteric veins, usually from ileum or jejunum, to gain access to the portal vein of calves. A loop of ileum or jejunum was brought through an incision and several inches of a vein in the mesentery were exposed. A transverse incision halfway across the vein was made, through which was introduced a small, blunt-pointed probe which served as a guide to the introduction of the polyethylene catheter. Retaining a loop of intestine outside the peritoneal cavity may cause an acute angulation of the veins leading into the larger radicles of the portal system. However, it was sometimes found necessary to change the position of the exposed intestine or sometimes partially to replace it into the cavity. Location of the end of the catheter was determined by palpation of the portal fissure. The desired location for the tip was approximately one inch before the major branching of the vein within the liver. After the catheter was in the desired location, several ligatures of non-absorbable material were tied around the vein and catheter near its point of entry, thereby ligating the small vein. Further anchor-

age of the catheter was accomplished by suturing it to the mesentery. The catheter was then brought through the abdominal wall and anchored to the skin. Catheters were left filled with a heparinised saline solution and were flushed daily. Blood samples were successfully collected for periods of up to 73 days. Roe, Bergman & Kon (1966) adapted the technique for use in sheep.

A different design of catheter was devised by Moodie, Walker & Hutton (1963) who used it in sheep. The mesenteric vein approach was used for its insertion but they attempted to avoid the problems associated with thrombus formation at the catheter tips with the different design. The catheter consisted of two flexible nylon tubes, one of which fitted inside the other. The inner tube had a nylon tap at its external end which fitted into a collar on the outer tube with a nylon tape to secure the fitting. After exposure of the common mesentery, a large anterior branch of the intestinal trunk of the mesenteric vein was freed from the accompanying artery and nerve for a length of about 1 cm. A small incision was made in the vein and the outer tube of the catheter passed in a forward direction between the points of forceps, which were used to dilate the vein, until the tip lay in the portal vein at the entrance to the liver. The inner tube was inserted into the catheter, filled with anticoagulant (heparin solution) and the tap closed. The position of the tip of the catheter was then confirmed by palpation and adjusted if necessary. The catheter was anchored to the common mesentery and the inner tube was kept filled with heparin solution and flushed with normal saline every 12 hours. Blood samples were usually withdrawn from the inner tube but if this was not possible the inner tube was removed and samples withdrawn through the outer tube. Small clots tended to act as a valve at the tip of the catheter, but another inner tube could be inserted into the outer tube so that its tip lay 1 to 2 cm beyond the end of the outer tube. The catheters remained useful for 43 to 161 days. At post mortem it was found that the catheter was embedded in the wall of the vein for most of its length and its tip was covered with tissue in the form of a papilla. Use of the longer inner tube enabled these obstructions to

be bypassed for a period of time but such growth eventually made even this procedure impossible. These double tube catheters were used by Moodie & Walker (1963) for estimating mineral absorption in sheep.

Carr & Jacobsen (1968) used calves in which a portal vein "Silastic" catheter was inserted via the gastroepiploic or the ileocoelic vein (mesenteric veins). In addition, a Doppler shift transducer was tied loosely about the portal vein to permit simultaneous portal vein flow measurements. Catheters remained patent for 15 to 97 days. Thrombi at the end of the catheters were again thought to be responsible for them becoming inoperative but no clotting was found inside the catheters. Harrison (1969) introduced a vinyl catheter into the portal vein of sheep via a small mesenteric vein and it was used by Paterson & Harrison (1972) to study the absorption of hydrocortisone. Polyvinyl chloride catheters were used by Elliott & Furneaux (1971) in catheterising the portal vein of a pig via the large anterior mesenteric vein. The catheter was inserted in the wall in the centre of a purse-string suture and was fixed to the omentum near to the site of entry. The use of the purse-string enables the flow in the anterior mesenteric vein to remain undisturbed. In addition these pigs had catheters inserted into the following vessels: jugular vein, carotid artery, hepatic vein, a mesenteric vein, bile duct and an intestinal lymph duct. The mesenteric vein catheter enabled an indicator to be infused so that portal vein blood flow could be determined by an indicator dilution technique.

The use of small mesenteric branches has been applied as a technique for portal catheterisation in rhesus monkeys (Faulkner, Czazkowski, Rayfield & Hickman, 1976). The right colic or ileocolic vein was used and careful placement of the catheter tip in the portal vein was found to be essential for patency because of the shift in organ position when sitting. However post-operatively, the monkey was required to be in a restraining chair. The mean life of catheters, which had a single end-hole, was 11 days. Multiple end-hole catheters were tried, but could not be maintained by daily flushing and required

a constant infusion of heparinised saline to prevent clotting. Faulkner & others (1976) described their technique as brief, relatively simple and successful. However their experiments required a catheter life of only about 7 days. Whilst using a mesenteric or splenic vein for catheterisation improves accessibility and anchorage, it suffers from the difficulty of accurate siting of the catheter tip in the portal vein. After recovery from surgery and movement of the animal, the tip may move away from the portal vein either up into the liver or be pulled down into the mesenteric veins.

(h) T-tube method

Mouzas & Smith (1969) withdrew portal blood using a "Silastic" T-tube which was inserted in the portal vein of dogs. Samples were able to be withdrawn up to 6 weeks after insertion. A T-shaped catheter was implanted into the portal vein of rats by Sable-Amplis & Abadie (1975). The portal vein of the rat was freed of connective tissue for a distance of 0.5 cm. Loose ligatures were passed under the vein at each end of the cleared area and the circulation was interrupted with haemostats. After hemisection of the vein, one end of the short portion (cross-bar of the T) of the catheter was inserted and tied in place. The vessel was then completely sectioned and the other end of the short piece inserted and tied leaving the long portion to be brought through the skin at the back of the neck. The catheters seemed to have a life of approximately one month.

The portal vein of the rat is small and fragile and Gallo-Torres & Ludorf (1974) have similarly inserted a T-tube piece with the flow diverted through it. The method required speed, otherwise engorgement and congestion of the mesenteric circulation occurred with liver hypoxia and clotting of blood within the portal vein. The method also seems to suffer from the disadvantage that too many other veins (the splenic, pyloric and coronary) are ligated and this affects portal haemodynamics; but the catheter life was long, up to 6 months. Gallo-Torres & Ludorf (1974) examined other methods of portal vein catheterisation in the rat but found none resulted in a long catheter life. However, the rat is unsuitable for most portal

vein studies due to its small size. The total number of blood samples are limited and their size must of necessity be very small and consequently very sensitive assay methods are required. There is also the possibility of restraint-stress, as rats need to be kept in a restraining cage following portal vein catheterisation.

(i) Umbilical vein route

Human patients with therapeutic portal anastomotic veins in the anterior abdominal wall have occasionally been used for absorption studies (Sherlock & Walshe, 1946; Bean, Franklin, Embick & Daum, 1951). However a significant finding was made by Bayly & Gonzales Carbalhaez in 1964 when they reported that the umbilical vein of an adult could be reopened and used to introduce a catheter into the portal vein. This had important clinical significance as it facilitated portography, diagnosis of acute gastrointestinal bleeding and treatment of portal hypertension. Major abdominal surgery was not required and the catheterisation could be performed using only a local anaesthetic. The umbilical vein makes an end to end junction with the left branch of the portal vein, the junction lying in the umbilical fissure of the liver.

Little advantage has been taken of using these catheters in patients for absorption studies. Dencker & co-workers (1972a,b,c, 1973) investigated the absorption of glucose, fructose and maltose using umbilical vein catheterisation. In addition, Andersson & co-workers (1975, 1977) studied the absorption of digoxin and proscillaridin. They removed samples of portal and peripheral blood simultaneously and constructed portal-peripheral difference curves.

McGilliard (1968) attempted to use the umbilical vein to introduce a catheter into the portal vein of calves. He was successful in calves 8 to 10 days old but after 25 days he found that the umbilical vein had degenerated and could not be used. Five-day old pigs were catheterised via the umbilical vein by Shimada & Zimmerman (1973); the catheters had a life of 1 to 2 weeks. They studied amino

acid absorption finding the concentrations in the portal vein higher than those in the vena cava during and immediately after feeding. Unfortunately, when the pigs grew the catheter was not long enough to reach the portal vein. Also, formation of a sheet of tissue at the tip of the catheter occurred, which allowed injection of substances but not withdrawal of blood. Faulkner & others (1976) tried to use the umbilical vein in monkeys, but found it difficult to re-establish the patency of the vein.

(j) Percutaneous transhepatic catheterisation

Recently the percutaneous transhepatic approach has gained use for portography in human patients (Burcharth, 1979). The method involves puncture of the abdominal wall under either general or local anaesthesia (Wiechel, 1979) and insertion of a guide-wire after a branch of the portal system is reached. The guide-wire is advanced under fluoroscopic guidance to the portal vein and a catheter is then inserted over the guide-wire. The method has been similarly used in the dog (Burgener, Gothlin & Gutierrez, 1978). However, until data on its long term use are available it can only be regarded as having potential. It suffers from a number of disadvantages. It is radiologically facilitated and skill is required for insertion without damaging other internal structures. The liver itself is punctured in any case during the procedure. Catheters have been left in place in humans for only a few days and Burgener & others (1978) only used the method as an acute procedure. Other complications encountered in humans have been thrombosis of smaller veins and internal bleeding (Wiechel, 1979).

(k) General

With the exception of the work of Paterson & Harrison (1972) and Andersson & others (1975, 1977), all of the studies for which portal vein catheters in conscious animals have been used have been concerned with sugars, amino acids and other nutrients, or with recording portal and hepatic blood flows. No studies in conscious animals have been reported dealing with drug absorption. All of the

methods so far devised and used have suffered from technical difficulties of one kind or another. The limited life of catheters is a serious nuisance when so much effort must be put into the surgical placement and post-operative maintenance. It is highly desirable that animals can be used long term so that several studies can be conducted in the same animal.

1.2.4 Summary of the major methods used to measure gastrointestinal absorption of drugs

Tables 1.1 and 1.2 are a summary of methods which are considered the major ones for the measurement of drug absorption. These are the methods that have been most commonly used, together with those explored and considered to have the most potential for future use.

1.3. Metabolism, Pharmacokinetics and Bioavailability of Prednisolone and Other Corticosteroids

Relatively little has been reported about the metabolism, kinetics and bioavailability of prednisolone (Thiessen, 1976) and much of the data that have recently appeared, especially with respect to the kinetics, are conflicting. Considerably more information is available about hydrocortisone, a naturally occurring corticosteroid which differs from prednisolone in not possessing the 1-2 double bond in ring A, but which might be expected to have a similar metabolic fate and kinetics. Hydrocortisone, like prednisolone, may be subject to bioavailability problems (Schneller, 1970). Prednisone and prednisolone have low solubilities in water which suggest that absorption may be dissolution-rate limited. It should be noted that in contrast to prednisone, bioinequivalence of prednisolone tablets has not been demonstrated (Thiessen, 1976). Prednisolone is used in tablet formulations and studies involving intravenous administration have used either prednisolone in a suitable solvent or the 21-phosphate ester which hydrolyses extremely rapidly *in vivo* (Kitagawa, Mohri & Kitagawa, 1972; Tse & Welling, 1977).

TABLE 1.1. Summary of the major *in vitro* methods used to measure gastrointestinal absorption of drugs. These methods are those most commonly used and those with the most potential for future use.

Method	Aspect studied	Relative use in drug absorption studies	Animals used	Notes
Fisher & Parsons (1949) circulation method	Mucosal-to-serosal transfer	little	rat intestine	Cumbersome
Wilson & Wiseman (1954) everted sacs	"	"	rat or hamster intestine	Serial sampling not possible
Crane-Wilson (1958) cannulated everted sacs	"	extensive	"	Probably best *in vitro* method in use
Dietschy & others (1966) perfusion method	Mucosal-to-serosal transfer or serosal-to-mucosal	some	"	Possible to use with everted or non-everted segments

TABLE 1.2. Summary of the major *in vivo* methods used to measure gastrointestinal absorption of drugs. These methods are those most commonly used and those with the most potential for future use.

Method	Aspect studied	Relative use in drug absorption studies	Animals used	Notes
Blood levels	Appearance in systemic blood	extensive	rabbit, dog, human	Most pharmacokinetic and biopharmaceutic studies use these methods
Urinary excretion	"	"	"	
Intubation	Disappearance from lumen	some	human	
Closed segments:				
Levine & others (1955)	"	"	rat	Serial sampling not possible
Schanker & others (1957)	"	"	"	
Doluisio & others (1969)	"	"	"	Serial sampling possible
Perfused segments:				
Schanker & others (1958)	"	"	"	Not good for slowly absorbing drugs. Closed loops best
Closed or perfused segments with complete mesenteric venous collection	" and appearance in mesenteric blood	little to date	rat, cat, dog, rabbit	Best *in situ* (anaesthetised animal) method to use
Fistulous animals	Disappearance from lumen	"	dog, monkey	Can be combined with portal vein or systemic blood sampling. Technically difficult
Portal vein:				
in situ sampling	Appearance in mesenteric blood	"	rat, cat, dog	Can be combined with closed or perfused loops
conscious animals	"	none	none	For best use should be combined with either intubation or a fistula-type method. Technically difficult. Use with patients has occurred recently

Prednisone is biologically inactive and is converted *in vivo* to its active metabolite, prednisolone.

1.3.1 Metabolism

(i) Metabolites in plasma

There have been few attempts to study systematically the metabolites of prednisolone occurring in blood, although levels of prednisolone and prednisone resulting from their interconversion have been examined. El Dareer, Struck, White, Mellett & Hill (1977) have investigated the metabolites of prednisone present in the blood of mice, dogs and monkeys. As well as unchanged prednisone, prednisolone and 20β-hydroxyprednisolone were detected in all three species. In the dog, Miyabo, Kishida & Hisada (1976) found the metabolites of ^{14}C-hydrocortisone in plasma 2.5 hours after intravenous injection to be 20 to 30 per cent unconjugated, 60 to 70 per cent as glucuronides and 8 to 9 per cent as sulphates. In contrast, more free (46 per cent) and less conjugated metabolites (approximately 44 per cent glucuronides, 2.5 per cent sulphates) were identified in human plasma under identical conditions (Miyabo & Kornel, 1974). Miyabo & Kornel (1974) also found that the metabolites of hydrocortisone in plasma were identical to those found in urine.

Interconversion between prednisolone and prednisone occurs. This interconversion is rapid and, regardless of whether prednisolone or prednisone is given, the plasma concentrations of prednisolone always exceed those of prednisone. The plasma concentration profiles of both compounds following oral administration of either prednisolone or prednisone are almost identical.

Following intravenous dosing of prednisolone to human subjects (Meikle, Weed & Tyler, 1975; Pickup & co-workers,1977), the concentrations of prednisone in the plasma rose to a maximum after one hour but were about one-sixth the prednisolone level at this time.

A similar pattern has been observed following oral dosing to humans (Davis & co-workers, 1978; Tanner, Bochner, Caffin, Halliday & Powell, 1979a) and beagle dogs (Colburn, Sibley & Buller, 1976). The prednisolone concentration peaks at approximately 1.5 hours whilst the prednisone reaches a maximum concentration at 2.5 hours. Prednisone is rapidly converted to prednisolone in man (Wilson, Ssendagire, May & Paterson, 1975; Sullivan, Hallmark, Sakmar, Weidler, Earhart & Wagner, 1976a; Schalm, Summerskill & Go, 1977; Davis & others, 1978; Matin & Amos, 1978) and dogs, mice and monkeys (Colburn & others, 1976; Schalm, Summerskill & Go, 1976; El Dareer & others, 1977; Newsome, Turley & Kennerly, 1977). After intravenous doses of prednisone, maximum prednisolone concentrations occur after approximately 5 minutes and are 3 to 5 times the prednisone concentrations. Following administration of oral doses of prednisone, the maximum concentration of prednisolone occurs after approximately one hour, whilst simultaneous measurement of the prednisone concentration shows it peaking at 1.5 to 2 hours after dosing. Meikle & others (1975) found about 70 per cent of a 10 mg oral dose of prednisone to man, was absorbed, metabolised to prednisolone and distributed to peripheral plasma within 8 hours.

The conversion of prednisone to prednisolone takes place predominantly in the liver (Meikle & others, 1975; Schalm & others, 1976; El Dareer & others, 1977), whereas the oxidation of the 11-hydroxyl group of prednisolone to form prednisone occurs in peripheral tissues (Meikle & others, 1975). It has been suggested that there is an interconversion equilibrium between the two compounds (Wilson & others, 1975; Colburn & others, 1976; Uribe, Go & Summerskill, 1976; Schalm & others, 1977). This is feasible because the compounds are metabolised <u>via</u> the pathways responsible for the interconversion of cortisone and hydrocortisone (Jenkins, 1966; Srivostava & co-workers, 1973). The equilibrium between prednisone and prednisolone following prednisolone administration is not influenced by variation of the dose (Tanner & others, 1979a).

There are conflicting reports concerning the conversion of prednisone to prednisolone in patients with liver disease. Some

investigators (Jenkins & Sampson, 1967; Schalm & others, 1977; Uribe, Schalm, Summerskill & Go, 1978) have found normal conversion whilst others (Powell & Axelsen, 1972; Davis & others, 1978) have found conversion to be delayed.

(ii) Metabolites in urine

Most of the study of the metabolism of prednisolone has been carried out on the metabolites found in urine. In man, it appears that the major pathway of metabolism for prednisolone is reduction of the Δ^1 double bond to form hydrocortisone and then via the route followed by hydrocortisone. Hydrocortisone is excreted in the urine of man mainly as 3β-D-glucuronides (Fotherby & James, 1972; Kornel & Saito, 1975). Saturation of ring A (Figure 1.18) occurs at the Δ^4-3-keto group forming the "tetrahydro" steroid followed by glucuronidation. Tomkins (1956) and Isselbacher (1956) found that prednisolone, after reduction of the Δ^1 double bond to form hydrocortisone, followed the hydrocortisone pathway in rat liver slices. However it has been shown in man that the $\Delta^{1,4}$-3-keto group of prednisolone is not reduced as rapidly as the Δ^4-3-keto group of hydrocortisone (Gray, Green, Holness & Lunnon, 1956; Sandberg & Slaunwhite, 1957; Vermeulen & Caspi, 1959; Slaunwhite & Sandberg, 1961).

Vermeulen (1959) found that 30 per cent of an oral dose of ^{14}C-prednisolone was eliminated unconjugated in human urine. The metabolites discovered in the urine included unchanged prednisolone, prednisone, the "tetrahydro" derivatives and 20β-hydroxy derivatives. The amount of unchanged prednisolone present in urine is approximately 10 per cent of an oral dose (Uete & Shimano, 1971; English, Chakraborty, Marks, Trigger & Thomson, 1975). A similar pattern of metabolites has been found in the horse (Moss & Rylance, 1967).

More than 90 per cent of an intravenous dose of ^{14}C-prednisolone was recoverable from the urine of patients, approximately half as glucuronides, one-tenth as "sulphates" and the rest unconjugated (Slaunwhite & Sandberg, 1961). Vermeulen (1959) suggest-

ed that conjugation of prednisolone occurs at both C-3 and C-21 but was slower at C-21. Prednisolone metabolism takes place predominantly in the liver and a diagrammatic summary of the major metabolic pathways of prednisolone in various species is given in Figure 1.18.

The nature and amounts of metabolites of prednisolone, hydrocortisone and other corticosteroids excreted in the urine of various species are found in Table 1.3 together with the data for biliary and faecal excretion where available. In the dog, the urinary excretion of metabolites of hydrocortisone, prednisolone and methylprednisolone is less than in man. No glucuronide conjugates of hydrocortisone were excreted in the urine of the hepatectomised dog, in contrast to the intact animal (Gold, 1961a,b) showing that glucuronidation of corticosteroids takes place solely in the liver.

El Dareer & co-workers (1977) studied the distribution and metabolism of prednisone in mice, dogs and monkeys. Five minutes after intraperitoneal injection of ^{3}H-prednisone to mice, it was found that only 8 per cent remained as unchanged prednisone in the liver. Fifty per cent of the radioactivity was accounted for as prednisolone. However after 15 minutes, most radioactivity in the liver was due to metabolites of prednisolone.

(iii) Metabolites in bile

Information about the biliary excretion of corticosteroids is scarce, but some data on the percentage of total metabolites excreted in bile and faeces are shown in Table 1.3. Slaunwhite & Sandberg (1961) found that less than 5 per cent of an intravenous dose of ^{14}C-prednisolone was excreted in the bile of patients with T-tube drainage. Significant amounts of sulphate conjugates as well as glucuronides were present.

Extensive uptake of ^{14}C-hydrocortisone and ^{14}C-cortisone in the liver and rapid biliary excretion of metabolites have been shown in mice (Hanngren, Hansson, Sjostrand & Ullberg, 1964). Other evidence also indicates that appreciable amounts of metabolites

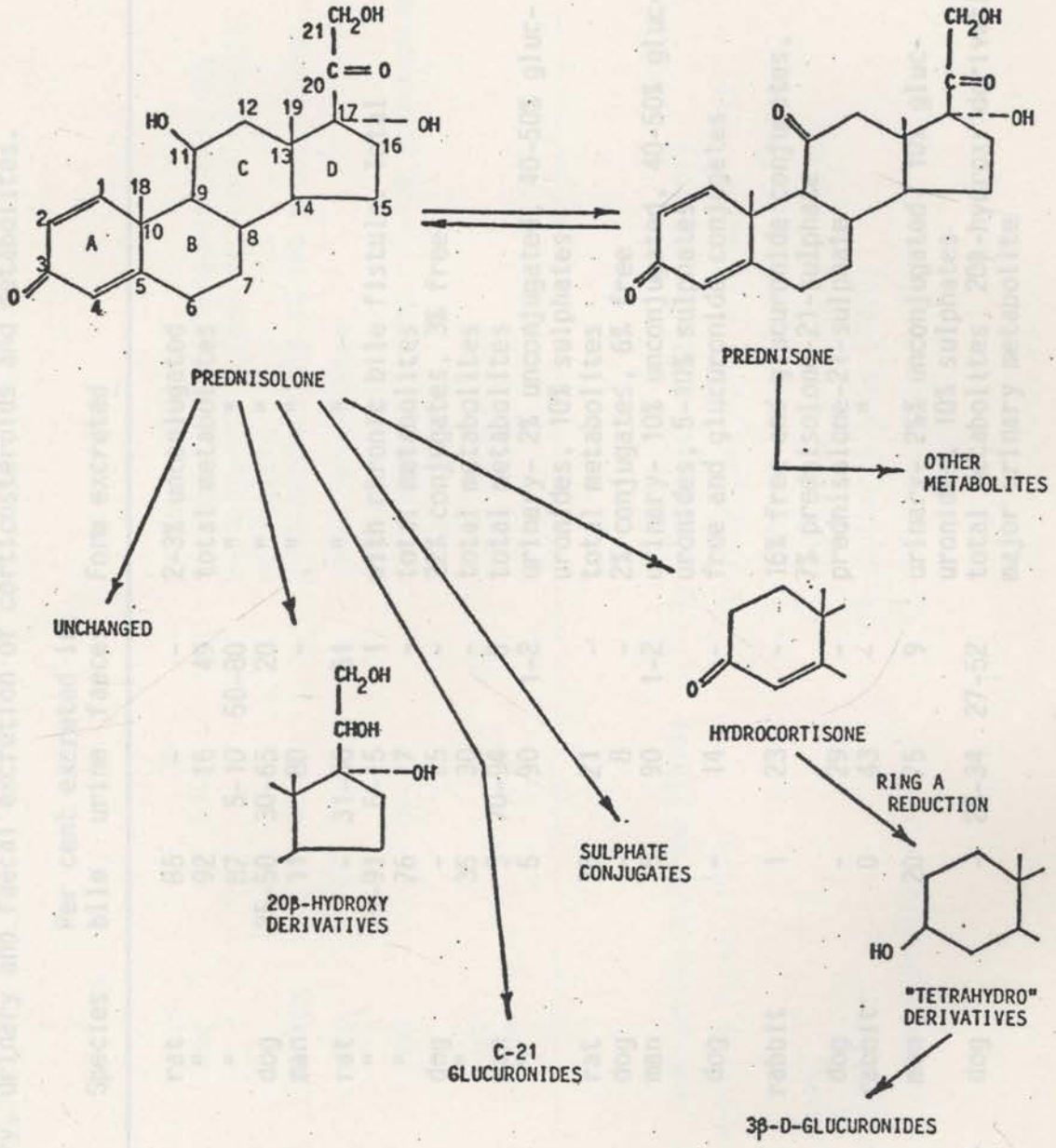


Figure 1.18. The major metabolic pathways of prednisolone.

TABLE 1.3. Biliary, urinary and faecal excretion of corticosteroids and metabolites.

Compound	Species	Per cent excreted in: bile	urine	faeces	Form excreted	reference
corticosterone	rat	85	-	-	2-3% unconjugated	1
	"	92	16	49	total metabolites	2
	"	87	5-10	50-80	" "	3
	dog	25-50	30-65	20	" "	3
	man	11	80	-	" "	3
hydrocortisone	rat	-	31-36	61	" "	3
	"	79-91	6-15	1	with chronic bile fistula. Total	3
	"	76	17	-	total metabolites	4
	dog	-	25	-	22% conjugates, 3% free	5
	"	35	30	-	total metabolites	6
	man	5	70-94	9	total metabolites	3
	"	5	90	1-2	urinary- 2% unconjugated, 40-50% glucuronides, 10% sulphates	7
prednisolone	rat	76	21	-	total metabolites	4
	dog	-	8	-	2% conjugates, 6% free	5
	man	5	90	1-2	urinary- 10% unconjugated, 40-50% glucuronides, 5-10% sulphates	7
prednisolone-21-phosphate	dog	-	14	-	free and glucuronide conjugates	8
	rabbit	1	23	-	16% free and glucuronide conjugates, 7% prednisolone-21-sulphate	8
prednisolone-21-sulphate	dog	-	29	-	prednisolone-21-sulphate	8
	rabbit	0	43	-	"	8
methylprednisolone	man	20	75	9	urinary- 2½% unconjugated, 10% glucuronides, 10% sulphates	7
methylprednisolone-21-acetate	dog	-	25-34	27-52	total metabolites, 20β-hydroxy derivative major urinary metabolite	9

cont.....

TABLE 1.3. (continued)

Compound	Species	Per cent excreted in: bile	urine	faeces	Form excreted	reference
prednisone	mouse	-	34	-	total metabolites	10
	dog	-	61-72	-	2% prednisone, 6% prednisolone, 20β-hydroxyprednisolone detected as major metabolite	10
	monkey	-	68-84	-	2-7% prednisone, 11-16% prednisolone	10
betamethasone	rat	61	28	54	total metabolites	2
	"	44	-	-	14% unconjugated	1
	man	-	58-79	-	14-31% unconjugated, 10-16% glucuronides, 2-7% sulphates	11
triamcinolone	rat	-	25	50	total metabolites	12
	dog	-	46	42	" "	12

Key to references;

1 Jones, Tredger, Chakraborty & Parke (1975)
2 Tredger, Chakraborty & Parke (1973)
3 Taylor (1971)
4 Ware & Combes (1973)
5 Silber & Morgan (1956)
6 Macarol, Morris, Baker & Bradley (1970)
7 Slaunwhite & Sandberg (1961)
8 Kitagawa, Mohri & Kitagawa (1972)
9 Buhler, Thomas & Schlagel (1965)
10 El Dareer & co-workers (1977)
11 Butler & Gray (1970)
12 Florini & others (1961a,b)

of corticosteroids are secreted into the bile of animals other than man. After both oral administration and intramuscular injection of 6α-methylprednisolone-21-acetate to dogs, faecal elimination of metabolites predominated (Buhler, Thomas & Schlagel, 1965), indicating major biliary excretion of methylprednisolone and/or metabolites. Hydrolysis of the acetate has been shown to occur in vivo (Garg, Ng, Weidler, Sakmar & Wagner, 1978). Following intravenous injection of ^{3}H-triamcinolone to rats, Florini & co-workers (1961a,b) found that 25 per cent was excreted in the urine over a period of 1 to 3 days and 50 per cent in the faeces. Large amounts of radioactivity were found in the intestinal contents between 30 minutes to 4 hours after injection. El Dareer & others (1977) dosed a monkey with ^{3}H-prednisone (1 mg per kg intravenously), killed it after 30 minutes and examined its tissues for radioactivity. The concentrations of prednisone, prednisolone and other metabolites were high in the kidneys and liver. However, the concentration of radioactivity in the bile was approximately 10 times that of serum. It consisted mainly of other metabolites together with some prednisolone and unchanged prednisone.

1.3.2 Pharmacokinetics

(i) Plasma concentration profile and half-life

In humans, oral doses of prednisolone are readily absorbed and show a peak plasma level at 1 to 2 hours. Similarly in the beagle dog, a peak prednisolone concentration occurs approximately one hour after administration of the dose (Colburn & Buller, 1973; Colburn & others, 1976; Tse & Welling, 1977). This is followed by continuous decline in the plasma levels suggesting absence of entero-hepatic circulation. The plasma half-life ($t_{½}$) of prednisolone in normal healthy man has been found to range from 2.1 hours to 5 hours. Table 1.4 lists the values obtained by various investigators. The $t_{½}$ of prednisolone in the dog is shorter than in man; half-lives ranging from 0.3 to 2.9 hours have been reported (Table 1.5). In contrast, the half-life of hydrocortisone in plasma following rapid intravenous injection in man is 1.5 to 2.0 hours (Peterson,

Wyngaarden, Guerra, Brodie & Bunim, 1955; Slaunwhite & Sandberg, 1961) and 44 to 52 minutes in the dog (Silber & Morgan, 1956; Kuipers, Ely, Hughes & Kelley, 1957). In both man and the dog, wide variations for the plasma half-life have been observed (see Tables 1.4 and 1.5), with the short value of 0.3 hour obtained in the male beagle dog by Tse & Welling (1977) being of particular note. There is also considerable variation in the peak plasma concentrations reported. A 10 mg dose of prednisolone has been shown to reach peak plasma levels ranging from approximately 100ng/ml (Davis & others, 1978) to approximately 300 ng/ml (Loo & Jordan, 1978), whilst a 30 mg dose was shown to approach levels of only 150 ng/ml (Hulme, James & Rault, 1975). In contrast, a peak level of 600 ng/ml has been found from a 30 mg dose of prednisone (Uribe & others, 1978). The differences in the peak plasma levels could be due to many causes, among them bio-availability problems, but as the detailed pharmacokinetics of prednisolone has still not been fully investigated, one can only speculate on the reasons for the differences in the peak levels as well as the half-lives. Pickup (1979) commented that the differences are difficult to explain in terms of variability in dose, body weight, plasma protein binding, food intake, drug formulation or half-life but may be a reflection of difficulties encountered with assay methods.

(ii) Pharmacokinetic models

Intravenous disappearance curves of prednisolone and hydrocortisone have mostly been described in terms of a single exponential (Peterson, 1959; Slaunwhite & Sandberg, 1961), but in a comparison of the kinetics of ^{3}H-prednisolone and ^{14}C-hydrocortisone in man (Araki & co-workers, 1966), it was shown that plasma disappearance curves after intravenous dosing of both drugs were best represented as the sum of two exponentials. A two-compartment open model has been used to describe the pharmacokinetics of prednisolone in most recently published work done in humans. For example, Pickup & others (1977) found that their data consisted of a rapid distribution phase which was complete within half an hour and a second slower terminal elimination phase and the model used to describe the

TABLE 1.4. Prednisolone half-life ($t_{½}$) in man.

Investigator	dose (mg) (oral unless otherwise stated)	$t_{½}$ (hours)
Tanner & others (1979a)	200	3.8
" " "	100 (i.v.)	3.6
D'Arcy & others (1971)	90	2.7
Colburn & Buller (1973)	60	3.5
Meikle & others (1975)	40 (i.v.)	5.0
Hsueh & others (1979)	30	4.2
" " "	30 (i.v.)	4.2
Miyachi & others (1979)	20	3.0-3.7
Wilson & others (1977)	20	2.1
Wilson & others (1975)	20	2.2
Leclercq & Copinschi (1974)	20	3.5
Sandberg & others (1970)	20	2.4
Pickup & others (1977)	~20 (i.v.)	3.9
Kozower & others (1974)	~20 (i.v.)	4.2
Tanner & others (1979a)	20 (i.v.)	3.6
English & others (1975)	15	3.5
English & others (1974)	15	2.5
Sullivan & others (1974)	10	2.9
Morrison & others (1977)	10	2.2
Davis & others (1978)	10	2.1
Meikle & others (1975)	10 (i.v.)	4.0
Tembo & others (1977b)	10	2.4
Pickup & others (1977)	~10 (i.v.)	3.3
Tanner & others (1979a)	10	3.7
Morrison & others (1977)	5	2.2
Pickup & others (1977)	tracer (i.v.)	2.5

TABLE 1.5. Prednisolone half-life ($t_{½}$) in the dog. All values were obtained from beagle dogs, except those of Kuipers & others (1957) and Kitagawa & others (1972) which were from mongrels.

Investigator	dose (mg)	$t_{½}$ (hours)
Kitagawa & others (1972)	~ 60 (i.v.)	1.2
Tse & Welling (1977)	60 (i.v.)	0.83
" " "	60 (oral)	0.85-1.2
Kuipers & others (1957)	40 (i.v.)	1.1
Kitagawa & others (1972)	~ 30 (i.v.)	1.2
Tse & Welling (1977)	30 (i.v.)	0.3
" " "	30 (oral)	0.82-1.9
Silber & Morgan (1956)	25 (i.v.)	1.0
Colburn & others (1976)	5 (oral)	1.8
Colburn & Buller (1973)	5 (oral)	2.9
" " "	2.5 (oral)	2.4

kinetics is shown in Figure 1.19.

A more complex two-compartment model was proposed to describe the kinetics of hydrocortisone (Tait & Burstein, 1964; Nugent, Warner, Estergreen & Eik-Nes, 1965) (see Figure 1.20) and has been used with prednisolone data obtained following intravenous injection in man (Kozower & others, 1974; Meikle & others, 1975). Meikle & others (1975) found the volume of distribution of the plasma (central) compartment to be similar for a 10 mg intravenous dose (12.5 litre/m^2) and a 40 mg intravenous dose (16 litre/m^2). However the volume of distribution of the peripheral (tissue) compartment was significantly larger at the higher dose, the volumes being 7.0 and 18 litre/m^2 respectively. The plasma clearances of prednisolone at the two doses were similar (82 and 100 litre/24 h/m^2).

To date, the application of a two-compartment model to the kinetics of prednisolone in the dog has not been reported. Tse & Welling (1977) claimed that their intravenous data were monoexponential. However the data points were not plotted on the curve, nor tabulated. The volume of distribution in the beagle dog was given as 12.3 litres

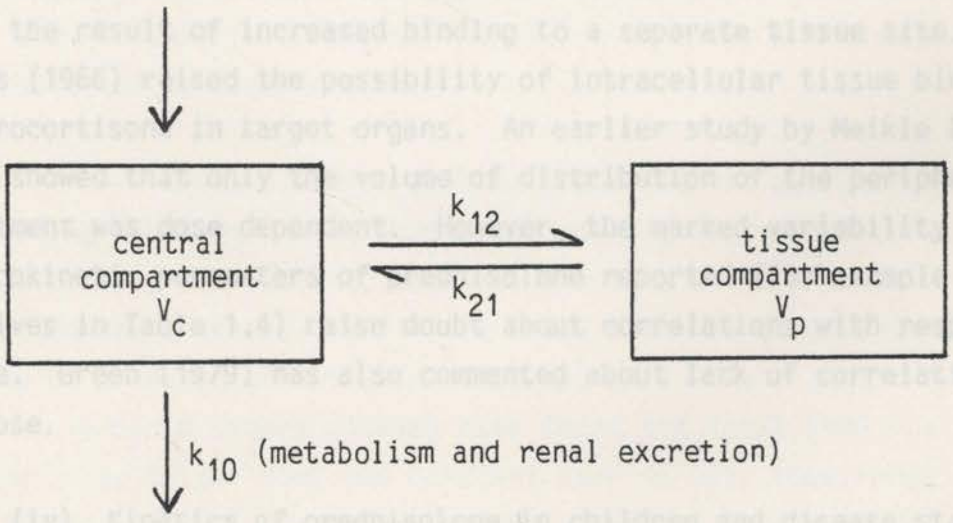


Figure 1.19. Two-compartment model used by Pickup & others (1977) to fit prednisolone kinetic data obtained from intravenous administration to humans.

or 69 per cent of the total body volume which is approximately equal to the total volume of body water.

(iii) Dose-dependency of prednisolone kinetics

It has been generally accepted that the kinetics of prednisolone are dose independent (English & others, 1975; Green & co-workers, 1978); however more recent studies (Pickup & others, 1977; Loo, McGilveray, Jordan, Moffat & Brien, 1978; Rose, Jusko & Nickelsen, 1979; Tanner & others, 1979a) indicate that prednisolone may have dose-dependent kinetics. Pickup & others (1977) found that increasing the dose increased the half-life as well as the volumes of distribution of the central and peripheral compartments and the plasma clearance. The total volume of distribution rose from 15.8 litres for a tracer dose to 45 litres for a dose of 0.3 mg/kg. The plasma clearance rose from 0.06 litre/kg/hour to 0.12 litre/kg/hour. Pickup & others (1977) proposed that the dose dependency of prednisolone that they observed was due to the saturable binding to plasma protein (CBG and albumin). However Tanner & others (1979a) found that only the volume of distribution ($V_{d\beta}$) was increased with increasing dose. They also found no alteration in plasma protein binding over a wide dosage range and suggested that the dose related increase in the volume of distribution may be the result of increased binding to a separate tissue site. Samuels (1966) raised the possibility of intracellular tissue binding of hydrocortisone in target organs. An earlier study by Meikle & others (1975) showed that only the volume of distribution of the peripheral compartment was dose dependent. However, the marked variability of pharmacokinetic parameters of prednisolone reported (for example see half-lives in Table 1.4) raise doubt about correlations with respect to dose. Green (1979) has also commented about lack of correlations with dose.

(iv) Kinetics of prednisolone in children and disease states

The half-life of prednisolone in children has been found by Green & others (1978) to be 2.2 hours and although they claim that the $t_{1/2}$ is shorter in children than adults, reference to

Table 1.4 shows that $t_{1/2}$'s slightly greater than 2 hours have also been found for adults.

It appears that the elimination half-life of prednisolone is increased in patients with liver disease. Davis & others (1978) found normal controls had a mean $t_{1/2}$ of 2.1 hours whilst patients with liver disease had a mean $t_{1/2}$ of 3.7 hours. Half-lives for prednisolone have been reported to be no different in patients with respiratory disease (Wilson & others, 1975) and in renal transplant recipients (Henderson, Wheatley, English, Chakraborty & Marks, 1979) from normal controls. No kinetic differences were found between asthmatic patients given high or low doses of prednisolone (May, Caffin, Halliday & Bochner, 1980).

(v) Plasma protein binding

Corticosteroids can bind both to CBG (corticosteroid-binding globulin, transcortin) and to albumin. CBG has a low capacity but high affinity for hydrocortisone, prednisolone and other corticosteroids. The binding capacity of CBG for prednisolone is equal to that for hydrocortisone, and like hydrocortisone, binding to albumin occurs when CBG capacity is saturated (Lewis, Jusko, Burke & Graves, 1971b). Albumin has a low affinity but high capacity for binding of corticosteroids.

Lewis & others (1971b) found that 90 per cent of circulating prednisolone was bound to plasma proteins at low doses, but the binding decreased to about 65 per cent at high doses. However Schalm & others (1977) have found that the total plasma protein binding of prednisolone at a low dose was only 80 per cent. By contrast Tanner & others (1979a) have found the total (CBG + albumin) binding to be 86 per cent and constant over an oral dose range of 5 to 120 mg. Angeli, Frajria, De Paoli, Fonzo & Ceresa (1978) found diurnal variation of both prednisolone and hydrocortisone binding to CBG in normal healthy volunteers, but in patients treated with prednisone over a long term, the diurnal variation did not exist.

Nugent & others (1965) proposed that CBG markedly affected the level of diffusible hydrocortisone - that is free hydrocortisone in the plasma able to diffuse into the extravascular tissues - and proposed the two-compartment model shown in Figure 1.20. Samuels (1966) suggested that corticosteroids may distribute preferentially into phospholipids and that this may account for adsorption of hydrocortisone on the surfaces of cells such as erythrocytes. The model of Nugent & others (1965) has recently been refined by Glantz, Luetscher, Day & Perloff (1976). This new model is more elaborate and assumes that free and albumin-bound (loosely) hydrocortisone are removed from the blood by the kidneys and the liver and that the CBG-bound (tightly) hydrocortisone is not extracted.

Plasma protein binding is a major factor inhibiting hepatic extraction of hydrocortisone (Zipser, Speckart, Zia, Edmiston, Lau & Horton, 1976). Aldosterone, which does not bind to CBG, has a splanchnic extraction or first-pass effect in humans of 92 per cent whereas for hydrocortisone it is 8 per cent (Zipser & others, 1976). It appears that the percentage of hydrocortisone extracted is similar to the percentage not bound to CBG. The CBG concentration in the dog is lower than in man and the extraction ratio of hydrocortisone is correspondingly higher (Glantz & others, 1976). McCormick, Herman, Lien & Egdahl (1974) showed that approximately 50 per cent of plasma hydrocortisone was removed in a single pass through the liver of the dog, whereas in man (Tait, 1963) only 7 to 25 per cent was extracted. The splanchnic extraction of hydrocortisone has also been related to plasma flow; at higher flows it is less efficient (Paterson & Harrison, 1972). However the clearance appeared to increase with increasing hepatic blood flow.

Using a two-compartment distribution model comprising a peripheral (tissue) compartment and a central (plasma) compartment, Lewis & others (1971b) calculated that with a steady-state total body pool of 1 mg of prednisolone, about 46 per cent of the steroid would be unbound in a normal subject. With a 5 mg body content of prednisolone, 64 per cent would be unbound. With decreased serum and extravascular protein levels, the amount unbound was calculated

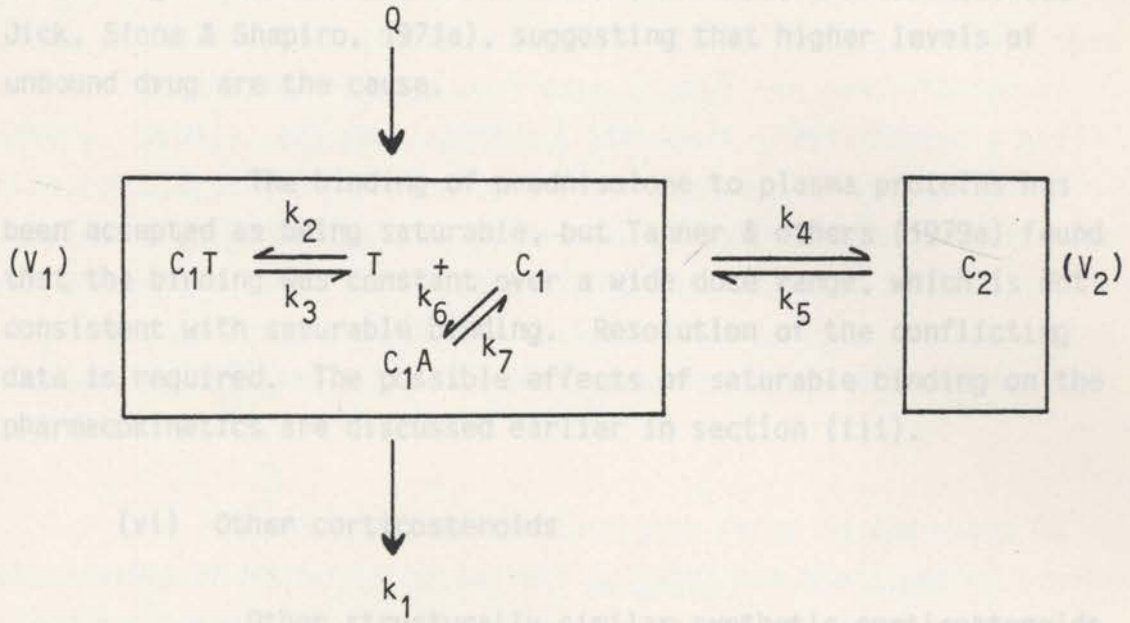


Figure 1.20. Two-compartment model proposed by Nugent, Warner, Estergreen & Eik-Nes (1965) for the distribution of hydrocortisone. Key; V_1 is the volume of the plasma (central) compartment, V_2 is the apparent size of the extra-plasma (peripheral) compartment, C_1 is the total hydrocortisone concentration in V_1, C_2 is the average total hydrocortisone concentration in V_2, T is the hydrocortisone binding capacity of CBG, A is the hydrocortisone binding capacity of albumin, Q is the rate of addition of hydrocortisone to the pool and k_1 to k_7 are first order rate constants.

to be higher. Powell & Axelsen (1972) confirmed that when serum albumin levels were low, a higher than normal proportion of the drug circulated as the free biologically active form. Clinical findings have revealed that patients with low serum protein levels have a higher incidence of side-effects from prednisolone (Lewis, Jick, Slone & Shapiro, 1971a), suggesting that higher levels of unbound drug are the cause.

The binding of prednisolone to plasma proteins has been accepted as being saturable, but Tanner & others (1979a) found that the binding was constant over a wide dose range, which is not consistent with saturable binding. Resolution of the conflicting data is required. The possible effects of saturable binding on the pharmacokinetics are discussed earlier in section (iii).

(vi) Other corticosteroids

Other structurally similar synthetic corticosteroids appear to exhibit kinetic profiles resembling that of prednisolone. For example, intravenous administration of ^{3}H-prednisone to beagle dogs produced plasma disappearance curves which could be described in terms of two exponentials (El Dareer & others, 1977). The apparent $t_{1/2}$ for the first phase of elimination was 15 minutes and that for the second phase was 82 minutes. Garg & others (1978) found that methylprednisolone administered orally to human subjects gave peak plasma concentrations at 0.5 to 2 hours with a half-life of 1.8 hours. Betamethasone gives a peak plasma concentration at 2 hours with a plasma half-life of 180 to 220 minutes (Miyachi & others, 1979). In patients with liver disease, the plasma clearance of betamethasone was delayed. A two-compartment model has also been fitted to the kinetic data for dexamethasone following intravenous administration (Brooks, Werk, Ackerman, Sullivan & Thrasher, 1972).

1.3.3 Bioavailability of prednisolone and prednisone dosage forms

(i) Prednisolone

Little has been reported concerning the bioavailability of prednisolone (Thiessen, 1976). It has been calculated that the bioavailability from an oral dose is 98.5 per cent (Tanner & others, 1979a). Isbister, Speros & Steinbeck (1969) tested a prednisolone preparation which was considered to be therapeutically inactive. No significant difference in plasma steroid concentrations was found in a comparison with a clinically effective tablet, although a non-specific assay was used. This is the only case where the bioavailability of clinically ineffective prednisolone tablets has been reported.

Marked differences have been found in the rates of dissolution of prednisolone tablets *in vitro* but there are no significant differences in plasma levels, peak times or areas under the curves of the tablets (Sullivan & others, 1974; Tembo, Hallmark, Sakmar, Bachmann, Weidler & Wagner, 1977b). Tembo & others (1977b) concluded that plots of the average plasma concentrations of the seven prednisolone tablets they tested were superimposable and that the tablets were bioequivalent. It appears that the absorption of prednisolone tablets is not controlled by the rate of dissolution or that the dissolution test is inappropriate.

In 1960, Wagner, Carpenter & Collins made a quantitative study of oral dosage forms of prednisolone in man and the dog. In both man and the dog, they found that administration of a conventional tablet gave peak plasma concentrations after one hour or less. Following dosage with a sustained-release granule formulation, the peak concentrations increased more slowly to a lower level but were approximately constant between 2 and 5 hours. The compressed tablet released the drug *in vitro* at a faster rate than did the sustained-release granules. A problem with this study of Wagner & others (1960) was the assay method, which measured total 17-hydroxycorticosteroids. The data obtained from humans contained

values that were lower than the apparent concentrations before dosage with prednisolone.

D'Arcy & others (1971) reported that the rate of dissolution in vitro of three sustained-release prednisolone formulations correlated with the area under the plasma level-time curve. English & others (1975) compared a sustained-release tablet with a conventional tablet. The plasma concentrations of prednisolone increased more slowly after the sustained-released form and maintained plasma levels longer though somewhat lower than with the conventional tablet. The in vitro dissolution rates of the two tablets were consistent with the time courses of plasma concentrations. These workers also observed that the plasma hydrocortisone level 24 hours after administration of sustained-release prednisolone was normal but in contrast it was reduced in subjects who received the conventional tablet. Whereas dissolution tests on conventional tablets do not correlate, those on sustained-release tablets correlate because the rate of release is carefully designed.

The bioavailability of prednisolone from enteric-coated tablets has been investigated in human subjects by a number of workers. Enteric-coated tablets have been found to be equivalent in therapeutic effect to plain tablets (West, 1959a,b). Studies in volunteers (Leclercq & Copinschi, 1974; Morrison & others, 1977; Wilson & others, 1977) have shown that enteric coating increases the lag time before prednisolone appears in the blood by about 1.5 to 2 hours but does not alter the total absorption compared with normal compressed tablets. The presence of food in the stomach at the time of administration does not affect the absorption of enteric-coated prednisolone tablets (Lee, Taylor, Walker & James, 1979a). A study in renal transplant patients (Hulme & others, 1975) showed delayed absorption and lower peak plasma concentrations after dosage with the enteric-coated tablets than with conventional tablets. However plasma sampling was not carried out for a sufficient length of time in this study for relative bioavailabilities to be assessed. Erratic plasma concentrations of prednisolone have been observed in other renal transplant patients who have received enteric-coated tablets

(Henderson & others, 1979; Fernando & Moorhead, 1979). A case of malabsorption of enteric-coated tablets in an asthmatic patient who absorbed conventional tablets normally has been noted (Mant, 1979). However, normal absorption for the enteric-coated tablet was shown in a volunteer.

The effect of the dosage form on bioavailability characteristics has been studied in the male beagle dog (Tse & Welling , 1977). A solution of a water-soluble prednisolone ester, a slurry and tablets were investigated. The slurry gave the best absorption with a significantly larger area under the plasma concentration-time curve than after dosage with the tablets or the solution of the prednisolone ester. Reduced absorption from the tablets, compared to the slurry, was probably due to tablet disintegration and dissolution characteristics. The reduced absorption from the solution of the prednisolone ester was suggested to be due to poor membrane permeability of the ionised drug. It is interesting to note that the conclusion regarding disintegration and dissolution of prednisolone tablets contrasts with that of Tembo & others (1977b) discussed above.

Concomitant administration of antacids has no effect on the absorption of prednisolone (Lee, Taylor, Walker & James, 1979b) and simultaneous administration of cholestyramine also has no influence on prednisolone bioavailability (Audetat & others, 1976, 1977).

(ii) Prednisone

As with prednisolone, it should be noted that no clinically ineffective tablet of prednisone has been shown to have low plasma levels when compared with a clinically effective one. Differences have only been found in <u>in vitro</u> dissolution tests (Campagna, Cureton, Mirigian & Nelson, 1963; Levy, Hall & Nelson, 1964). DiSanto & DeSante (1975) found a prednisone tablet that did not meet dissolution rate specifications of the U.S.P. was bioequivalent to a tablet with almost a 25-fold faster rate. Sullivan,

Sakmar, Albert, Blair & Wagner (1975) tested a clinically ineffective prednisone tablet *in vivo* along with two clinically effective products. Two of the preparations did not pass the U.S.P. dissolution test, only 12.3 and 11.4 per cent respectively being released in twenty minutes. However as estimated by the area under the plasma concentration curves, the amounts absorbed *in vivo* for all three preparations were not significantly different. The times of peak plasma concentrations were different and significant differences occurred only in the initial absorptive phase. Sullivan & others (1975) concluded that dissolution rate correlated with rate of absorption but examination of the plasma level-time curves shows that the 11.4 per cent release preparation achieved higher plasma levels more rapidly than the 12.3 per cent release preparation, which gave peak plasma levels almost identical to those of the preparation that passed the dissolution test. Analysis of the data from a further study of eight different commercial prednisone tablets in man (Sullivan & others, 1976a) showed significant differences in the rate of appearance of prednisolone in plasma, but not in the amount converted to prednisolone. Only two of the tablets passed the U.S.P. dissolution test. The slowest-dissolving tablet, with an average of 4.8 per cent dissolved in twenty minutes, gave similar areas under the plasma level-time curves (for both prednisone and prednisolone) to those of the tablet with complete dissolution in twenty minutes. However the times to reach the peak plasma levels were significantly different. The slowly dissolving tablet took 155 minutes to reach a peak prednisolone level whereas the other tablet needed only 75 minutes to reach the peak level. The *in vitro* dissolution rates of the prednisone tablets correlated with the rate of appearance of prednisolone but not with the amount of prednisolone which reaches the circulation (Sullivan, Sakmar & Wagner, 1976b). Also, it has been noted that the plasma prednisolone levels achieved in healthy volunteers following administration of prednisone are lower and more variable than after prednisolone itself (Tse & Welling, 1979; Davis & others, 1978). It is interesting to note that El Dareer & others (1977) claimed that oral doses of prednisone given to dogs and monkeys appear to be absorbed erratically. However the data were not presented.

Only one study has been reported concerning the concurrent administration of other drugs with prednisone. Tanner, Caffin, Halliday & Powell (1979b) found that antacids had no effect on the serum prednisolone concentrations attained.

(iii) Conclusion

The reliance on dissolution tests to predict therapeutic failures for either prednisone or prednisolone does not appear convincing. Whether the correlation of prednisone dissolution with the rate of appearance of prednisolone (Sullivan & others, 1976b) relates to therapeutic efficacy will need further study. Therapeutic failures have had extremely slow dissolution rates. However, slow dissolution rates do not necessarily indicate therapeutic ineffectiveness. Clearly, further study is required in order to develop satisfactory dissolution tests for tablets of prednisone and prednisolone.

Reports on the effect of food on the absorption of prednisone are conflicting while there has been only one study on the influence of food on the absorption of prednisolone. Food has been found not to affect the rate of absorption of slowly or rapidly dissolving prednisone tablets (Tembo, Sakmar, Hallmark, Weidler & Wagner, 1976) but a liquid meal delayed the peak plasma concentration by approximately 2 hours (Uribe, Schalm, Summerskill & Go, 1976). Clearly, further work is required on the effect of food on the absorption of both prednisone and prednisolone.

1.3.4 Absorption of corticosteroids

Administration of hydrocortisone to humans using intestinal tubes has shown that once in solution its absorption was directly proportional to concentration, suggesting that absorption occurred by simple diffusion (Schedl, 1965). About 50 per cent of hydrocortisone was absorbed from solution in 10 minutes in the small intestine.

Using anaesthetised rats with the intestine perfused at a flow rate of 1.7ml/min, Schedl & Clifton (1961) found that the concentration of prednisolone in the outflowing perfusion solution was 16.6 per cent lower than in the inflowing solution. Similar values were found for prednisone (15.5 per cent) and hydrocortisone (21 per cent); however, the loss of progesterone from the perfusion solution was almost complete (93.9 per cent). The absorption was independent of concentration. Hayton & Levy (1972a) have studied prednisolone absorption by measuring its disappearance from the lumen of rats using the technique of Doluisio & others (1969). In 60 minutes, 40 per cent of the prednisolone had disappeared, and in the presence of N,N-di-n-propylpropionamide, approximately 70 per cent had disappeared. N,N-di-n-propylpropionamide forms a complex with prednisolone in organic solvents and may also form a complex in the lipoid environment of membranes and thereby alter the rate of absorption. Absorption of prednisolone was found not to be a simple exponential process and this was attributed to accumulation of drug in the intestinal wall. However, the absorption of prednisolone followed monoexponential kinetics after 60 minutes. In a similar study, Hayton (1975) showed that the absorption of prednisolone was not significantly affected by ethanol and n-butanol but was increased by n-hexanol. The mechanism of the interaction could not be established, but the effect was concentration dependent and rapidly reversible.

1.3.5 Biliary excretion and entero-hepatic recycling of corticosteroids

Recent reviews (Smith, 1974; Klaassen, 1975; Plaa, 1975; Levine, 1978; Rollins & Klaassen, 1979) have dealt extensively with the biliary excretion and entero-hepatic circulation of steroids, as well as drugs and other foreign compounds. It is intended here to elaborate only on certain aspects pertinent to corticosteroids. In man and animals, a variety of steroids are known to be excreted in the bile, among them both natural and synthetic oestrogens, testosterone metabolites, corticosteroids and cardiac glycosides. However, the relative extent of biliary excretion varies quite markedly in different species for the same compound and for different compounds in the same species.

Among the factors which determine biliary excretion, one of the most important appears to be molecular weight. A specific molecular weight is required for a molecule to be excreted into bile. In the rat and the dog the threshold value is approximately 325 ± 50 (Smith, 1974; Hirom, Millburn & Smith, 1976). The threshold values in the guinea-pig and rabbit are 400 ± 50 and 475 ± 50, respectively (Hirom, Millburn, Smith & Williams, 1972), while in man the value is 500 to 600 (Levine, 1978). Compounds with molecular weights less than the threshold are excreted principally in urine (Levine, 1978). Both urinary and biliary excretion may take place with compounds whose molecular weights lie in the range from the threshold to 100 to 150 daltons greater, and above this range excretion is predominantly via the bile. Compounds excreted in the bile are frequently anions at physiological pH (Smith, 1974). If a compound does not already possess an ionisable group, it may be added through formation of conjugates such as glucuronides or sulphates. In addition conjugation increases the molecular weight of a compound, glucuronidation increasing the molecular weight by 176. It is the size of the resultant metabolite not the original molecule that must exceed the threshold. Glucuronides are quantitatively the most important conjugates involved in biliary excretion. Earlier studies have indicated that separate pathways exist for the biliary excretion of organic anions and unchanged polar molecules such as cardiac glycosides and neutral steroids. However the different pathways may not be as distinct as considered previously (Levine, 1978).

Compounds that are eliminated in the bile are delivered to the small intestine where they may be modified by the action of intestinal bacteria. Glucuronide and sulphate conjugates for example are known to be hydrolysed releasing the original compound which may then be re-absorbed and re-enter the portal blood from where it again will be subject to biliary excretion (Williams, 1972; Scheline, 1973). This process is known as entero-hepatic circulation or recycling. Entero-hepatic circulation is responsible for conservation of substances within the body such as bile salts (Smith, 1971).and results in the prolongation of action of many drugs. Of the substances that are eliminated in the bile, some may not be re-

absorbed but excreted through the faeces. Faecal excretion of a compound and its metabolites frequently tends to be higher in the rat and the dog, which are classed as good biliary excretors (Smith, 1971). Entero-hepatic circulation has been demonstrated for oestrogenic and progestational steroids, testosterone metabolites and cardiac glycosides. Bile salts are the classical example of entero-hepatic circulation. In man it has been estimated that two circulations of the bile acid pool are completed with each meal (Borgstrom, Dahlqvist, Lundh & Sjovall, 1957). The release of bile into the duodenum is intermittent in man, the dog and other animals (although not the rat) due to the presence of the gallbladder for storage. As a result, the entero-hepatic circulation of a foreign compound will be observed as a series of discrete recycling peaks. For example, after oral administration of proscillaridin A to man, a peak plasma level occurred at 20 to 45 minutes followed by a rapid decline and then a second peak after 5 to 12 hours (Belz, Stauch & Rudofsky, 1974).

Proof of an entero-hepatic circulation can be obtained by interruption of the cycle. Thus, if the gut microflora is eliminated, compounds which are excreted as glucuronide conjugates are not re-absorbed since deconjugation is prevented. In the rat, mestranol and 17β-oestradiol are rapidly excreted in the bile as glucuronide conjugates. However pretreatment with neomycin caused a 50 per cent reduction in the entero-hepatic circulation of the steroids (Brewster, Jones & Symons, 1977). A similar result was noted for ethinyloestradiol and norethisterone using a bile donor-recipient linked preparation (Back & co-workers, 1978). More than 92 per cent of the androgens excreted in the faeces of the male beagle dog were unconjugated (Martin, Bhargava & Adlercreutz, 1977). After the oral administration of ampicillin, the total faecal excretion of androgen increased. This increase was due to increased conjugates of androgens as a result of the decreased ability of the gut microflora to deconjugate the glucuronides excreted in bile.

An alternative interruption of the entero-hepatic cycle can be achieved by collecting the bile via T-tube drainage or a biliary fistula. In normal patients, plasma radioactivity after

administration of ^{3}H-spironolactone was found to be prolonged for 4 to 5 days before an exponential decline with a half-life of 1.8 days was observed. In patients with complete drainage of bile following surgery, no prolongation of plasma radioactivity was noted (Abshagen, Grodzicki, Hirschberger & Rennekamp, 1977). The half-life of methylproscillaridin, which is 40 to 50 hours in normal patients, was decreased to 17 to 30 hours in patients with biliary fistulas (Staud, Rietbrock & Fassbender, 1975).

Information about the biliary excretion and entero-hepatic circulation of corticosteroids is scarce (see Table 1.3). Specific reference to prednisolone shows that the biliary excretion in man is small and there is no evidence of significant entero-hepatic cycling. Biliary excretion of prednisolone has not been studied in the dog. However, the urinary recovery following intravenous injection of both hydrocortisone and prednisolone is low (Silber & Morgan, 1956), suggesting that biliary excretion of hydrocortisone and prednisolone in the dog is extensive and possibly constitutes the major excretory pathway. Consistent with this hypothesis, the faecal (and therefore biliary) excretion of methylprednisolone exceeds urinary excretion in the dog (Buhler & others, 1965).

It should be noted that formation of prednisolone glucuronide increases the molecular weight to 536.5 and adds the required anionic group. However the extent of biliary excretion of prednisolone in man would be difficult to predict, since the molecular weight of the glucuronide is within the threshold range (500 to 600). Experimentally, only minimal excretion has been found (Table 1.3). Since the threshold value in the dog is approximately 350, appreciable biliary excretion of prednisolone glucuronide as well as excretion of some unchanged prednisolone would be expected to occur. The rat, which has a similar molecular weight threshold to the dog, is a good biliary excretor of corticosteroids (see Table 1.3) and hydrocortisone undergoes an entero-hepatic circulation in the rat (Hyde & Williams, 1957; Taylor, 1971).

CHAPTER TWO

EXPERIMENTAL METHODS

2.1 Acute (in situ) Experiments

2.1.1 Animals

Mongrel dogs with weights ranging from 9 to 30 kg were used. They were starved for 24 hours prior to experimentation but allowed water ad libitum.

2.1.2 Catheters

Polyethylene catheters (Dural Plastics) were used for the catheterisation of the femoral artery and the femoral vein. Dimensions were: 90 cm x 1.40 mm i.d., 1.90 mm o.d. Silicone rubber ("Silastic") was used for portal vein and biliary catheters with dimensions of 90 cm x 1.02 mm i.d., 2.16 mm o.d.

2.1.3 Surgical instruments and equipment

See Appendix A1.

2.1.4 Anaesthetic

The dogs were anaesthetised with intravenous pentobarbitone sodium ("Nembutal", Abbott Laboratories), 30 mg/kg.

2.1.5 Surgical procedures

(i) Portal vein sampling

Catheters were inserted into a femoral artery and femoral vein so that the catheter tips resided in the aorta and inferior vena cava respectively. Arterial blood pressure was monitored with a Statham P23 pressure transducer connected to the heparin-

ised saline-filled femoral artery catheter. Lead II ECG was measured with a Grass Model 7P4 EKG Tachograph. Both were recorded on a Grass Model 7 Polygraph. Endotracheal intubation was routinely carried out so that positive pressure respiration could be initiated quickly. The animal was maintained at 38^{o} by heating the operating table.

A midline abdominal incision was made, the spleen located, a branch of the splenic vein selected and a catheter inserted and guided until its tip lay in the portal vein at the entrance to the liver (page 122). The portal vein catheter was exteriorised through the abdominal incision which was then clamped. The doses of prednisolone were administered as a saline solution either by intravenous or intraportal bolus, or by injection into the stomach or duodenum. Blood samples were removed via the catheters as required.

(ii) Stomach ligation

The midline incision was extended so that the pylorus could be reached and ligated. Ligation at the cardia was not carried out. The dose of ^{3}H-prednisolone was injected into the stomach as a saline solution and the contents were injected and withdrawn into the syringe several times to ensure thorough mixing with the stomach contents. The puncture made by the needle was clamped with a haemostat to prevent leakage of the stomach contents. The midline incision was then closed by clamping. Blood samples were withdrawn via the portal vein catheter and the plasma assayed for ^{3}H levels (page 134).

(iii) Portal vein blood flow measurements

An upper midline abdominal incision extending to the xiphoid was made. The incision was retracted laterally and the intestines and stomach reflected and held using saline-soaked gauze so that the portal vein was exposed. The portal vein was cleared by blunt dissection of fatty tissue, lymph nodes and ducts for a distance of 1 cm between the branching at the porta hepatis and the junction between the portal vein and the gastroduodenal vein. The section between the junctions with the gastroduodenal and gastrosplenic veins and a section of the gastroduodenal vein were also cleared. A cannulating electromagnetic

flowmeter probe with an attachment for blood sampling (Figure 2.1) was used. The larger of the two branching arms was inserted into the portal vein between the gastroduodenal and gastrosplenic veins and the smaller inserted into the gastroduodenal vein (Figure 2.2).

Once the blood flow was re-established, 5,000 units of heparin was injected intravenously. It was necessary to keep the time from ligation of the portal vein to restoration of portal flow through the flowmeter below 90 seconds to avoid massive splanchnic oedema and death within 10 minutes. The intestines and other abdominal structures were replaced in their normal positions and the incision closed with clamps. The animal was maintained at 38^{o}. A period of 15 to 30 minutes was allowed for recovery and stabilisation of blood flow. Blood loss occurring during insertion of the flow probe was replaced by an intravenous infusion of saline.

Mean blood flow was measured using an EMI Type 28 Model SFMB-1 electromagnetic flowmeter and recorded on a Grass Model 7 Polygraph. Blood pressure and Lead II ECG were also recorded. For zero flow readings, the incision was reopened briefly and the cannula clamped downstream from the probe with a haemostat for a few seconds.

(iv) Biliary excretion

A midline incision to the xiphoid was made and retracted laterally. The gallbladder and bile duct were located and the intestines retracted with saline-moist gauze. The common bile duct was isolated by blunt dissection and then ligated proximal to the duodenum. An incision was made transversely in the bile duct distal to the duodenum and a "Silastic" catheter was inserted a few centimetres towards the liver and secured. Once bile flow commenced, the gallbladder was emptied by gentle expression and the incision was closed by clamping. ^{3}H-Prednisolone was administered in a saline solution via the femoral vein catheter. Bile flow rates up to 17 ml/hour were observed and accumulated bile was collected at 0.5, 1, 2, 3 and 4 hours. At the end of each experiment, the gallbladder was checked for any accumulated bile; none was recovered. Aliquots of bile samples were analysed

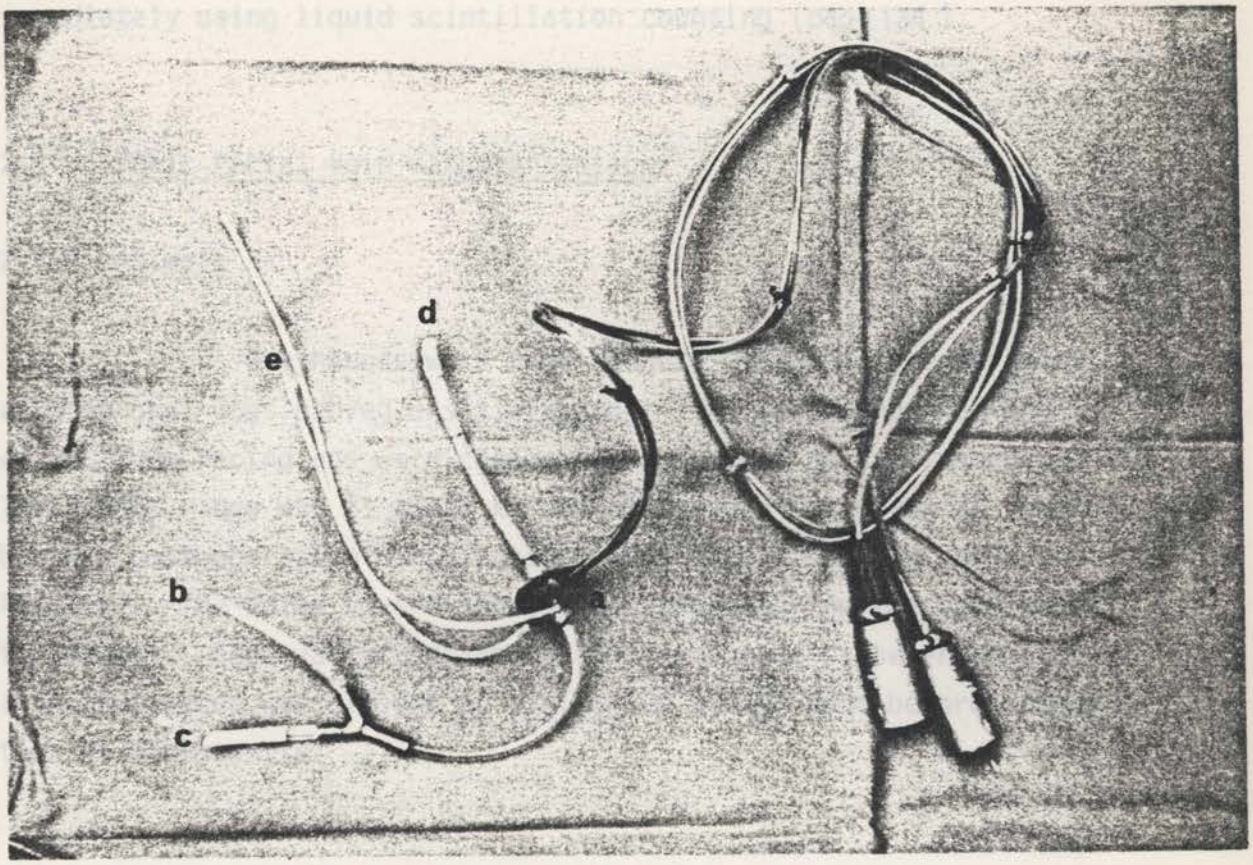


Figure 2.1. Cannulating electromagnetic flowmeter probe. Key: a, probe; b, cannula for insertion into gastroduodenal vein; c, cannula for portal vein upstream; d, cannula for portal vein downstream; and e, catheters for sampling the portal blood. Made by J.A. Angus, Department of Pharmacology, University of Sydney.

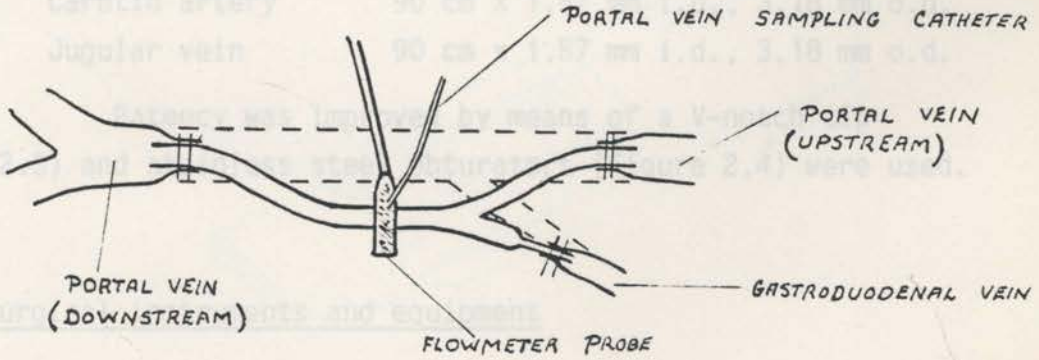


Figure 2.2. Diagram showing the arrangement of the flowmeter probe in place for portal vein measurements. Total blood flow from the portal and gastroduodenal veins is diverted through the probe.

immediately using liquid scintillation counting (page 134).

2.2 Chronic Portal Vein Catheterisation

2.2.1 Animals

Greyhounds were used because exploratory experiments with mongrel dogs proved unsatisfactory. The greyhounds had been culled from racing and were consequently in good physical condition. The weight range was 21 to 27 kg and preference was given to females because post-operative complications were less likely to arise (see Results). The animals were thoroughly washed during the week prior to surgery. Food was withdrawn 24 hours prior to catheter implantation but water was allowed ad libitum up to one hour before induction of anaesthesia.

2.2.2 Catheters

Silicone rubber catheters ("Silastic", Dow Corning International Ltd.) were used since they provided adequate elasticity and could be tightly secured. The dimensions of the catheters were:

Portal vein	120 cm x 1.02 mm i.d., 2.16 mm o.d.
Carotid artery	90 cm x 1.57 mm i.d., 3.18 mm o.d.
Jugular vein	90 cm x 1.57 mm i.d., 3.18 mm o.d.

Patency was improved by means of a V-notch tip (Figure 2.3) and stainless steel obturators (Figure 2.4) were used.

2.2.3 Surgical instruments and equipment

See Figure 2.5. Details of equipment are given in Appendix A1.

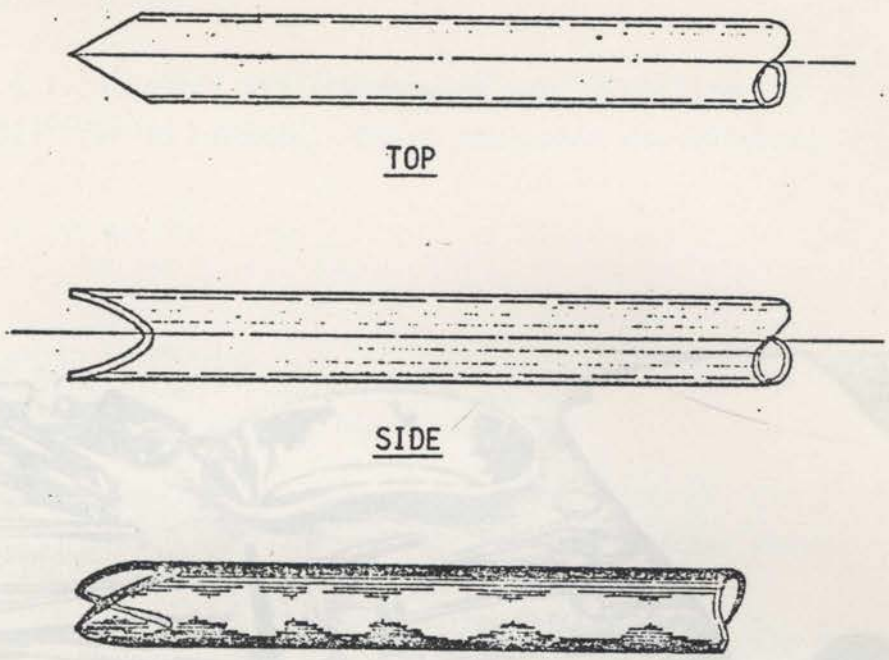


Figure 2.3. V-notch tip used on catheters inserted into dogs for chronic experiments.

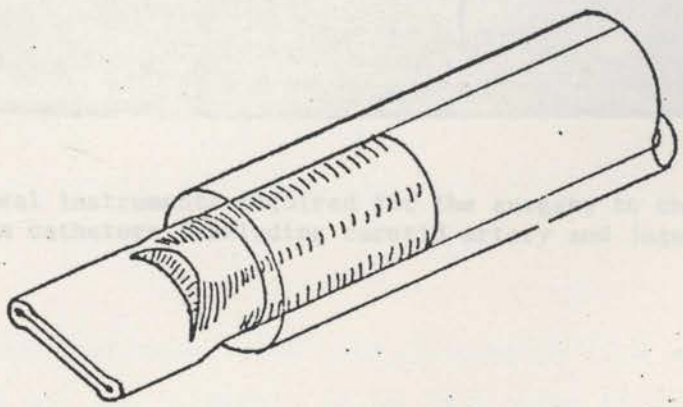


Figure 2.4. Sketch of an obturator sealing the exterior end of a catheter (14-gauge for carotid artery and jugular vein catheters, 17-gauge for portal vein catheter).

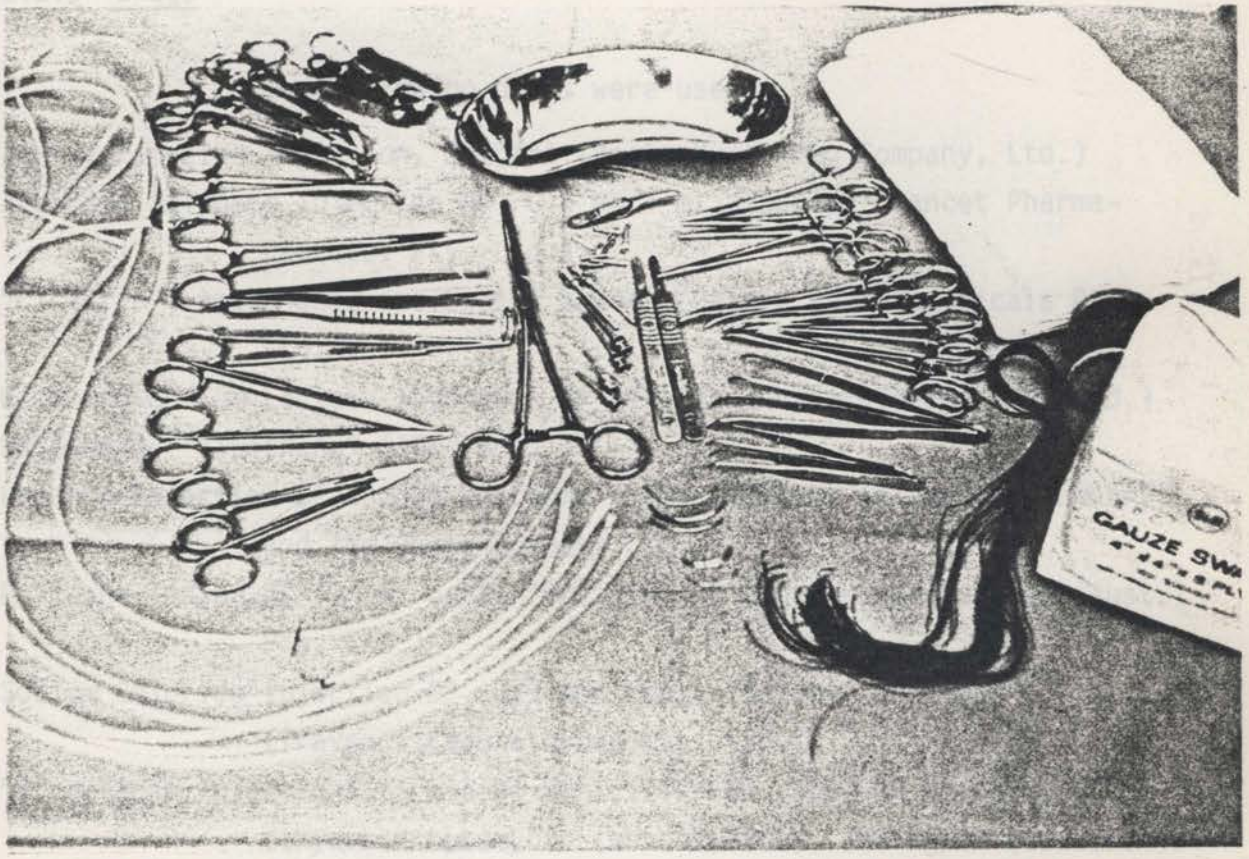


Figure 2.5. Surgical instruments required for the surgery to chronically implant portal vein catheters (including carotid artery and jugular vein catheters).

2.2.4 Sterilisation procedure

All catheters and instruments were sterilised by autoclaving at 121° for 30 minutes. Other equipment was obtained pre-sterilised.

2.2.5 Drugs

The following drugs were used:

Acetylpromazine Injection, 2 mg/ml (Boots Pure Drug Company, Ltd.)

Atropine Sulphate Injection BP, 0.4 mg/1 ml ampoules (Lancet Pharmaceutical Pty. Ltd.)

"Cicatrin", amino acid antibiotic powder (Calmic Pharmaceuticals Pty. Ltd.)

Compound Sodium Lactate Injection BP (Travenol Laboratories Pty. Ltd.)

Halothane, "Fluothane" (ICI Ltd.)

Heparin Injection BP, 5,000 units/ml - 5ml ampoules (Allen and Hanbury's and The Boots Company Ltd.)

Povidone-iodine NF 10 per cent, "Povidine K" (Knoll Laboratories (Aust.) Pty. Ltd.)

Sodium Chloride Injection BP, 0.9 per cent, "Saline, Solu-pac" (Travenol Laboratories Pty. Ltd.)

"Streptopen Injection", procaine penicillin BP, 250,000 units/ml and dihydrostreptomycin (as sulphate), 250 mg/ml (Glaxovet).

2.2.6 Anaesthesia

The dogs were dosed with acetylpromazine (0.4 mg) and atropine sulphate (0.4 mg) approximately 10 to 15 minutes prior to induction of anaesthesia. The anaesthetic gas mixture for induction was 5 per cent halothane in nitrous oxide (3 litre/min) and oxygen (1 litre/min) reducing to 1.5 per cent halothane in nitrous oxide (1 litre/min) and oxygen (0.5 litre/min) for maintenance. A closed circuit rebreathing anaesthetic machine was used and after induction,

the trachea was intubated to facilitate administration of the anaesthetic.

When induction was complete, electrodes for recording heart rate and Lead II ECG were inserted and a continuous visual record was obtained during surgery on a slow scanning oscilloscope.

2.2.7 Surgical procedure

A direct approach to the carotid artery and jugular vein was used. Since the portal vein was relatively inaccessible, the catheter was inserted into a branch of the splenic vein and guided through into the portal vein. The actual length of catheter required was determined from measurements in greyhounds at autopsy and the distance from the hilus of the spleen to the branching of the portal vein at the entrance of the liver was found to be relatively uniform. Dogs weighing 18 to 25 kg had a spleen-portal vein distance of 14 to 15 cm and dogs 25 to 30 kg, 16 to 17 cm.

Strict asepsis of the operating area was maintained during the operation. The dog was draped, leaving one area about 3 cm wide by 15 cm long along the midline of the abdominal region and another about 3 cm by 5 cm on the neck above the right jugular vein exposed for incisions. The first part of the operative procedure was the placement of catheters into the carotid artery and the jugular vein. The jugular vein was exposed and the catheter was filled with heparinised saline (500 u/ml) and tied in position with silk ligatures (2/0). The catheter was then used to administer a slow intravenous infusion of Compound Sodium Lactate Injection BP at a rate of 100 ml/hour for the duration of the operation in order to replace fluid and electrolytes. The carotid artery catheter was then introduced, advanced some 30 to 40 cm and tied in place. The patency of the tube was tested and the ends of the back tie were secured around the tubing as an anchorage.

A midline incision about 13 cm long was made in the abdomen (Figure 2.6). On exposure of the peritoneal cavity, the falciform ligament was separated and tied if necessary. The spleen was located and, with very gentle handling, lifted out through the incision (Figure 2.7) and placed on large gauze swabs moistened with warm sterile saline (Figure 2.9). The spleen was reflected to the dog's left side to expose the splenic blood vessels and then completely covered in moist swabs. The general arrangement of blood vessels in the reflected spleen is shown in Figures 2.8 and 2.10. A small branch (Figure 2.10) was chosen and approximately 0.75 cm of the vessel was carefully cleared of adhering tissue and ligated proximal to the spleen with a silk tie. The catheter was then introduced and advanced so that its tip would lie in the splenic vein. At autopsy, the catheter tip was invariably found to be located at the junction of the left and right branches of the portal vein. The catheter was tested for patency (Figure 2.11) and secured in position. The spleen was carefully replaced in position and the location of the catheter tip checked by palpation (Figure 2.12). Replacement of the spleen caused the stretched vessels to retract, thus pushing the tip of the catheter into the portal vein. The catheter was again checked for patency and left filled with heparinised saline. A loop of catheter was left in the abdominal cavity to allow for movement of the animal. The falciform ligament with associated fatty tissue was ligated with absorbable sutures at the abdominal wall and excised. The abdominal wall incision was closed leaving the catheter in the centre of the suture line, after which it was passed subcutaneously and exteriorised just below the shoulders (Figures 2.13 to 2.15). The venous and arterial catheters were brought to the surface at the back of the neck (Figure 2.16).

All wounds and incisions were dusted with "Cicatrin" powder before suturing and 5 ml of "Streptopen" suspension was given by intramuscular injection. Combine dressing (20 cm wide) was wrapped tightly around the body of the dog covering the abdominal incision sites and was held firmly in place with an overlay of elastic adhesive bandage ("Elastoplast"). The neck was covered using a crepe bandage which also enclosed the catheters.

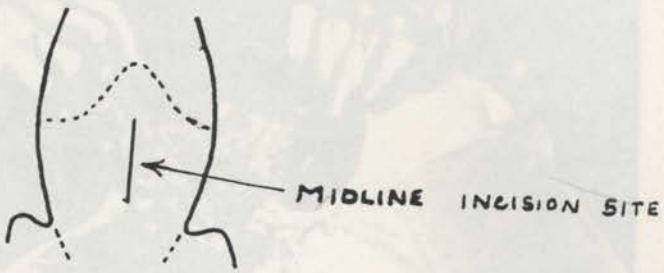


Figure 2.6. Position of the midline incision for portal vein catheter insertion.

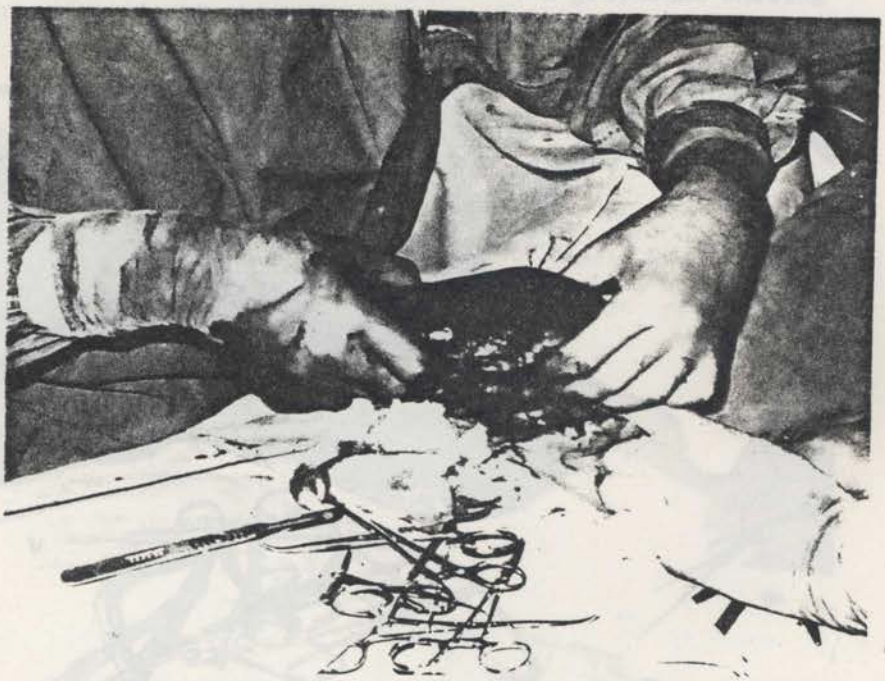


Figure 2.7. Careful moving of the spleen through the midline incision. The enlarged spleen resulting from general anaesthesia with halothane tears easily, necessitating very gentle handling.

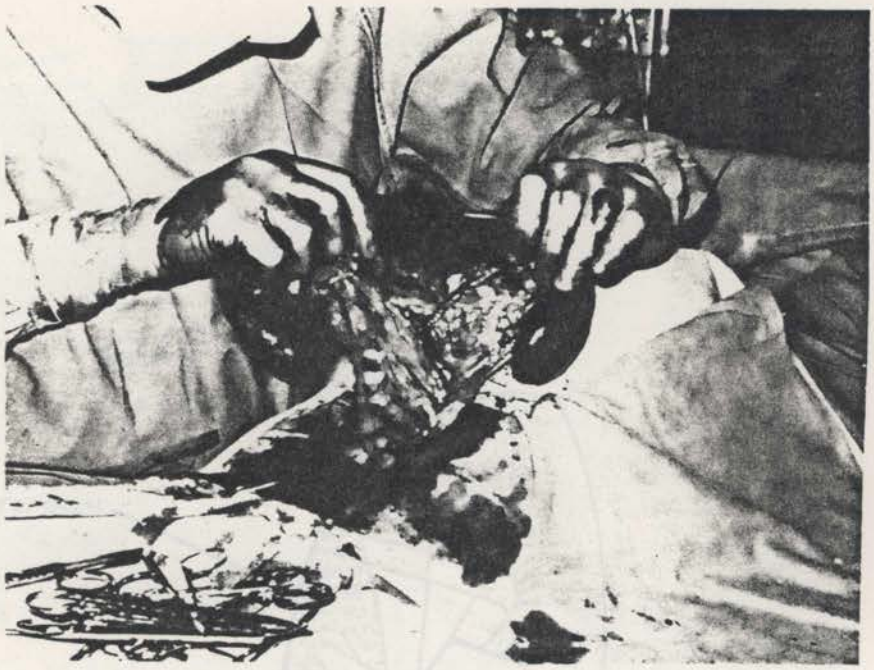


Figure 2.8. Exteriorised spleen of a greyhound showing readily visible blood vessels.

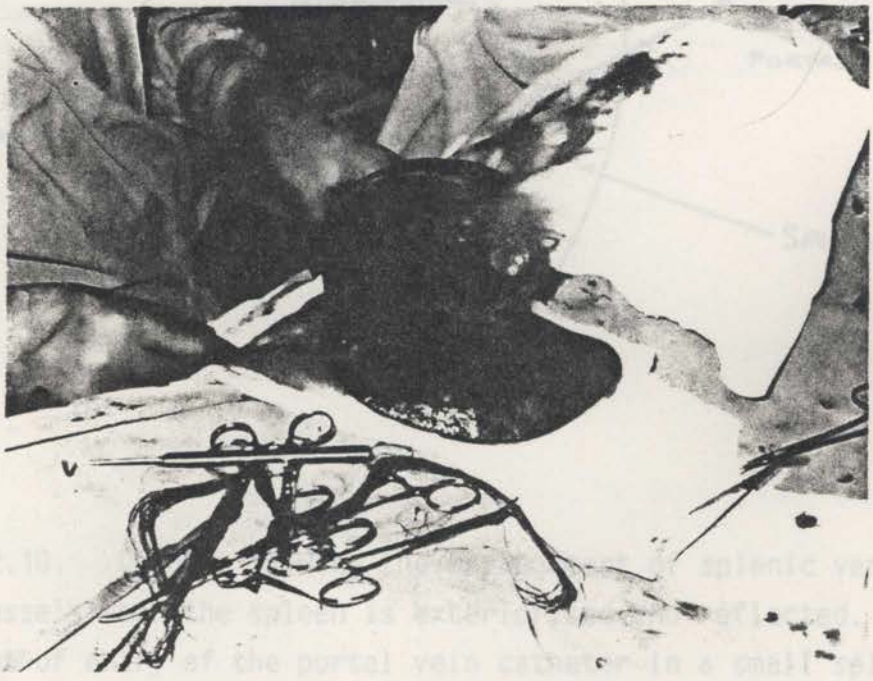


Figure 2.9. Spleen resting on a large saline-moist gauze pad, ready to be prepared for the introduction of the portal vein catheter.

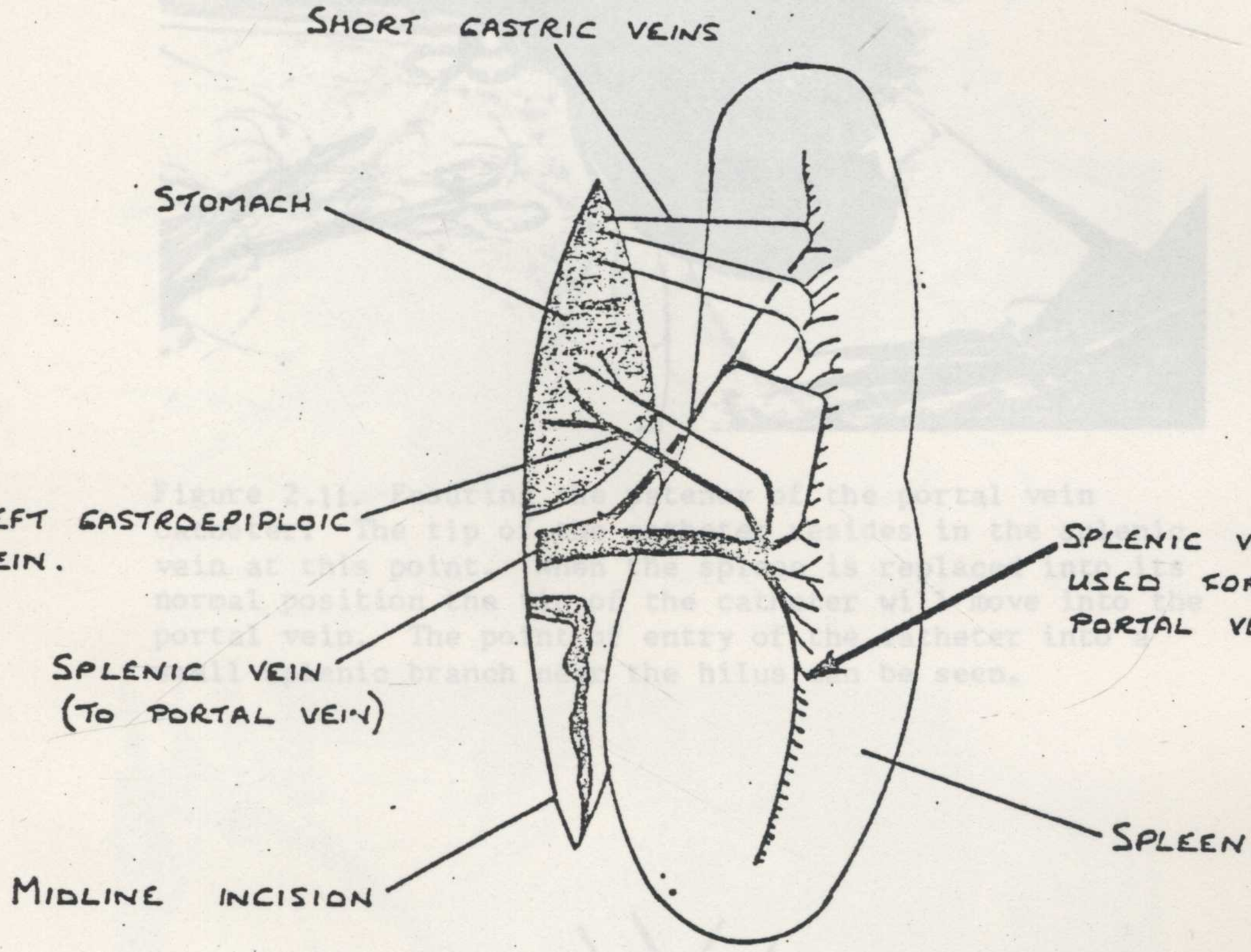


Figure 2.10. Diagram showing the arrangement of splenic venous blood vessels when the spleen is exteriorised and reflected. The point of entry of the portal vein catheter in a small splenic branch vein close to the hilus is indicated with an arrow.

Figure 2.11. Ensuring the patency of the portal vein catheter. The tip of the catheter resides in the splenic vein at this point. When the spleen is replaced into its normal position the tip of the catheter will move into the portal vein. The point of entry of the catheter into a small splenic branch near the hilus can be seen.

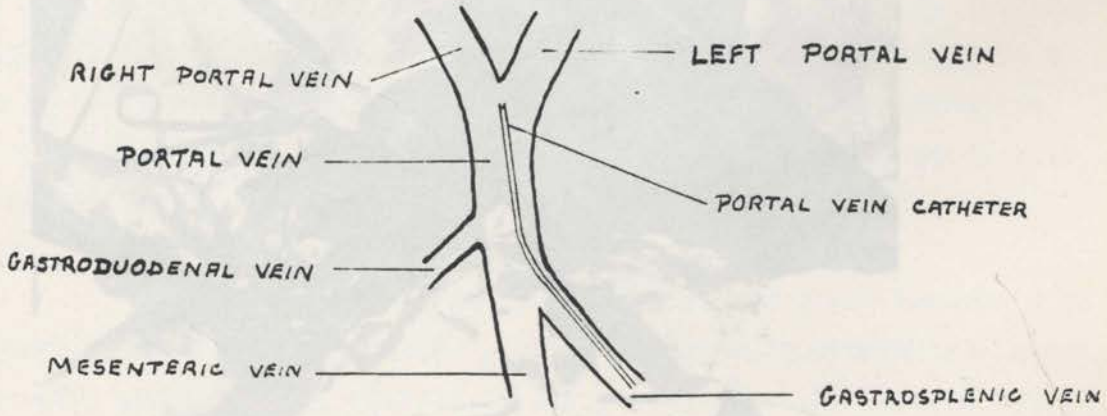


Figure 2.12. Diagram indicating the desired location of the tip of the portal vein catheter at the junction of the left and right portal branches.

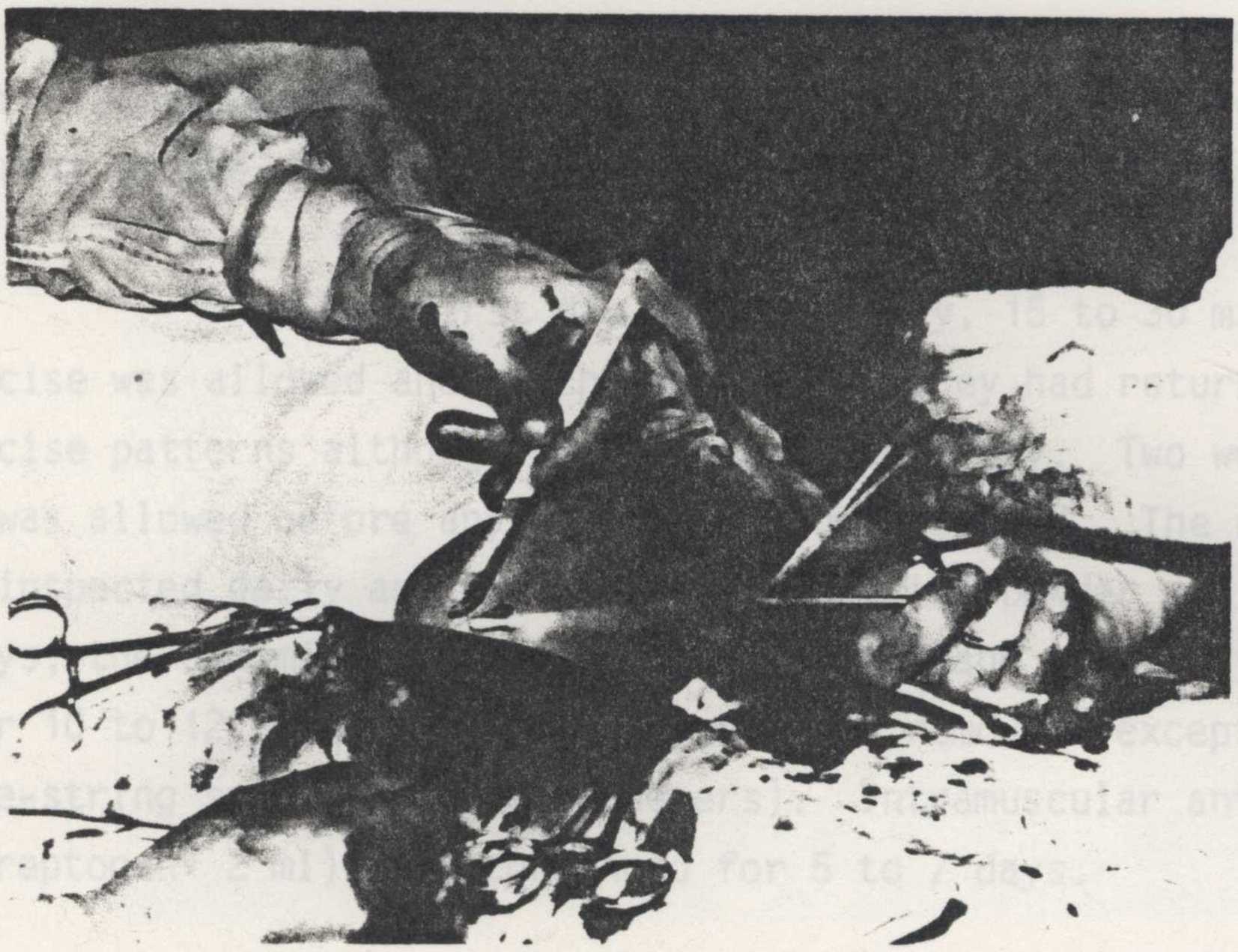


Figure 2.13. Preparation of the first section of the subcutaneous tunnel.

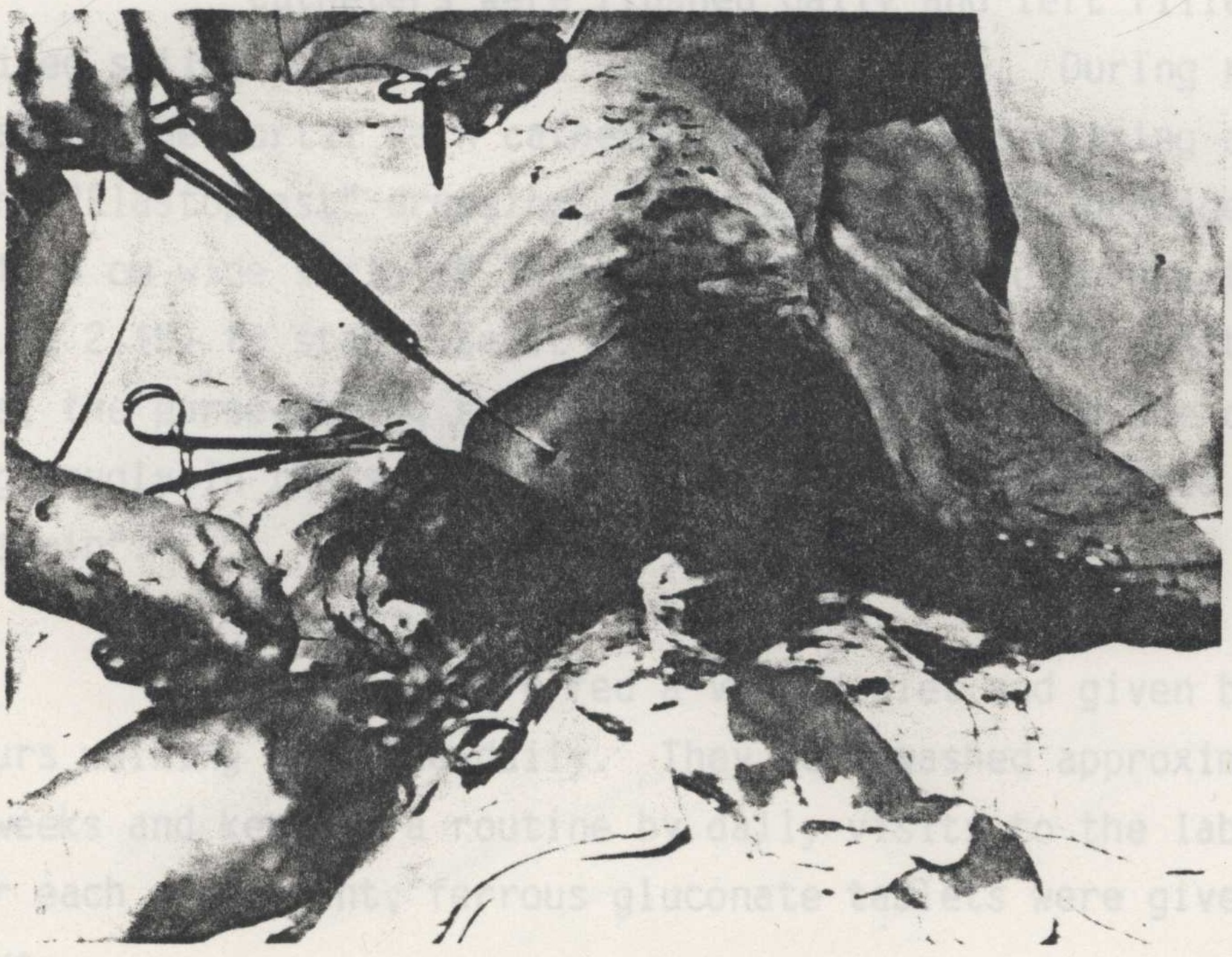


Figure 2.14 Catheter being drawn through the first section of the tunnel.

2.2.8 Post-operative recovery and maintenance of animals

(i) Post-operative recovery

The animals regained consciousness 30 to 60 minutes after discontinuation of anaesthetic and were observed frequently for the rest of the day and during the night after surgery.

On the first day after surgery, 15 to 30 minutes exercise was allowed and by the second day they had returned to usual exercise patterns although restrained in activity. Two weeks recovery was allowed before any drugs were administered. The neck incision was inspected daily and dusted with "Cicatrin" powder and, after 4 to 5 days, the abdominal incision was inspected and similarly treated. After 10 to 12 days, the skin sutures were removed (except for the purse-string sutures around catheters). Intramuscular antibiotics ("Streptopen" 2 ml) were continued for 5 to 7 days.

(ii) Maintenance of animals

Catheters were flushed daily and left filled with heparinised saline solution (500 units heparin/ml). During recovery, access to the portal vein catheter was gained by raising a small area of the "Elastoplast" dressing. When the abdominal bandage was removed, a 2 to 3 cm wide strip of "Elastoplast" was taped across the catheter (Figure 2.15) to stabilise it when it was not in use. After 2 to 3 weeks, the purse-string sutures sloughed off leaving the catheters lying snugly in the scar tissue. These areas were dusted daily with "Cicatrin".

The dogs were fed a varied diet and given between 1 to 2 hours walking exercise daily. They were washed approximately every two weeks and kept in a routine by daily visits to the laboratory. After each experiment, ferrous gluconate tablets were given for 2 to 3 days.

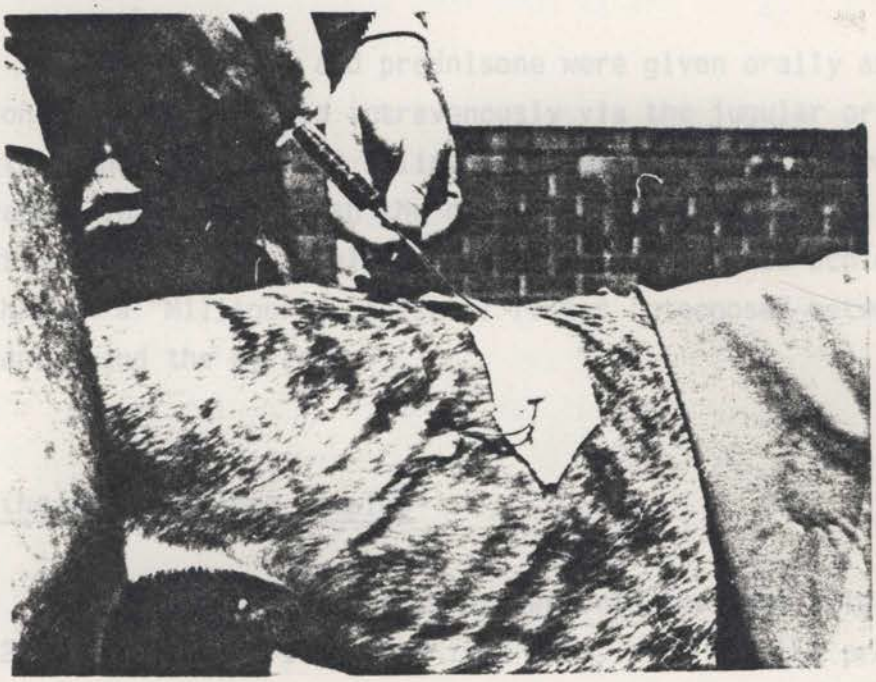


Figure 2.15. Blood sampling from the portal vein catheter (approx. four months after implantation).

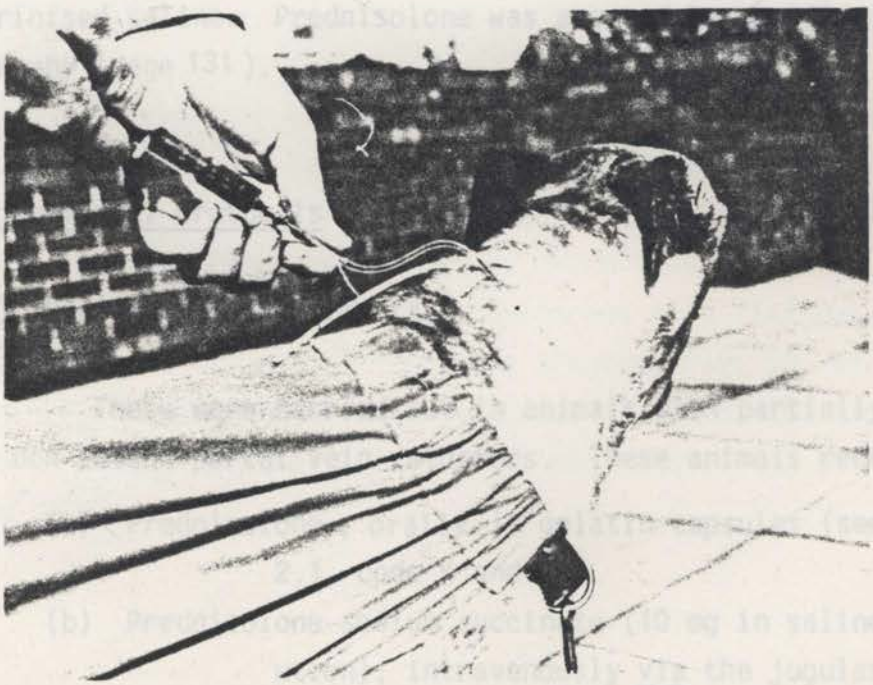


Figure 2.16. Blood sample being withdrawn from a jugular vein catheter, after 4 months implantation.

2.3 Chronic Experiments

2.3.1 Prednisolone administration

Prednisolone and prednisone were given orally and prednisolone was administered intravenously *via* the jugular or the portal vein catheters. Ten ml saline was given after each parenteral administration in order to wash the drug out of the catheter. Final sterilisation of parenteral solutions of prednisolone was achieved by passage through a "Millipore" bacterial filter interposed between the needle adaptor and the syringe.

2.3.2 Withdrawal of blood samples

Blood samples were withdrawn into syringes (Figures 2.15 and 2.16) and placed in heparinised tubes with aseptic precautions. When frequent sampling was required, the catheters were flushed with sterile saline in place of heparinised saline. At the end of each experimental session the catheters were left filled with heparinised saline. Prednisolone was assayed by gas-liquid chromatography (page 131).

2.3.3 Experimental protocols

(i) Preliminary experiments

These were carried out in animals with partially patent or non-patent portal vein catheters. These animals received:

(a) Prednisolone , orally in gelatin capsules (see Table 2.1, code K and N).

(b) Prednisolone sodium succinate (10 mg in saline solution), intravenously *via* the jugular vein catheter.

(c) Prednisolone hemisulphate sodium (10 mg in saline solution), intravenously *via* the jugular vein catheter.

(ii) Main study

Three female greyhounds were used weighing 23, 23 and 25 kg respectively, the catheters remaining patent for 2, 3 and 5.5 months. The procedure was as follows:

Food was withdrawn from 24 hours before dosing until completion of the blood sampling, but water was allowed ad libitum. After oral administration, simultaneous samples were taken from the portal vein and either the carotid artery or jugular vein catheters at 30, 45, 60, 90, 120 and 150 minutes for Dog I, and at 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 180 and 240 minutes for Dogs II and III. After intravenous doses (via the jugular vein catheter), samples were withdrawn at 4 (approximately), 10, 15, 20, 30, 40, 50, 60, 75, 90, 120, 150 and 180 minutes.

The dosage forms were randomly administered and after initial administration this pattern was repeated to create a cycle. When possible after three cycles, various other formulations were also administered. An interval of 4 to 5 days was allowed between doses. The dosage forms administered and the number of times each formulation was given are shown in Table 2.1.

Analysis of variance (ANOVA) with repeated measures and unequal cell sizes was carried out on the portal and arterial plasma concentrations, the P - A differences and the areas under the time courses of the plasma concentrations and differences of products A,B and C. This analysis was performed on a Tektronix Model 4051 using the analysis of variance programs Plot 50, volume 2 (Searle, 1971). Areas under the time courses of the plasma concentrations and differences from 0 to 4 hours were calculated using the trapezoidal rule.

TABLE 2.1. Dosage forms administered to chronic dogs.

Dosage form				Code	The number of times each formulation was given to the three dogs of the main study.		
					Dog I	Dog II	Dog III
5 mg PREDNISOLONE TABLETS	"DELTASOLONE"	Knoll Labs. Batch	13070L	A	5	3	2
" "	"ADNISOLONE"	Adam Drug Co.	297	B	3	3	2
" "	"DELTASOLONE"	Knoll Labs.	10075*	C	2	3	2
" "	"DELTA-CORTEF"	Upjohn	A5024	D	-	-	5
" PREDNISONE TABLETS	"DECORTISYL"	Roussel	069928Y1	E	-	-	3
" PREDNISOLONE CAPSULES	"DELTASOLONE CAPS"	Knoll Labs.	10092	F	-	1	3
" "	Extemporaneous ‡		03	G	-	3	2
" "	"		04†	H	-	-	2
" INTRAVENOUS BOLUS via JUGULAR VEIN CATHETER (0.9% NaCl solution, 15 ml volume)				I	-	3	3
50 mg PREDNISOLONE CAPSULES	Extemporaneous ‡		02	J	1	1	2
10 mg "	"		01	K	1	1	1
" INTRAVENOUS BOLUS via JUGULAR VEIN CATHETER				L	-	-	1
" INTRAPORTAL BOLUS via PORTAL VEIN CATHETER				M	-	1	-
2 x 10 mg PREDNISOLONE CAPSULES	Extemporaneous ‡		01	N	2	-	-

* via National Biological Standards Laboratory, Canberra. These had been claimed by a physician to have been a therapeutic failure.

† Contained ^{3}H-prednisolone, 2.065×10^{7} d/min.

‡ Hand filled into gelatin capsules after diluting to 200 mg with lactose.

2.4 Human Intubation Experiments

Five healthy male volunteers aged 20 to 22 years received solutions of prednisolone and prednisolone together with salicylic acid in a random order. Each solution was studied once in each subject on different days. The solutions contained 160 mEq/litre HCl, radioactive chromium chloride ($^{51}CrCl_3$, 25 μC/litre) as non-absorbable indicator and 10 mg prednisolone with or without salicylic acid (20 mM).

Subjects were fasted for 12 hours prior to the study. The subject was placed in the left lateral decubitus position and 200 ml of test solution was instilled into the stomach through a naso-gastric tube*. The gastric contents were mixed for 60 seconds by rapid withdrawal and reinjection with a syringe and a 30 ml control sample aspirated immediately. Fifteen minutes later, the rest of the gastric contents were aspirated as fully as possible and the residual volume was recovered by rinsing four times with 50 ml of HCl (160 mEq/litre). The four wash aspirates were then combined. This procedure was repeated 3 or 4 times with the same prednisolone solution. Samples from the first 15 minute period were discarded because of gastric mucosal adsorption of the indicator (Ivey & Schedl, 1970).

All samples were tested for bilirubin by "Ictotest" tablets and those with more than a trace of bilirubin were discarded. The volume secreted and the volume passing through the pylorus (volume emptied) were calculated from the concentration of ^{51}Cr (Hunt, 1951). Prednisolone in gastric samples was measured by gas chromatography (page 132). The fraction of the prednisolone dose absorbed from the stomach during each test period was calculated by the formula:

$$\text{Fraction of prednisolone absorbed} = \frac{\left| V_3C_3 + V_4C_4 + V_p \cdot \left(\frac{C_2 + C_3}{2} \right) + V_2C_2 - V_1C_1 \right|}{V_1C_1}$$

equation (2.1)
(Ivey & others, 1975)

* Intubation was carried out by Dr.K.J.Ivey, A.W.Morrow Department of Gastroenterology, Royal Prince Alfred Hospital, Sydney.

where: V_1 is the volume of the test solution (200 ml),
C_1 is the concentration of prednisolone in the test solution,
V_2 is the volume of the sample after initial mixing (30 ml),
C_2 is the concentration of prednisolone after initial mixing,
V_3 is the volume aspirated after 15 minutes,
C_3 is the concentration of prednisolone in the 15 minute aspirate,
V_4 is the combined wash volume,
C_4 is the concentration in the combined wash of prednisolone and
V_p is the volume lost through the pylorus per 15 minutes.

2.5 In vitro Dissolution

A dissolution apparatus conforming to U.S.P. XVIII (1970) was used. A"Thermomix"was used to maintain the bath temperature at 37 $\pm$ 0.1^{o} and it was possible to house 5 dissolution vessels of 1000 ml capacity. The cylindrical stainless steel dissolution baskets were rotated by a variable speed electric motor. Tablets or capsules of prednisolone and prednisone were placed in the baskets, immersed in 900 ml of dissolution medium (freshly distilled water at 37^{o}) in the vessels and the baskets rotated. The rotation of the baskets was normally 50 revs/min (Kendall, 1972), but comparative runs at a speed of 100 revs/min were also carried out. Five ml samples of dissolution fluid were taken for analysis at 1 minute, 5 minutes, then every 5 minutes from 10 to 30 minutes, and every 10 minutes until 70 minutes or until the concentration of drug in the fluid reached a constant value. After the measurement of absorbance at 246 nm (page 134), the samples were returned to their respective dissolution vessels to maintain the volume.

2.6 Analytical Methods

2.6.1 Gas-liquid chromatography

(i) Determination of prednisolone in plasma

Gas-liquid chromatography (glc) was carried out using a Hewlett-Packard Model 5711A Dual Column instrument fitted with flame ionisation detectors. Glass columns having an internal diameter of 3 mm and a length of 150 cm were used with the samples for analysis being injected directly onto the column. The columns were packed with 5 per cent OV-1 on Gas-Chrom Q (100-120 mesh). The OV-1 (5 per cent) was coated on Gas-Chrom Q by the filtration method of Horning, Vanden Heuvel & Creech (1963) and conditioned by heating at 280^{o} for 12 hours with nitrogen flowing through the column. For the analysis of blood samples, the carrier gas (nitrogen) flow rate was 60 ml/min at 414 kPa. The hydrogen gas flow rate to the detector was 60 ml/min at 212 kPa and the air flow, 240 ml/min at 166 kPa. The detector and injection port were maintained at 300^{o} and the column at 265^{o}. Attenuation settings of 2 x 1 to 8 x 10 were used.

A method based on that of Bacon & Kokenakes (1969) was used. 6α-Methylprednisolone was chosen as the internal standard. Under the above conditions, it has a retention time of 15.3 minutes compared to 13.1 minutes for prednisolone. Plots of peak height against amount injected were linear over the range from 0.01 to 100 μg for both prednisolone and methylprednisolone. Solutions containing equal amounts of the two steroids were found to have a constant peak height ratio of 1.02 $\pm$ 0.01.

Blood samples of approximately 2 ml were collected in heparinised tubes. They were centrifuged at 2500 revs/min for 10 minutes, about 1 ml of plasma withdrawn and maintained at -20^{o} until required for analysis. One ml of plasma was diluted to 4 ml with distilled water and the internal standard (6α-methylprednisolone) solution added. The mixture was extracted 3 times with 8 ml of chloroform: tetrahydrofuran (3:1) and the combined extracts were evaporated to

dryness using a rotary evaporator. The residue was redissolved in chloroform and then concentrated to a volume of approximately 20 to 30 µl. One to 5 µl aliquots were then injected into the chromatograph. The injection volume or the attenuation setting was adjusted so that the peak height of methylprednisolone was approximately 100 mm. The amount of prednisolone present in the sample was calculated from the equation:

$$\text{Amount of prednisolone in sample} = \frac{\text{prednisolone peak height}}{\text{methylprednisolone peak height}} \times \frac{1}{1.02} \times \text{amount of methylprednisolone added} \qquad \text{equation (2.2)}$$

Figure 2.17 shows a typical chromatogram which indicates the separation of prednisolone and methylprednisolone from other lipid-soluble materials extracted from plasma achieved by the use of 5 per cent OV-1 columns. The peak with retention time of 26.7 minutes corresponds to cholesterol. It was established that prednisolone plasma concentrations down to 50 ng/ml could be estimated with acceptable accuracy. For example, replicate analyses of a plasma sample nominally containing 100 ng/ml prednisolone gave 110 ± 6 ng/ml (S.D. n = 5).

Prednisolone was obtained from Pfizer Laboratories and Lancet Pharmaceuticals and 6α-methylprednisolone was obtained from Upjohn Pty. Ltd.

(ii) Determination of prednisolone in gastric samples

Gastric fluid samples of 1 ml were diluted with water to 4 ml and internal standard solution (6α-methylprednisolone) added. This mixture was extracted 3 times with 8 ml of chloroform:tetrahydrofuran (3:1) and the combined extracts evaporated under vacuum. The residue was dissolved in approximately 50 µl of ethanol and an aliquot injected into the gas chromatograph.

The conditions for chromatography differed slightly from those used for the analysis of plasma samples. The differences were:

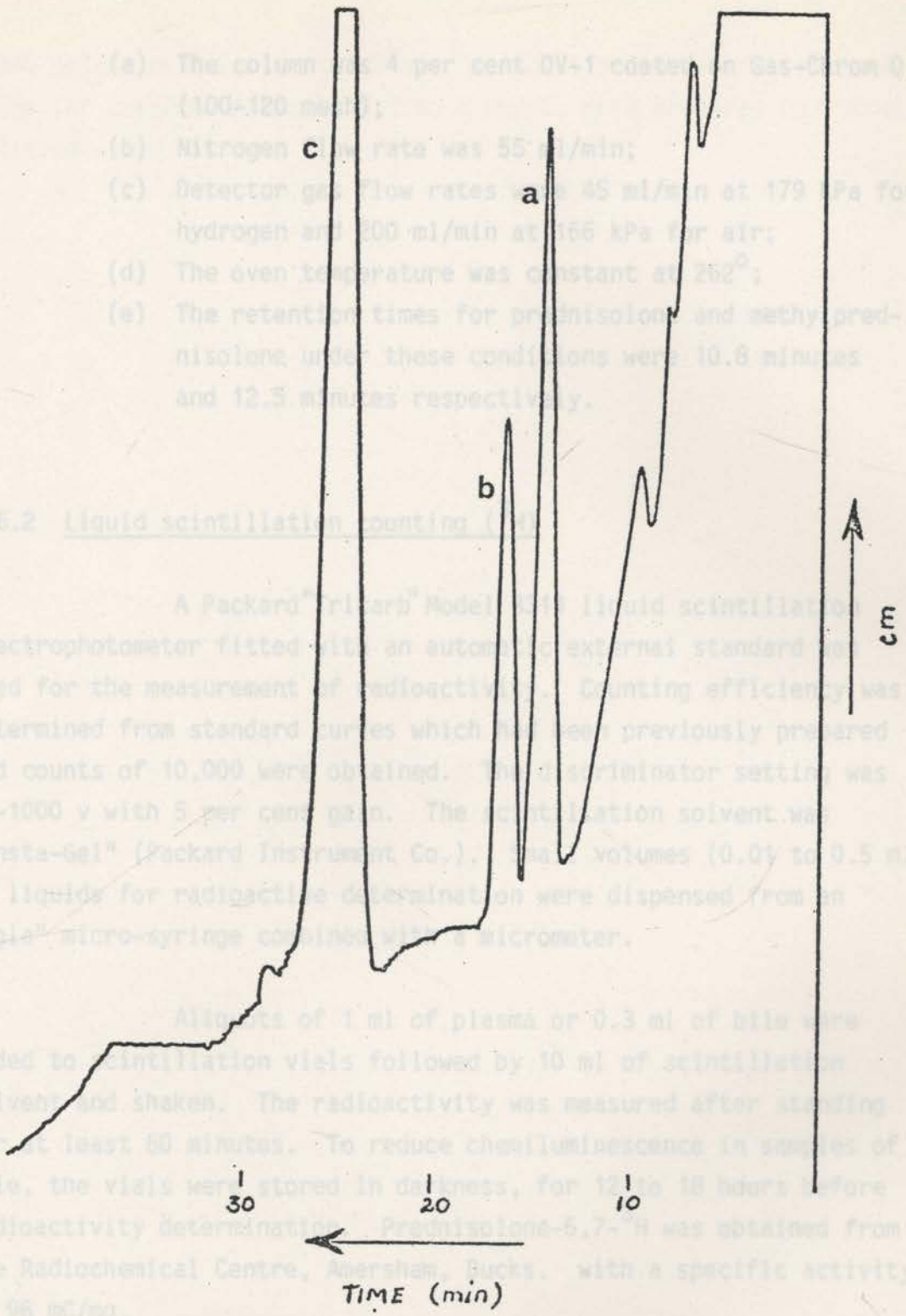


Figure 2.17. Chromatogram of a plasma sample showing the separation of prednisolone and 6∝-methylprednisolone (internal standard) from the lipid soluble materials extracted from the plasma on a 150 cm 5% OV-1 column. a=prednisolone; b=methyl-prednisolone; c corresponds to cholesterol. The retention times of peaks a,b and c were 13.1, 15.3 and 26.7 minutes respectively. The plasma prednisolone concentration measured was 388 ng/ml and the attenuation setting was 8 x 1.

(a) The column was 4 per cent OV-1 coated on Gas-Chrom Q (100-120 mesh);
(b) Nitrogen flow rate was 55 ml/min;
(c) Detector gas flow rates were 45 ml/min at 179 kPa for hydrogen and 200 ml/min at 166 kPa for air;
(d) The oven temperature was constant at 262°;
(e) The retention times for prednisolone and methylprednisolone under these conditions were 10.8 minutes and 12.5 minutes respectively.

2.6.2 Liquid scintillation counting (^3H)

A Packard "Tricarb" Model 3314 liquid scintillation spectrophotometer fitted with an automatic external standard was used for the measurement of radioactivity. Counting efficiency was determined from standard curves which had been previously prepared and counts of 10,000 were obtained. The discriminator setting was 50-1000 v with 5 per cent gain. The scintillation solvent was "Insta-Gel" (Packard Instrument Co.). Small volumes (0.01 to 0.5 ml) of liquids for radioactive determination were dispensed from an "Agla" micro-syringe combined with a micrometer.

Aliquots of 1 ml of plasma or 0.3 ml of bile were added to scintillation vials followed by 10 ml of scintillation solvent and shaken. The radioactivity was measured after standing for at least 60 minutes. To reduce chemiluminescence in samples of bile, the vials were stored in darkness, for 12 to 18 hours before radioactivity determination. Prednisolone-6,7-^3H was obtained from the Radiochemical Centre, Amersham, Bucks. with a specific activity of 96 mC/mg.

2.6.3 Ultraviolet spectroscopy

A Varian "Techtron" Ultraviolet Spectrophotometer Model 635 with a Marconi Instrument Ltd. Digital Multimeter TF2670 was used. Prednisolone absorbance was measured in 1 cm path cells at a wavelength

of 246 nm and prednisone at 242 nm. Calibration curves, which were linear for concentrations of 0.2 to 5 µg/ml, were prepared in freshly distilled water.

CHAPTER THREE

RESULTS

3.1 Chronic Portal Vein Sampling Model in the Dog

A series of experiments were required to develop a successful method for the chronic portal vein catheterisation. The sequential steps in this process are described in this section in a general fashion.

3.1.1 The umbilical vein route

A series of 5 fully grown mongrel dogs, weighing 25 to 31 kg was used to determine the feasibility of the umbilical vein route. They were anaesthetised with pentobarbitone sodium (30 mg/kg, intravenously) and from descriptions of the technique in humans (Christophersen & Jackson, 1967; Braastad, Condon & Gyorkey, 1967; Malt, Corry & Chavez-Peon, 1968), a white cord-like structure corresponding to the appearance of the umbilical vein in the human was found. Hemisection revealed a lumen and indicated that it was the collapsed umbilical vein which could be traced for approximately 6 to 7 cm from the umbilicus through the peritoneal wall and into the falciform ligament. After about 3 to 4 cm on the free border of the falciform ligament it disappeared together with the corresponding portion of the falciform ligament. In the human, the falciform ligament is continuous to the umbilical fissure of the liver and the umbilical vein is found on the free margin. According to Miller, Christensen & Evans (1964), the falciform ligament in a dog degenerates as it leaves the puppy stage. The middle portion disappears completely, leaving the section 12 to 14 cm long from the umbilicus and the section from the umbilical fissure of the liver. This latter section was found in two of the dogs. It was concluded that when the middle section of the falciform ligament, which supports the umbilical vein, degenerates and disappears, it takes with it the corresponding portion of the umbilical vein. Therefore, this route could not be used in the dog.

3.1.2 Breed of dog used

Originally mongrel dogs were used on the assumption that they would be representative of the dog population as a whole in the metabolism of drugs. However they were found to be unsuitable for practical reasons. Their state of health when received from animal pounds was variable. All were infested with worms, necessitating eradication which was a prolonged and time wasting process. Excitable behaviour in some dogs was a nuisance after they had been catheterised and sampling was being attempted. Most mongrels seemed unwilling to leave the portal vein catheter undisturbed, despite head restraints and back leg hobbles. The practical problems experienced with mongrels led to the trial use of a greyhound, which was successful.

Greyhounds were found to be very co-operative dogs. This is probably due to their discipline for racing and was of great advantage in an experimental animal for long-term use. Greyhounds were easily adaptable to the laboratory routine and working with them was far easier than with mongrels. For example, during blood sampling they would stand still for long periods. Between samples they lay quietly until again required. Most importantly, greyhounds were not perturbed in any way by having a coat covering the portal vein catheter, or by having the neck bandage.

The change to greyhounds brought other unexpected advantages. Their leanness made for easier surgery. Fatty tissue is a hindrance during surgery and its almost complete absence in greyhounds, especially in the area surrounding the tributaries leading from the spleen to the main splenic vein, improved visibility and made orientation, identification and selection of a suitable vessel for catheter entry much easier.

Greyhounds were received from the pound immediately following disposal by owners and were in good health. Vaccination for canine distemper and infectious hepatitis was not necessary as this is a condition of registration and they were free of worms. This was advantageous, as most anthelmintics have adverse effects on the

liver, which could adversely effect metabolic or kinetic studies. The good health of greyhounds improved prospects of successful surgery and recovery.

It should be noted that the dogs had no blood tests carried out to detect diseases not revealed by physical examination. This was considered unlikely but the possibility exists. Haematocrits were measured frequently after the implantation of catheters and found to be satisfactory.

3.1.3 Operational procedure for the implantation of catheters

(i) Recovery

Pentobarbitone sodium was initially used for anaesthesia during surgery. However the prolonged recovery time and emergence excitement was considered liable to predispose to self-inflicted injuries. An inhalation anaesthetic such as halothane which allows consciousness to be regained in 30 to 60 minutes and a quiet recovery was therefore used. Overall recovery was improved following halothane anaesthesia.

The preference for the use of females arose from the abdominal bandaging of the dogs following the surgery. The urinary function of males needed to be accommodated and this complication was absent if females were used.

(ii) Maintenance of animals

No infections occurred at the exits of the catheters through the skin. With time, the purse-string sutures fell away and the skin closed around the catheters. However, movement of a catheter in or out was possible and gave additional flexibility. The aseptic sampling procedures were successful and no infections were evident in any dog.

The minimum frequency of flushing of the catheters with heparinised saline necessary to maintain patency was not determined. However, they could be left for up to two days between flushing, but were flushed daily to allow for a margin of safety. Arterial catheters occasionally filled with blood, but it was prevented from clotting by the anticoagulant solution.

Exercise of greyhound dogs was essential to maintain physical and mental health. The dogs became disinterested and listless if left in a kennel and consequently they were brought to the laboratory daily.

3.1.4 Catheters

(i) Tubing

Polyethylene (PE) was used in the early developmental experiments. It was successful for the jugular vein and carotid artery catheters, but being rigid, it was not suitable for the portal vein catheter. It was difficult to tie a catheter securely into a vessel and to leave sufficient loose catheter in the abdomen to allow for movement of the animal. Even securely fasted PE catheters could be pulled out of blood vessels if enough force was applied and on kinking the tubing splits along the crease formed.

PE catheters remained patent in the portal vein of greyhounds for only 10 to 14 days. Clotted blood was not found within the catheters but thrombi around the catheter tips in the vein were found, which also caused partial obstruction of the portal vein. This rapid formation of thrombi is probably caused by the freely floating rigid end of the PE tubing tearing the intima of the vessel wall. Intimal damage within a blood vessel results in thrombus formation.

"Silastic" tubing was found to be the most successful for portal vein catheters. Its major advantage is its flexibil-

ity. It enabled catheters to be very tightly secured in vessels by slight squeezing of the tubing with a tie. Such catheters could not be pulled from blood vessels unless the vessel itself gave way and kinking of "Silastic" is of no consequence. It was considered that intimal damage to vessel walls is minimised with the soft flexible end of the tubing and "Silastic" portal vein catheters had much longer periods of patency than PE catheters.

The diameter of the tubing used for the portal vein catheter was a compromise. Ideally, very small diameter tubing should be used so as not to restrict the blood flow in the vein. However it has the practical disadvantage that it blocks easily. Large diameter tubing caused thrombosis of the splenic vein down to its junction with the portal vein. Venous blood from the spleen was shunted via the gastroepiploic vein and gastroduodenal vein to the portal vein. No effect on the health of the dog was seen but the alteration to gastrointestinal blood flow may result in a changed pattern of absorption. The size of the carotid artery and jugular vein catheters was not critical. Their tips were located in much larger vessels and did not impede blood flow. The vessels used for insertion were ligated.

(ii) Geometry of the catheter tip

The V-notch tip was superior to a 45 degree bevel tip. The bevelled tip has the advantage of easier insertion into a blood vessel but when placed in postion in a vessel it is possible for the bevelled side to become temporarily embedded in the wall. Bevelled tip catheters frequently became unusable for 1 to 2 hours and then returned to patency. It appeared that once thrombus formation starts at a bevelled tip, a valve-like flap of fibrin is drawn across the opening when withdrawal of blood is attempted. It was possible to inject material down these catheters for 2 to 3 weeks after which it became impossible to withdraw blood. When a fibrin flap formed on the end of a V-notch tip, it was possible to withdraw blood from around the sides of the flap into the notches (Figure 3.1).

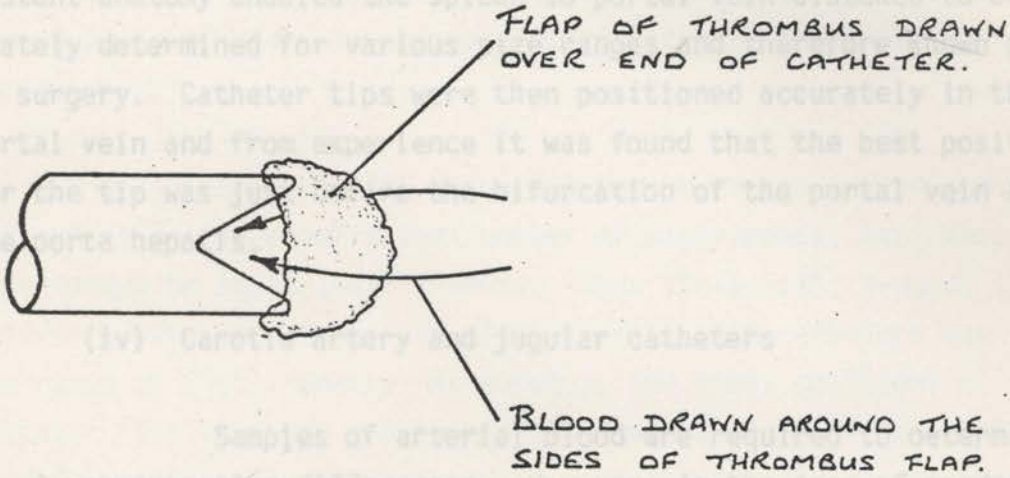


Figure 3.1. Diagram showing how V-notch type catheter tip enables blood withdrawal when a valve-like flap of thrombus covers the end of the catheter.

(iii) Position of catheter tip in the portal vein

The distance between the point of introduction of the catheter into the splenic vein branch and the portal vein was estimated at the time of insertion into the mongrel dogs and the early greyhounds. The position of the catheter tip was checked by palpation, however at post mortem the positions of the tips were found to be extremely variable. The use of greyhounds with their more consistent anatomy enabled the spleen to portal vein distance to be accurately determined for various size ranges and therefore known prior to surgery. Catheter tips were then positioned accurately in the portal vein and from experience it was found that the best position for the tip was just before the bifurcation of the portal vein at the porta hepatis.

(iv) Carotid artery and jugular catheters

Samples of arterial blood are required to determine P - A concentration differences. However, in the case of prednisolone, the plasma concentrations in the samples from the carotid artery and jugular vein were identical at all times measured and therefore either could be used for sampling. The jugular vein catheter was not essential but is an advantage because it serves as a back-up to the carotid artery catheter and enables easy intravenous administration of drug. The V-notch tip was used on carotid artery and jugular vein catheters after its success with portal vein catheters was demonstrated.

3.1.5 Termination of the useful life of catheters

(i) Carotid artery and jugular vein

The patency of carotid artery and jugular vein catheters exceeded that of portal vein catheters, and no difficulty was experienced with them. The tip of the carotid artery catheter was located in the aorta and that of the jugular vein catheter at the

junction of the superior and inferior vena cavas. Blood from these catheters therefore, was arterial and mixed venous respectively.

(ii) Portal vein

Great difficulty was experienced initially in achieving useful lives from the portal vein catheters. Success was achieved as a result of improvements made to the technique. These were (a) changing from polyethylene tubing to "Silastic" tubing, (b) adoption of the V-notch tip and (c) better positioning in the portal vein.

For a portal vein catheter to be considered successful and allow for a sufficient number of experiments, its useful life needed to be at least 6 weeks. When "Silastic", V-notch tip catheters were used, the life of the portal vein catheters was in the range of 2 to 3 weeks. At autopsy, the final positions of the catheter tips in these greyhounds was found to range from in the splenic vein 3 cm from its junction with the portal vein to wedged in a small branch of the portal vein deep in the liver. The catheter with its tip in the splenic vein caused complete thrombosis of that vein. When the distance from the spleen to the portal vein bifurcation at the entrance to the liver was determined accurately, the time of the useful lives of catheters rose. Two months life was attained (Dog I), followed by a life of 3 months (Dog II) and in the last dog (Dog III) the portal vein catheter remained patent for 5½ months and was patent at the time the dog was sacrificed. The fourth dog in the final series suffered a stroke two weeks after insertion of the catheters.

When the useful life of a portal vein catheter terminated, the sequence that occurred was: cessation of sample withdrawal capability but continuing ability to inject down the catheter and then 1 to 2 weeks later complete blockage. Attempts to clear the blocked catheters were made but all were unsuccessful. Nylon fishing line, flexible catheter wire and thin copper wire were used to attempt to push a clot away but the route of the catheter was too

tortuous for any effective force to be exerted at the tip.

During the period of "half-blockage", when injection down the catheter could be made, an inner catheter* was threaded through the portal vein catheter, but could never be advanced past the portal vein catheter tip. When total blockage occurred to the tip, insertion and flushing down an inner catheter with saline solution resulted at times in some dilute red coloured fluid with solid pieces of clot flushing back up the outside in the portal vein catheter. Attempts to dissolve the thrombi at the ends of the catheters with a fibrinolytic agent were made. An inner catheter was inserted (after complete blockage) and a solution of urokinase (Leo Pharmaceutical Products, Denmark), 5,000 Ploug units in 2 to 3 ml of plasma instilled into the catheter and left for several hours. This was done in two dogs but was unsuccessful.

(iii) X-ray fluoroscopy

The position of catheters in the portal vein could only be manually checked at insertion and at autopsy. An X-ray fluoroscope was used to visualise the position of portal vein catheters in conscious animals. The dog was laid in a left lateral recumbent position, resulting in fluoroscopy being performed in a plane at an angle of approximately 30 degrees to the lateral. A syringe containing a 60 per cent solution of sodium and meglumine diatrizoates ("Urografin", Schering, A.G., Berlin) was connected to the portal vein catheter. "Urografin" solution was then forced down the catheter with considerable pressure and the liver area was oriented using the fluoroscope screen. Films were then taken while "Urografin" was injected down the catheter rapidly and with force. Figures 3.2 and 3.3 show the portograms resulting from a dog with a correctly positioned portal vein catheter (Dog III, catheter inserted 24/12/74, X-rays, 14/2/75). The portograms show clearly the position of the tip of the catheter just before the branching of the portal

* Polyethylene tubing, SP8, 0.20 mm i.d., 0.50 mm o.d. Dural Plastics & Eng. Pty. Ltd.

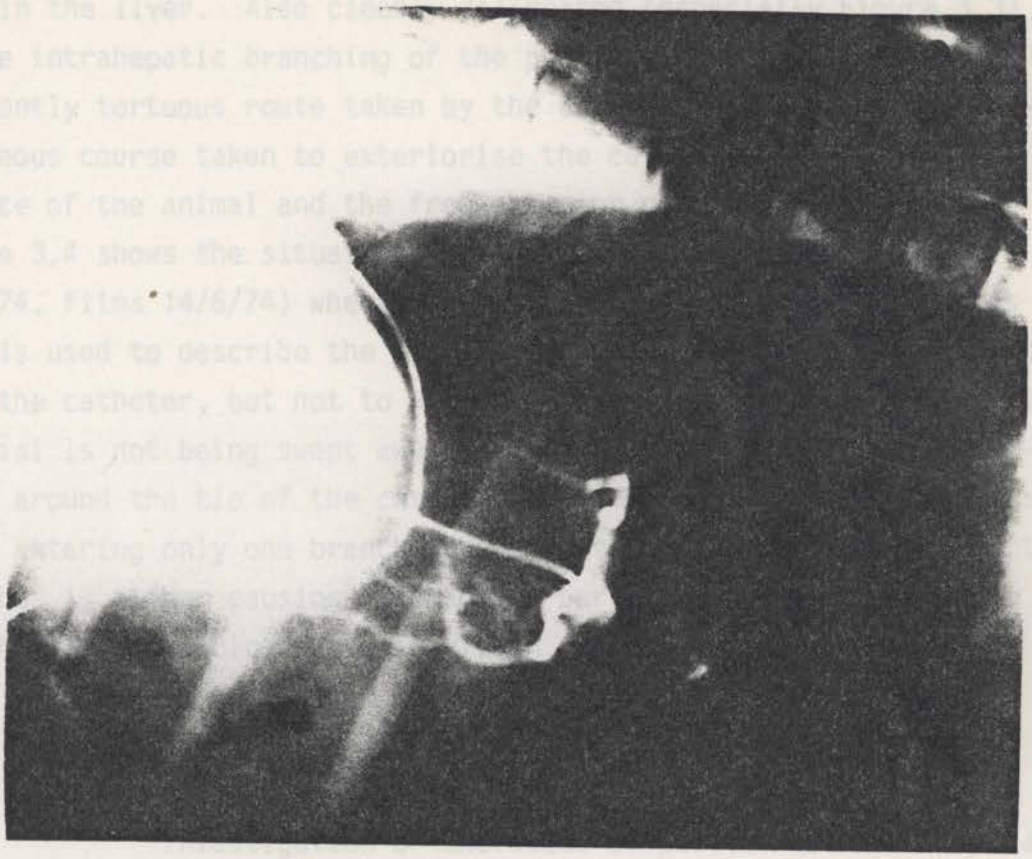


Figure 3.2. Portogram in Dog III with a correctly positioned portal vein catheter. The position of the tip of the catheter just before the branching of the portal vein into left and right branches can be seen.

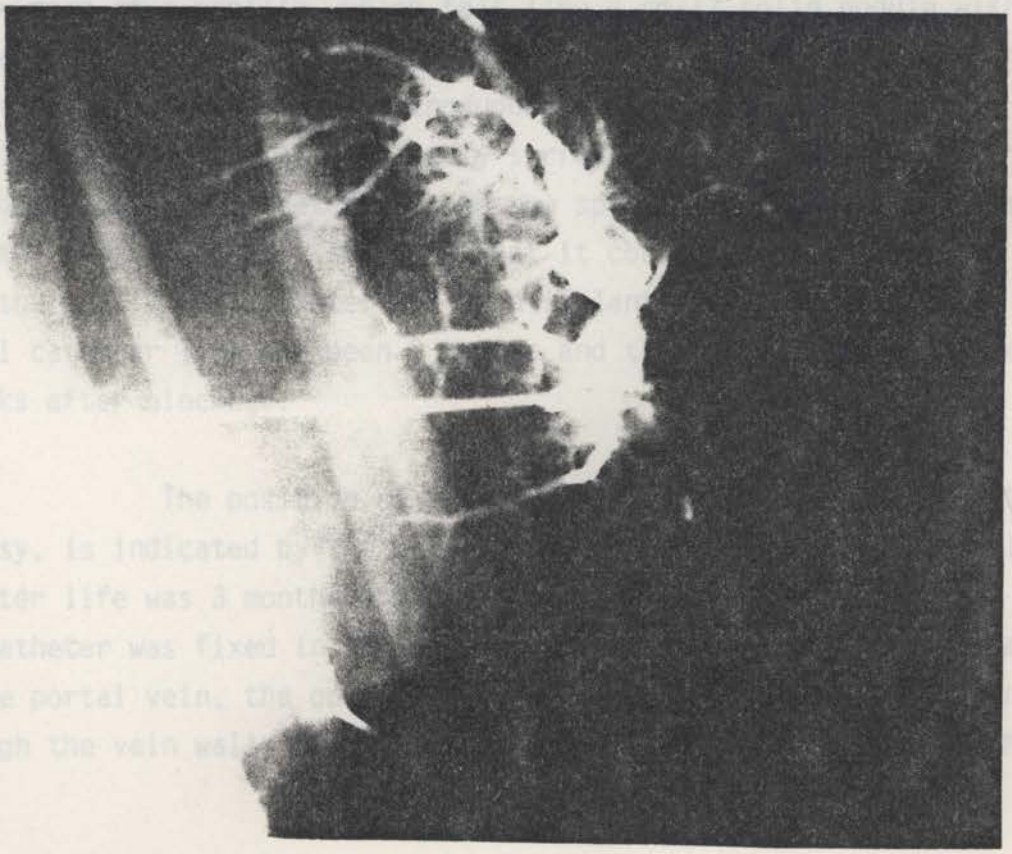


Figure 3.3. Portogram in Dog III , similar to Fig. 3.2. The intrahepatic branching of the portal venous system within the liver can clearly be seen.

vein in the liver. Also clearly delineated (especially Figure 3.3) is the intrahepatic branching of the portal venous system vessels. The apparently tortuous route taken by the catheter arises from the subcutaneous course taken to exteriorise the catheter at the dorsal surface of the animal and the free exterior portion of catheter. Figure 3.4 shows the situation with Dog I (catheter implanted 12/4/74, films 14/6/74) when the catheter was "half-blocked". This term is used to describe the situation when it was possible to inject down the catheter, but not to withdraw samples from it. The contrast material is not being swept away but is clinging to a thrombus formation around the tip of the catheter. The contrast material appears to be entering only one branch of the portal vein, suggesting that the thrombus is either causing blockage or partially blocking the other branch. Also the blood vessels within the liver cannot be defined.

(iv) Autopsy

Investigation of the state of portal vein catheters at autopsy revealed the following. On no occasion was any thrombus formation found inside a "Silastic" catheter. On all catheters fibrous tissue growth was only found surrounding the tip. This was usually in the form of a papilla, which felt like a small solid nodule within the vein. In Dog I, the tip of the catheter was at the junction of the portal vein and the gastroduodenal vein. It adhered to the portal vein wall and was completely surrounded by fibrous tissue which extended along the catheter back to the splenic vein junction. The catheter was so strongly embedded that it could not be pulled away from the wall. The catheter had been implanted for 3½ months, the useful catheter life had been 2 months and the autopsy was performed 6 weeks after blockage.

The position of the tip of the catheter in Dog II at autopsy, is indicated by the white arrow in Figure 3.5. The useful catheter life was 3 months and the autopsy followed 2 weeks later. The catheter was fixed in position at the entrance to the right branch of the portal vein, the outline of the catheter can be faintly seen through the vein wall for a short distance from the arrow tip to the

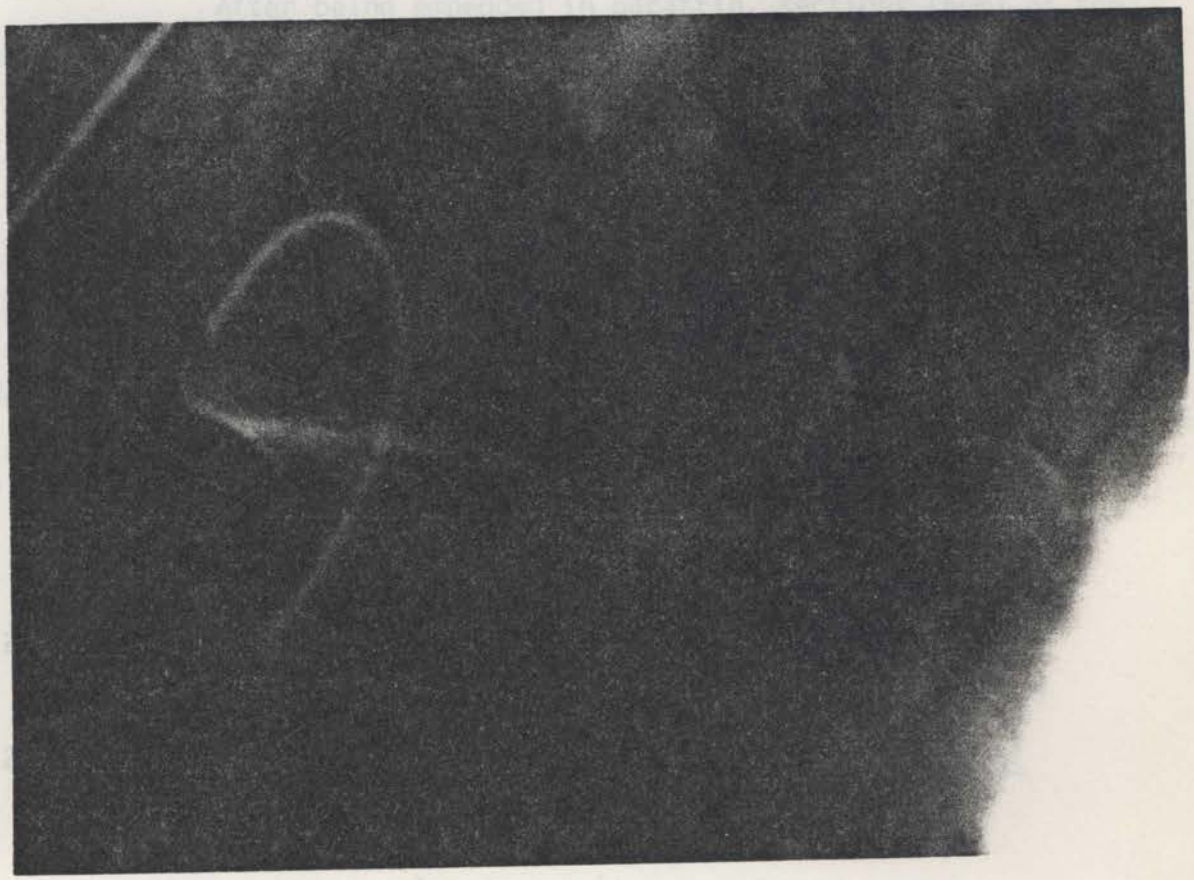


Figure 3.4. X-ray fluoroscope film taken during injection of contrast material down a "half-blocked" portal vein catheter in Dog I. The contrast material is not being swept away rapidly but is clinging to a thrombus formation around the tip of the catheter.

lymph duct crossing over the vein. Figure 3.6 shows the section of vein wall where the catheter was attached. The branching into right and left portal veins can be seen. The catheter tip broke free quite easily from its attachment to the fibrous tissue growth. A plaque was also present on the wall.

(v) Histological sections

After being embedded in paraffin, sections (6μm) of the fibrous tissue growth and the plaque in Dog II were taken and stained with haematoxylin and eosin. The thrombus (Figure 3.7) was long-standing, the zone of organisation suggesting that it was at least 2 to 3 months old. The edge of the clot can also be seen. Figure 3.8 is an enlargement of the broad zone of organisation of the thrombus. Figure 3.9 shows the plaque which was of recent origin. A section of the catheter tip lumen showed no presence of thrombus formation and sections of the liver of the dog were normal showing no areas of tissue damage. Gross histological examination of the spleens of all dogs revealed them to be normal.

3.2 Studies with Prednisolone

3.2.1 Measurement of portal vein and systemic concentrations of prednisolone in anaesthetised dogs

During preliminary experiments to develop the method to measure the portal vein blood flow, intravenous bolus doses of prednisolone (16.7 mg/kg) were administered into the femoral vein. Intravenous injections in two dogs with uninterrupted portal veins gave elimination half-lives for prednisolone of 7.5 and 22 minutes (Table 3.1). In both cases the arterial blood pressure fell for approximately 1 to 1½ minutes after the dose was administered. In the one case where portal vein as well as arterial samples were obtained, the plasma concentrations from both sites were very similar.

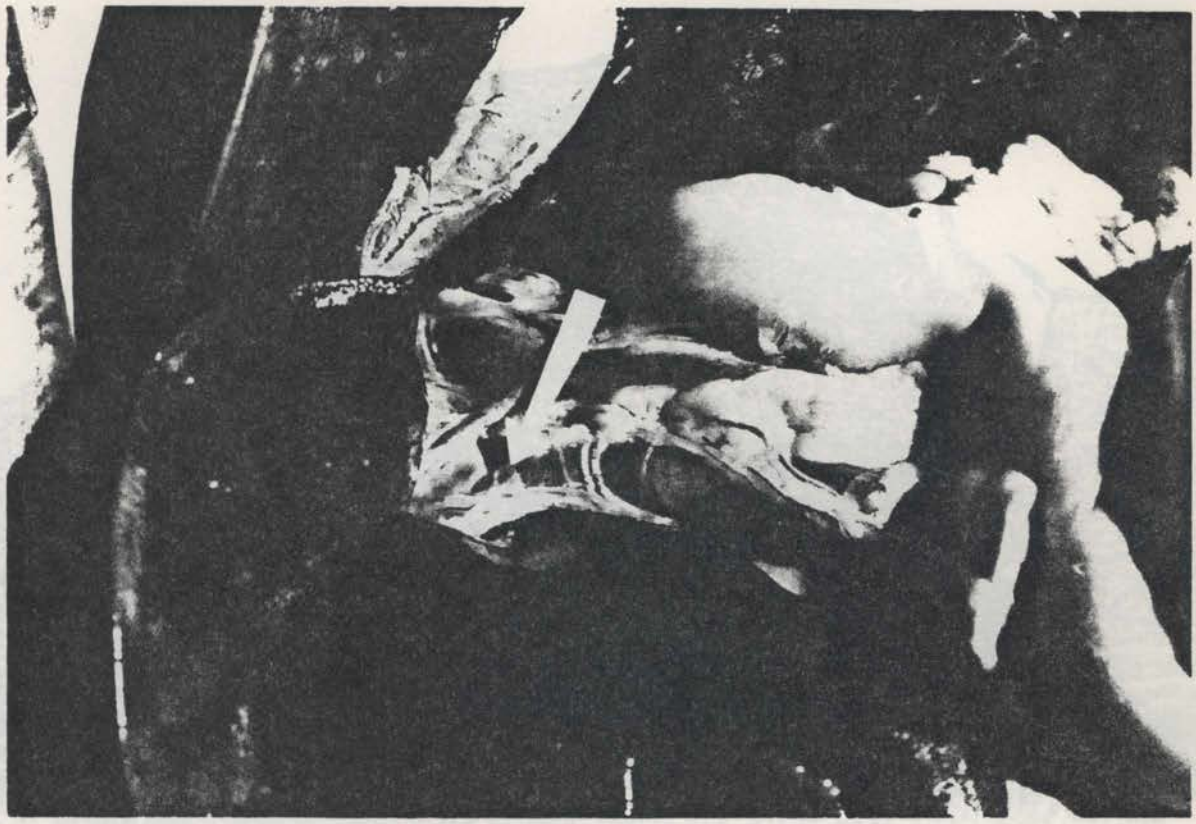


Figure 3.5. Position of the catheter tip of the portal vein catheter in Dog II found at autopsy.

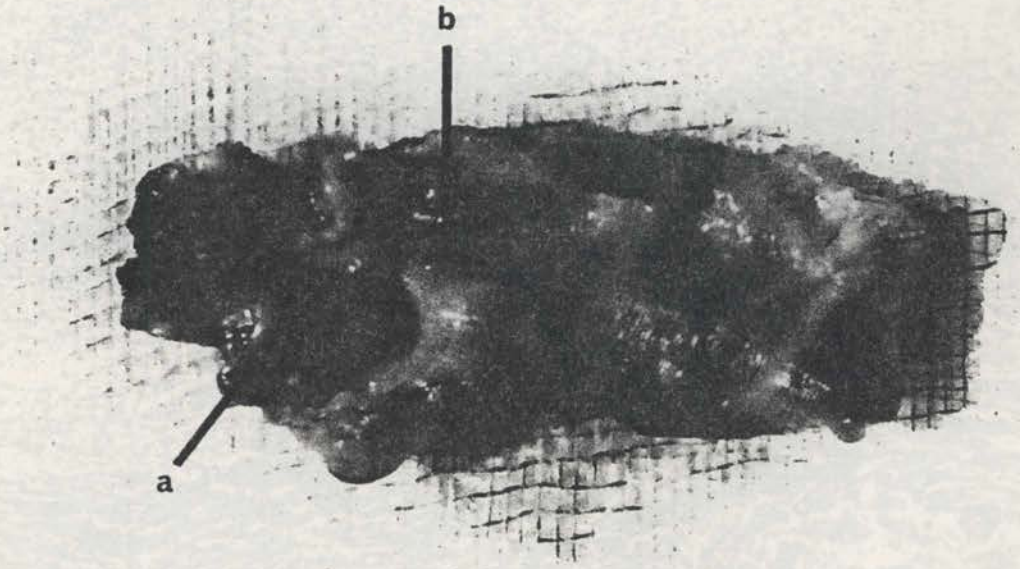


Figure 3.6. Section of the portal vein wall from Dog II showing (a) the thrombus at the site of attachment of the catheter tip, and (b) a plaque.

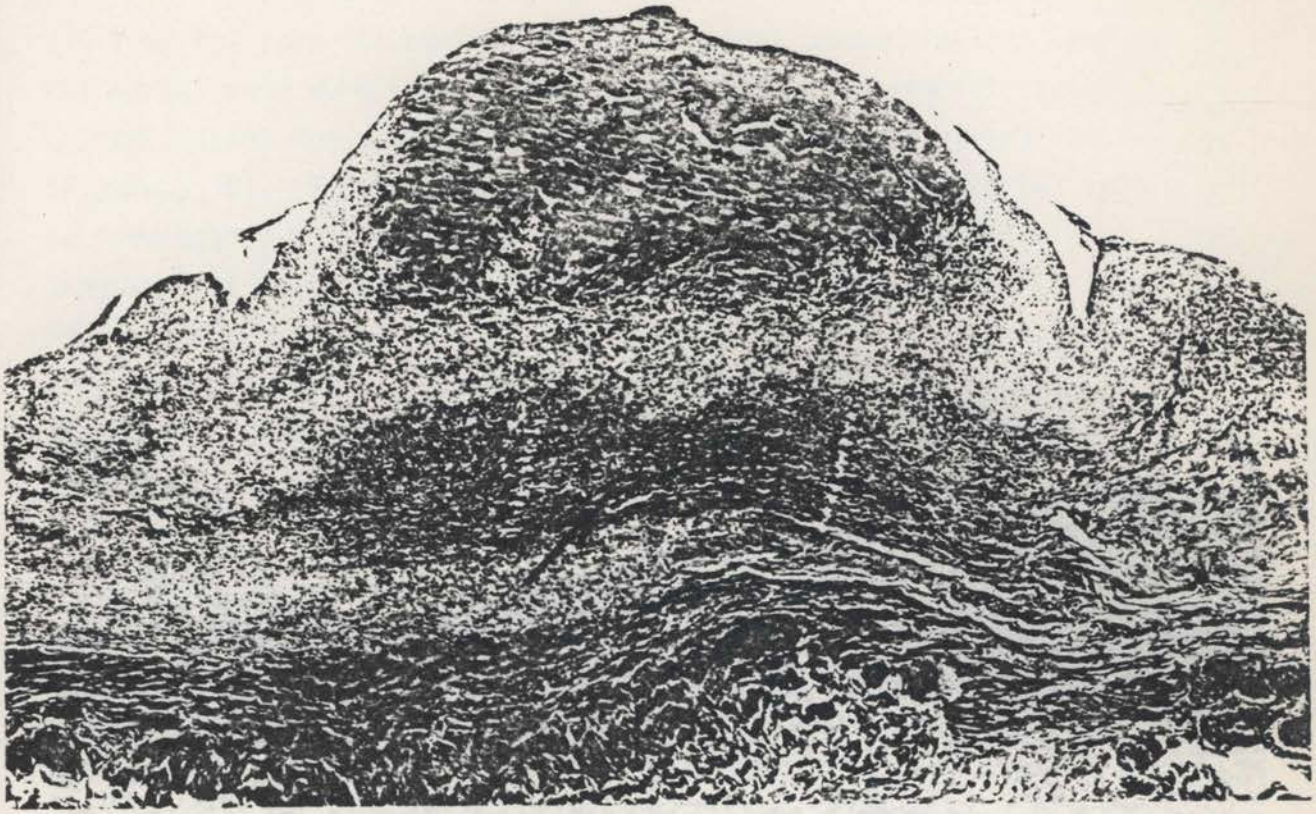


Figure 3.7. Section (6 μm) of the thrombus indicated in Fig. 3.6, x 25. Note the zone of organisation (arrowed).

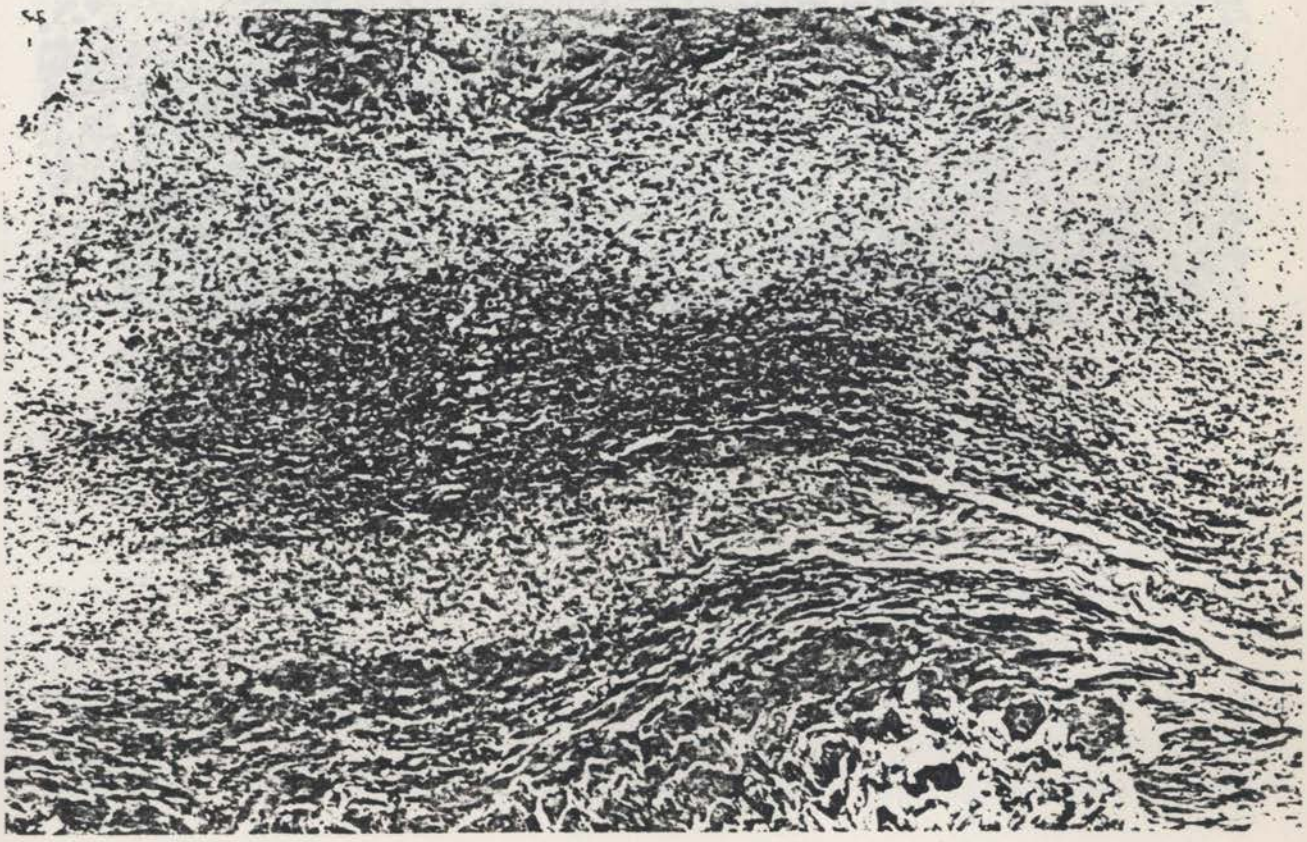


Figure 3.8. Zone of organisation of the thrombus found in the portal vein of Dog II (Figure 3.7). x 40.

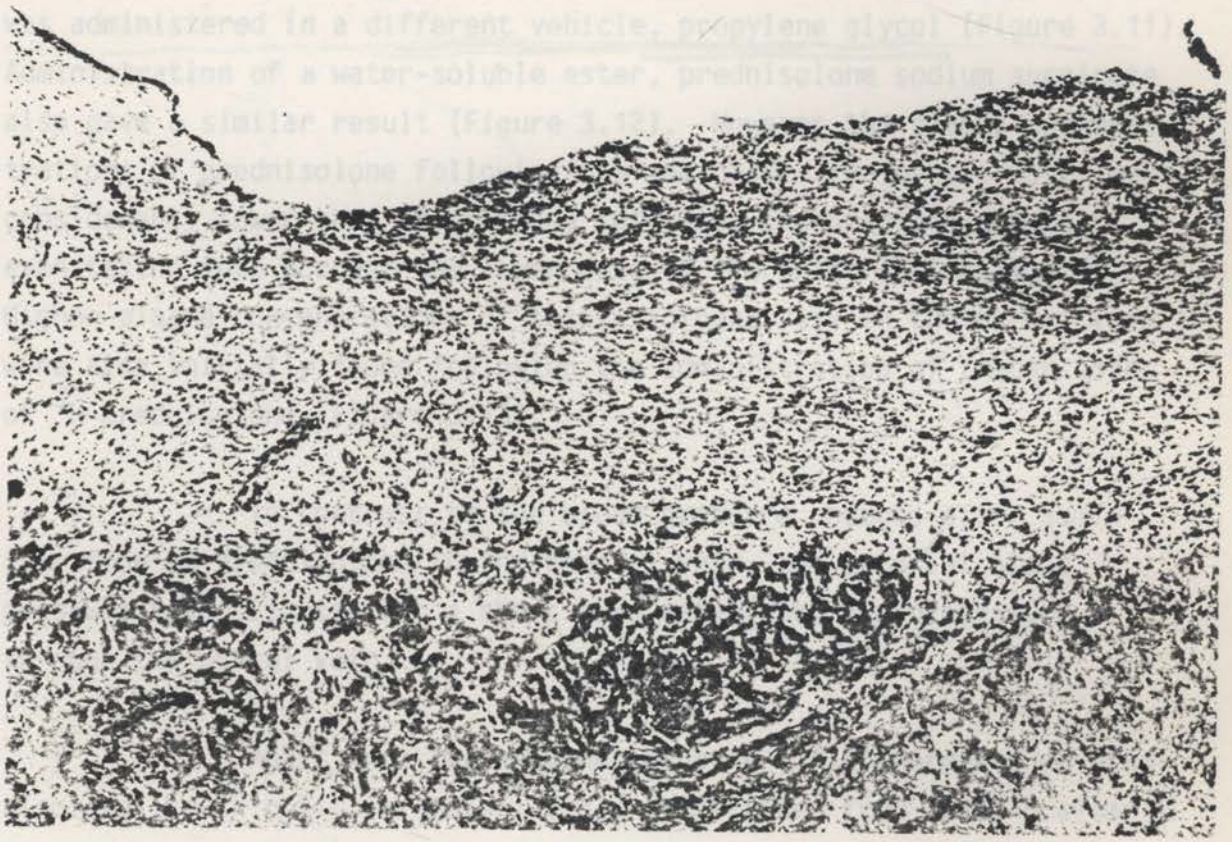


Figure 3.9. Plaque found in the portal vein of Dog II (Figure 3.6). It was considered to be of recent origin. x 25.

However, following injection of prednisolone (16.7 mg/kg) into the portal vein, the plasma concentrations from the portal vein were considerably higher than the arterial plasma concentrations for approximately 30 minutes after administration (Figure 3.10). Further doses were administered via the portal vein to investigate this effect. The trend for the concentrations of prednisolone to be higher initially in the portal vein than in the femoral artery was confirmed in a dog given two doses of prednisolone into the portal vein. A similar result was found when prednisolone was administered in a different vehicle, propylene glycol (Figure 3.11). Administration of a water-soluble ester, prednisolone sodium succinate also gave a similar result (Figure 3.12). However the plasma concentrations of prednisolone following the administration of the ester were considerably lower than those found following prednisolone alcohol, even taking into account the lower dose of the ester (7.5 mg/kg). Higher plasma concentrations of total radioactivity in the portal vein were also initially found following the administration of tracer doses of ^{3}H-prednisolone (Figure 3.13).

In contrast to the blood pressure changes after administration of prednisolone in the femoral vein, the arterial blood pressure rose slightly for a short time after each administration through the portal vein.

Apart from the examination of P - A differences of prednisolone, few kinetic parameters could be obtained from these preliminary experiments. The half-life of prednisolone between 10 and 60 minutes after dosage ranged from 7.5 to 110 minutes (Figures 3.10 to 3.13, Table 3.1). Analysis of the curves reveals biexponential kinetics (Figures 3.10 to 3.13). The variation of the values for $t_{\frac{1}{2}\beta}$ may be due to the irregularities in the curves between 40 and 60 minutes. In many cases, there was a small increase in plasma concentration at this time.

The terminal half-lives were very long after the administration of tracer doses of ^{3}H-prednisolone. The tracer doses were sampled for a longer period of time, 120 minutes as against only 60 minutes for the

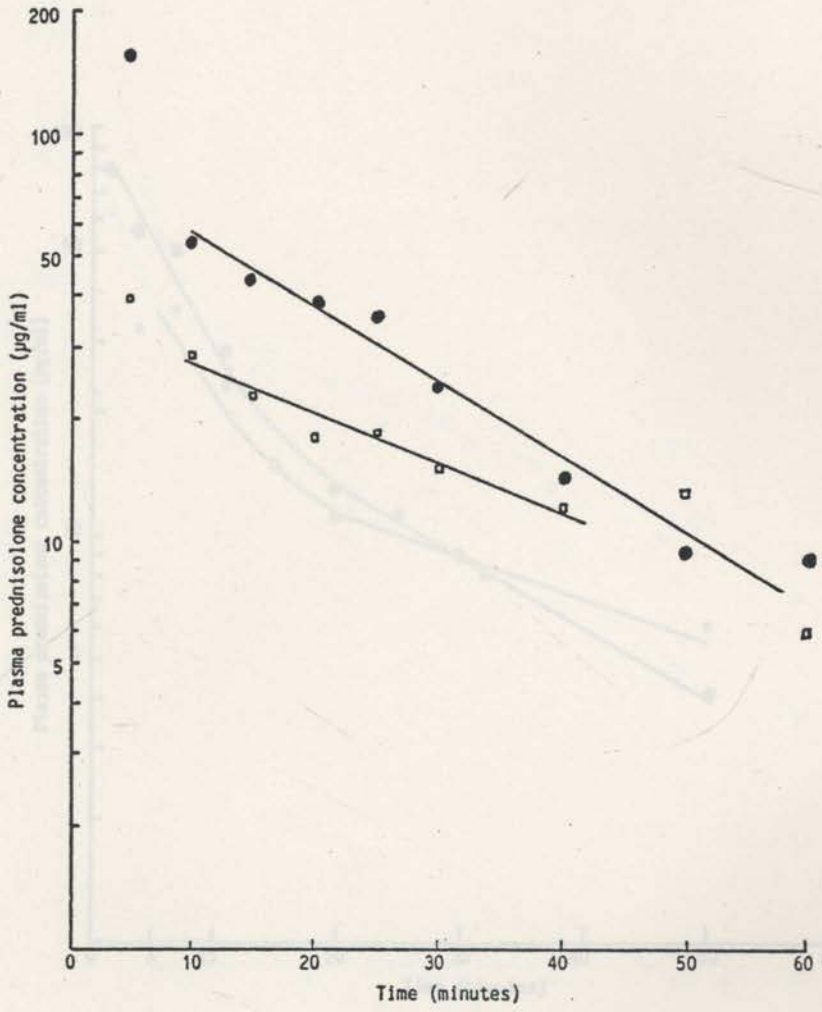


Figure 3.10. Plasma prednisolone concentrations (portal vein, ● ; femoral artery, □) after an intraportal bolus injection of 450 mg to an anaesthetised mongrel dog (27 kg). $t_{1/2}$portal = 14.5 minutes, $t_{1/2}$arterial = 25 minutes.

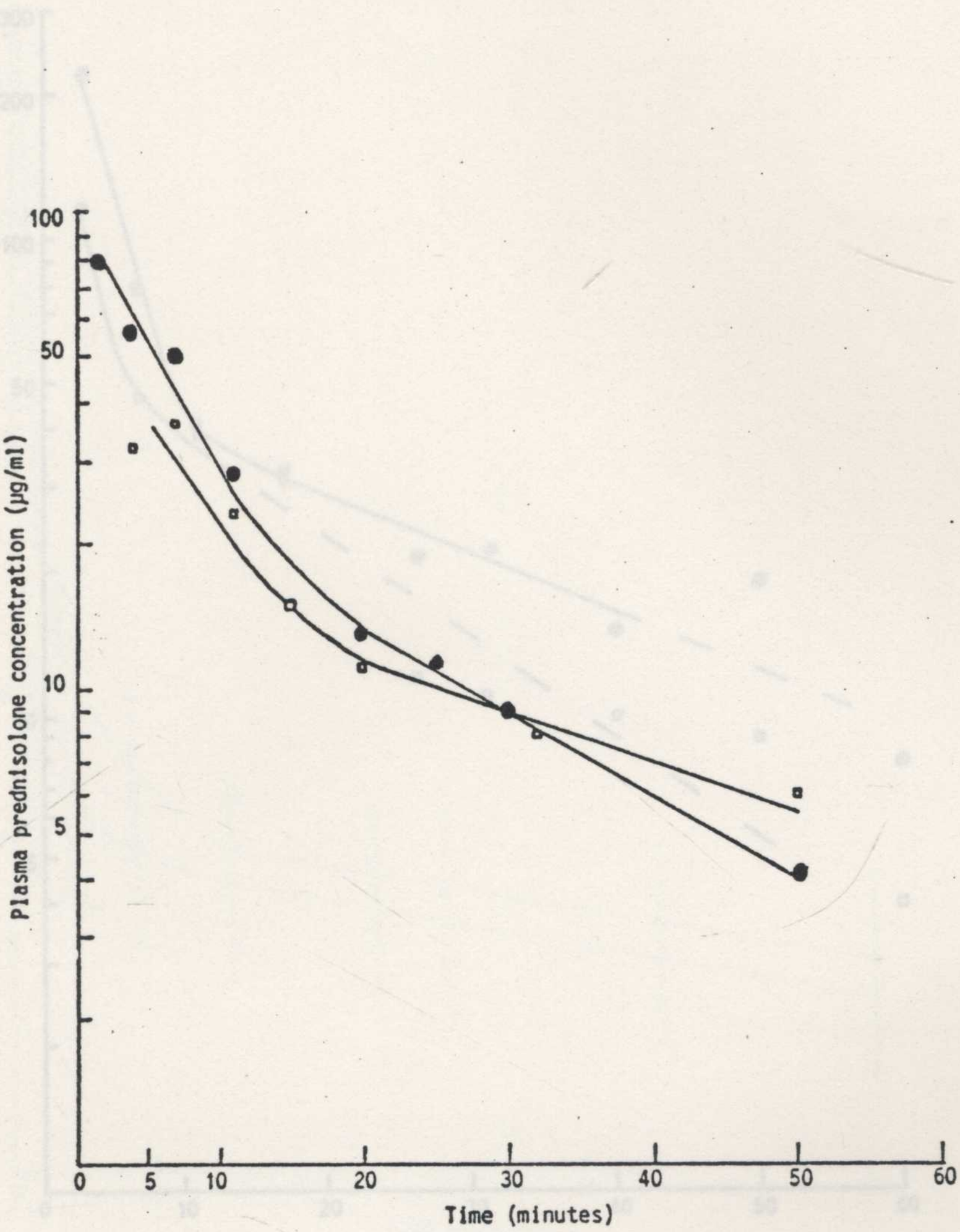


Figure 3.11. Plasma prednisolone concentrations (portal vein, ● ; femoral artery, □) after an intraportal bolus injection of 266 mg to an anaesthetised mongrel dog (16 kg) using propylene glycol as the vehicle. Portal $t_{½\alpha}$, 4 minutes, $t_{½\beta}$, 19 minutes; arterial $t_{½\alpha}$, 3.5 minutes, $t_{½\beta}$, 34.5 minutes.

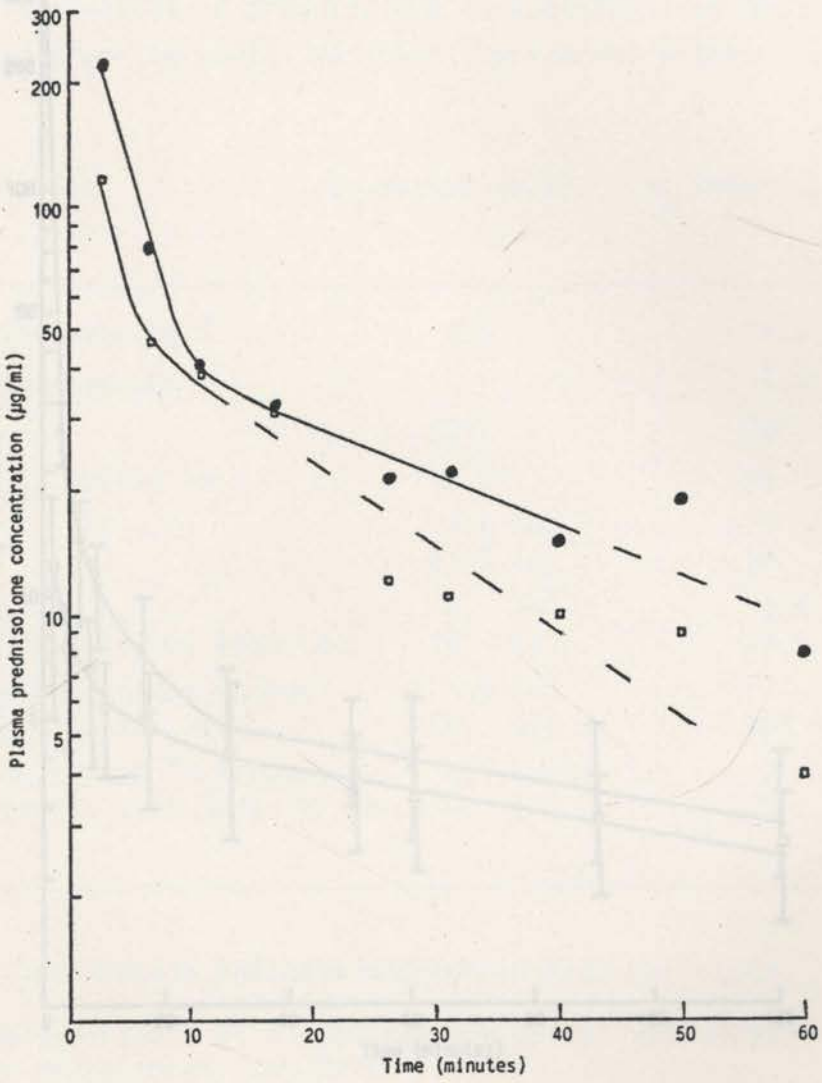


Figure 3.12. Plasma prednisolone concentrations (portal vein, ● ; femoral artery, ◘) after an intraportal bolus injection of 75 mg of prednisolone sodium succinate in an anaesthetised mongrel dog (10 kg). Injection vehicle, 0.9% NaCl (1 ml). $t_{½\alpha}$ portal = 1.5 minutes, $t_{½\beta}$ portal = 26 minutes, $t_{½\beta}$ arterial = 15 minutes.

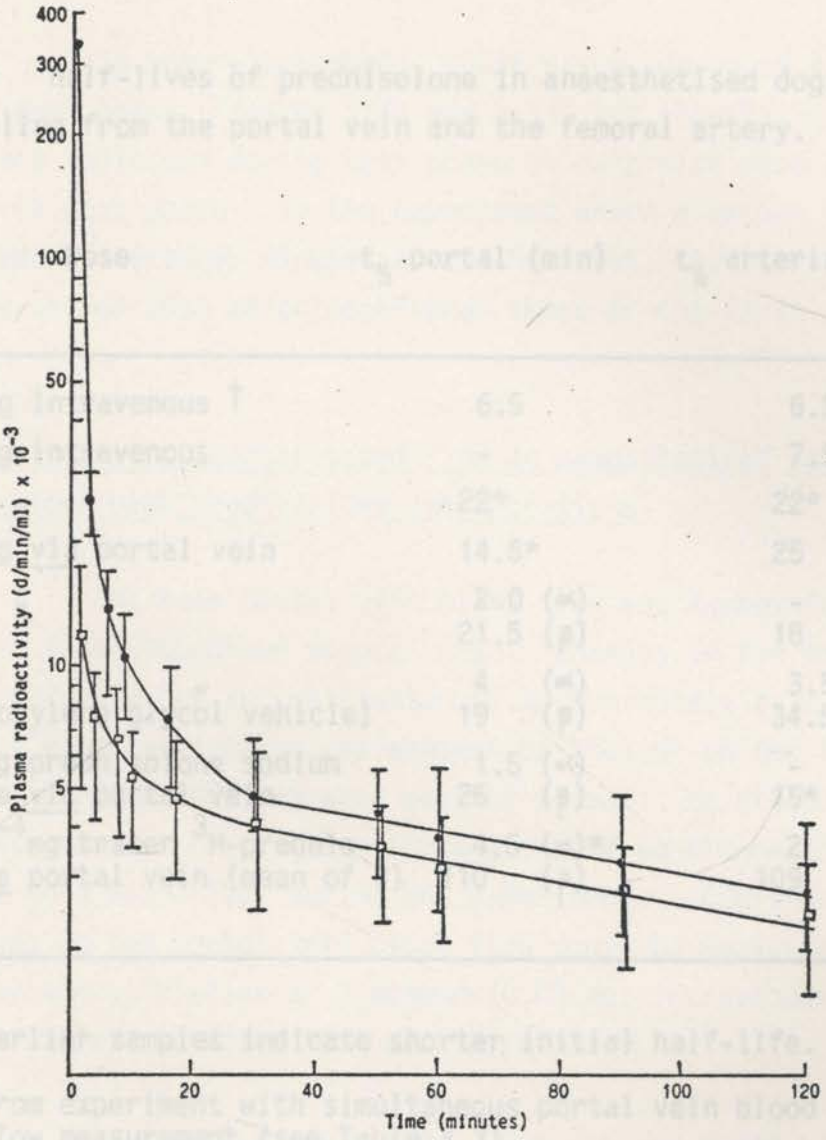


Figure 3.13. Mean plasma radioactivity concentrations following intraportal bolus injection of ^{3}H-prednisolone (1.29×10^{-4} mg, 2.755×10^{7} d/min) in 3 anaesthetised dogs (mean weight 17.3 kg). ●, represents portal vein concentrations; □, represents vena cava concentrations (from femoral vein catheters). Injection vehicle, 0.9% NaCl solution (0.4 ml). Vertical bars show the range of the 3 values. $t_{½\alpha}$portal = 4.5 minutes, $t_{½\beta}$portal = 110 minutes; $t_{½\alpha}$arterial = 2 minutes, $t_{½\beta}$arterial = 109 minutes.

TABLE 3.1 Half-lives of prednisolone in anaesthetised dogs with sampling from the portal vein and the femoral artery.

Dose	$t_{½}$ portal (min)	$t_{½}$ arterial (min)
0.5 mg/kg intravenous †	6.5	6.5
16.7 mg/kg intravenous	-	7.5
"	22*	22*
16.7 mg/kg via portal vein	14.5*	25
"	2.0 (∝)	-
	21.5 (β)	18
" "	4 (∝)	3.5
(with propylene glycol vehicle)	19 (β)	34.5
7.5 mg/kg prednisolone sodium	1.5 (∝)	-
succinate via portal vein	26 (β)	15*
1.29×10^{-4} mg tracer ^{3}H-prednis-	4.5 (∝)*	2
olone via portal vein (mean of 3)	110 (β)	109

* Earlier samples indicate shorter initial half-life.

† From experiment with simultaneous portal vein blood flow measurement (see Table 3.3).

other curves. However, the specificity of the radiochemical estimation was not determined. Metabolites of prednisolone may have contributed to the total radioactivity in plasma.

In some experiments, there was evidence of a faster distributional phase (Figures 3.10 and 3.13) but insufficient plasma samples were collected during this phase to determine accurately the half-life of this phase. In the experiment where a second intraportal dose was administered 65 minutes after the first, the half-lives following the second dose were longer than those of the first (Table 3.2).

3.2.2 Measurement of portal blood flow in anaesthetised dogs together with prednisolone concentrations

The mean portal vein blood flow was successfully measured in 4 anaesthetised mongrel dogs. Changes in the mean flow occurred gradually but an oscillation of approximately ± 10 ml/min in parallel with respiration was present as "noise" on the flow trace when the flowmeter response was not damped. Maintenance doses (30 mg, intravenous) of pentobarbitone produced no alterations in the portal vein blood flow during the experiments. Confirmation that changes in the portal vein blood flow could be measured, was obtained by administration of glucagon (0.03 mg, intravenous). The resulting increase in blood flow was 57 per cent.

Of the four experiments, one dog was dosed with 200 mg prednisolone (as a suspension in water) into the stomach, the second with 50 mg prednisolone into the stomach, the third with 50 mg into the duodenum and the fourth with 10 mg (in ethanol solution) intravenously *via* the femoral vein catheter. Table 3.3 summarises the data from these experiments.

Portal vein concentrations were higher than the arterial concentrations when prednisolone was instilled, indicating absorption of prednisolone from the gastrointestinal tract. In contrast, the concentrations following the intravenous dose were

TABLE 3.2 Half-lives of prednisolone in an anaesthetised mongrel administered 16.7 mg/kg through the portal vein and repeated 65 minutes later. The plasma sampling was carried out from the portal vein and the femoral artery.

Time of administration (minutes)	$t_{1/2}$ portal (min)	$t_{1/2}$ arterial (min)
0	2.0 (α) 21.5 (β)	- 18
65	7 (α) 71 (β)	10.5 71

TABLE 3.3 Portal vein and arterial plasma concentrations[a], and portal vein blood flow rates following administration of prednisolone to anaesthetised dogs. The P - A differences are also included.

Dog 1 17 kg, dosed 200 mg prednisolone into stomach
" 2 16 kg, " 50 mg " " "
" 3 20 kg, " 50 mg " " duodenum
" 4 19 kg, " 10 mg " intravenous

Dog	Time (min)	Plasma prednisolone concentrations (ng/ml) Portal vein	Femoral artery	Portal vein blood flow (ml/min)	(P - A) (ng/ml)
1	0	-	-	115	-
	25	450	356	112	94
	35	403	310	96	93
	45	477	360	77	117
	55	365	294	77	71
	70	406	210	65	196
2	0	-	-	92	-
	7	nd	nd	92	0
	15	132	66	92	66
	23	830	156	73	674
	30	1147	673	84	474
	45	510	210	92	300
	60	250	81	96	169
	70	nd	nd	99	0
3	0	-	-	108	-
	20	1322	785	80	537
	25	4312	800	73	3512
	35	830	546	69	284
	45	2740	681	62	2059
	55	1320	760	53	560
	65	1054	822	65	232
	80	1957	1945	53	12
	105	974	981	53	-7
4	0	-	-	78	-
	10	2633	2440	65	193
	15	1217	1178	53	39
	20	523	472	50	51
	35	263	267	47	-4
	50	nd	nd	35	0
	60	nd	nd	32	0
	70	nd	nd	20	0
	85	nd	nd	16	0

[a] nd represents values below the limit of detection of the assay method (50 ng/ml).

similar. The P - A differences for absorption from the stomach (experiments 1 and 2) appeared to show different absorption patterns. In the first, small peaks occurred at 45 and at or after 70 minutes, however in the second experiment, a single peak occurred at 23 minutes (which coincided with a decrease in blood flow) and absorption ceased by 70 minutes. Absorption from the duodenum (experiment 3) also appeared erratic with peaks occurring 25 and 45 minutes following instillation of the dose. The portal and arterial curves indicate an absorption peak at approximately 80 minutes, however this peak was not revealed in the P - A differences.

The fall in the portal vein blood flow with time in three of the four experiments was probably caused by thrombus formation within the flow probe. At the termination of the experiments, thrombus formation was found in the probe and the probe tubing at joints, which restricted the cross-sectional area of the flow space of the probe. Further, oedema within the splanchnic area occurred after approximately one hour with a corresponding fall in the arterial blood pressure.

The blood flow trace indicates that turbulent flow occurs during the withdrawal of blood (Figure 3.14).

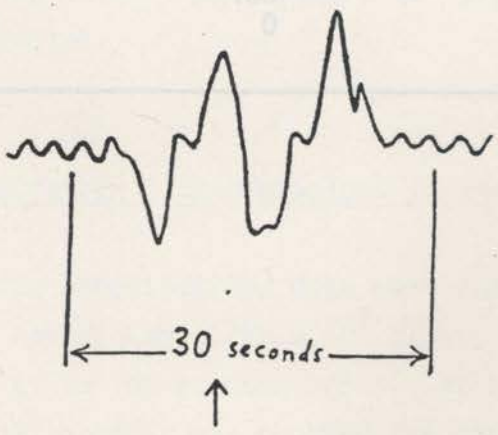


Figure 3.14. Flow turbulence during portal vein sampling, as recorded from an electromagnetic flowmeter probe (3x). Blood taken at (↑).

TABLE 3.4 Amount of prednisolone absorbed per time interval in the acute dogs listed in Table 3.3.

Dog	Time interval (Minutes)	Amount of prednisolone absorbed (ug)	Total amount of prednisolone absorbed (ug)
1	0 - 25	268	
	25 - 35	98	
	35 - 45	91	
	45 - 55	72	
	55 - 70	143	672
2	0 - 7	0	
	7 - 15	49	
	15 - 23	246	
	23 - 30	317	
	30 - 45	511	
	45 - 60	331	
	60 - 70	83	1,537
3	0 - 20	1,010	
	20 - 25	780	
	25 - 35	1,348	
	35 - 45	762	
	45 - 55	760	
	55 - 65	234	
	65 - 80	108	
	80 - 105	0	5,000

The amount of prednisolone absorbed (M) between successive sampling times was calculated from the relationship

$$M = \frac{Q\,[(P - A)_t + (P - A)_{t'}]\,(t' - t)}{2}$$

where Q is the mean portal vein blood flow rate over the period t to t', and $(P - A)_t$ and $(P - A)_{t'}$ are the differences between the portal and arterial concentrations at times t and t'. For the first sampling time when prednisolone could be detected, the portal vein concentration was used instead of (P - A) since prednisolone had to be absorbed from zero initially to reach the portal vein concentration. It is assumed that the red blood cell - plasma concentration distribution ratio for prednisolone is 1 (see page 226).

Table 3.4 shows the amounts absorbed per time period, calculated as above, and the estimate of the total amount absorbed to the conclusion of sampling. The total amounts of prednisolone absorbed represent 0.34 per cent of the dose instilled in Dog 1, 3.1 per cent in Dog 2 and 10 per cent in Dog 3. Of the two animals dosed with 50 mg prednisolone, instillation into the duodenum gave greater absorption than instillation into the stomach over the 70 minute period of measurement. Also, the absorption from the duodenum is more rapid than from the stomach and appears to have ceased by approximately 70 to 80 minutes.

3.2.3 Biliary excretion of prednisolone in anaesthetised dogs

Four anaesthetised dogs were administered ^{3}H-prednisolone (1.5 mg containing 2.755×10^{7} d/min, approximately 0.08 mg prednisolone/kg) as an intravenous bolus. At the conclusion of each experiment, the gallbladder was checked for the presence of bile. None was found indicating that all bile produced during the experiments had flowed out via the cannula and had been collected.

The total biliary excretion of radioactivity after four hours represented 47 per cent of the dose administered. The rate of elimination of radioactivity in the bile was greatest between 30 and 60 minutes after administration and remained high between 60 and 120 minutes (Figure 3.15).

The disappearance curve for plasma radioactivity was biexponential with the half-lives of the α and β phases being 4 and 55 minutes respectively.

3.2.4 Gastric absorption of prednisolone

(i) In anaesthetised dogs with ligated stomachs

The plasma levels of prednisolone found in the portal vein following instillation of ^{3}H-prednisolone (1.5 mg dose containing 2.755×10^{7} d/min), calculated assuming all radioactivity present represents prednisolone, are found in Table 3.5. The figures are based on the average found from four dogs.

Although it was not possible to quantitate the amount absorbed, the concentrations of prednisolone (and/or metabolites) in the portal vein indicate that a significant quantity of prednisolone was absorbed from the stomach. The rate of absorption was greatest at 30 minutes after instillation and absorption continued after that time because the plasma concentrations remained level and did not fall.

(ii) In conscious humans

The ratio of the concentrations of ^{51}Cr indicator to prednisolone after each 15 minute period of study did not change significantly and the fraction of prednisolone absorbed during instillation of a 10 mg dose, as determined from equation (2.1), was less than 0.02 in all periods of each study. There was no change in this absorption pattern for prednisolone when salicylic acid was

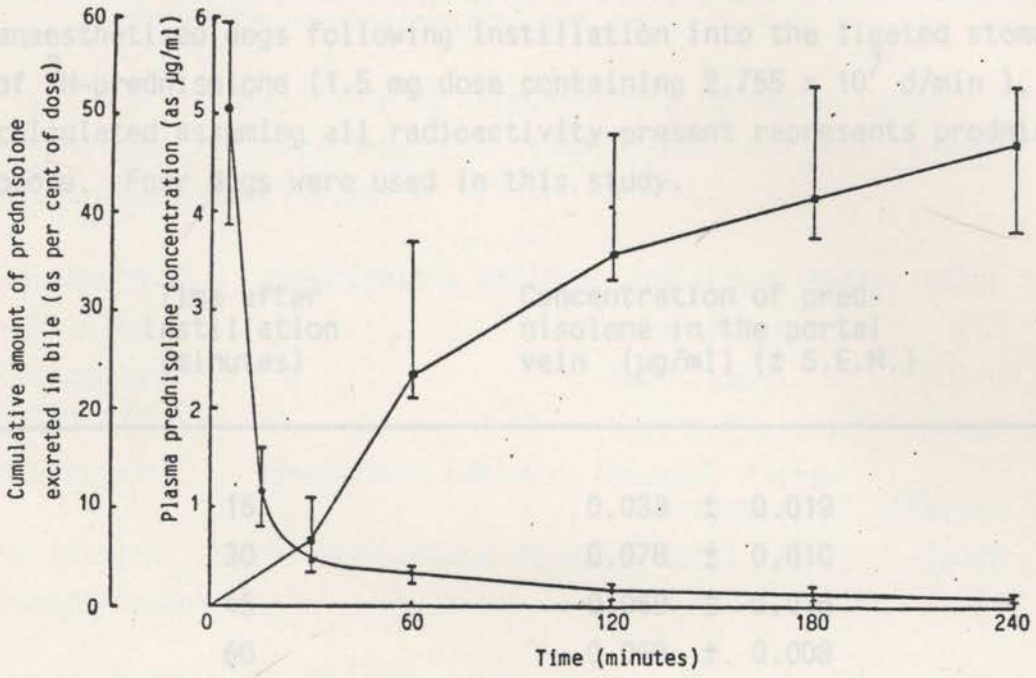


Figure 3.15. Biliary excretion of ^{3}H-prednisolone. ● is the venous plasma concentration of prednisolone calculated assuming all radioactivity present as prednisolone. ■ is the cumulative amount of radioactivity excreted in the bile, as a percentage of the dose administered. The dose was 1.5 mg prednisolone containing 2.755×10^{7} d/min as an intravenous bolus to anaesthetised dogs. Four dogs were used. Vertical bars show the range of the four values obtained.

TABLE 3.5 Concentration of prednisolone in portal vein plasma of anaesthetised dogs following instillation into the ligated stomach of ^{3}H-prednisolone (1.5 mg dose containing 2.755×10^{7} d/min), calculated assuming all radioactivity present represents prednisolone. Four dogs were used in this study.

Time after instillation (minutes)	Concentration of prednisolone in the portal vein (μg/ml) (± S.E.M.)
15	0.033 ± 0.019
30	0.078 ± 0.010
45	0.069 ± 0.016
60	0.060 ± 0.008
75	0.050 ± 0.007
90	0.064 ± 0.008
105	0.041 ± 0.010
120	0.060 ± 0.008

present in the test solution. The gastric absorption of prednisolone was insignificant and was within the limits of error of the method.

3.2.5. *In vitro* dissolution of prednisolone and prednisone preparations

The following preparations, stated to contain 5 mg of prednisolone or prednisone were investigated for their *in vitro* dissolution characteristics:

"Deltasolone"	Prednisolone tablets	Knoll Labs.	Batch 13070L	Code A
"Adnisolone"	" "	Adam Drug Co.	" 297	" B
"Deltasolone"	" "	Knoll Labs.	" 10075*	" C
"Delta-Cortef"	" "	Upjohn Pty.Ltd.	" A5024	" D
"Decortisyl"	Prednisone tablets	Roussel Pharmaceuticals	" 069928Y1	" E
"Deltasolone Caps"	Prednisolone capsules	Knoll Labs.	" 10092	" F
Extemporaneous	" "	Hand-filled	" 03	" G

* *via* N.B.S.L. These had been claimed a therapeutic failure by a clinician.

All of the preparations passed the U.S.P. XVIII Dissolution Test for either prednisolone or prednisone, that is, 60 per cent of the stated amount dissolved within 20 minutes, at a basket speed of 100 revs/min. At the U.S.P. XVIII basket rotation speed of 100 revs/min, there was no discrimination between products and in each case maximum dissolution was reached by 5 minutes.

Figure 3.16 shows the cumulative amounts of prednisolone or prednisone dissolved against time with the speed of rotation of the baskets at 50 revs/min. Five examples of each preparation were evaluated. All of the preparations with the exception of Knoll prednisolone capsules (F) displayed satisfactory dissolution behaviour, although they all appear to contain less than the stated amount of drug. When the rotation speed was increased to 100 revs/min for a further 10 minutes after the final samples had been taken, only the Knoll capsules showed an increase in the amount dissolved.

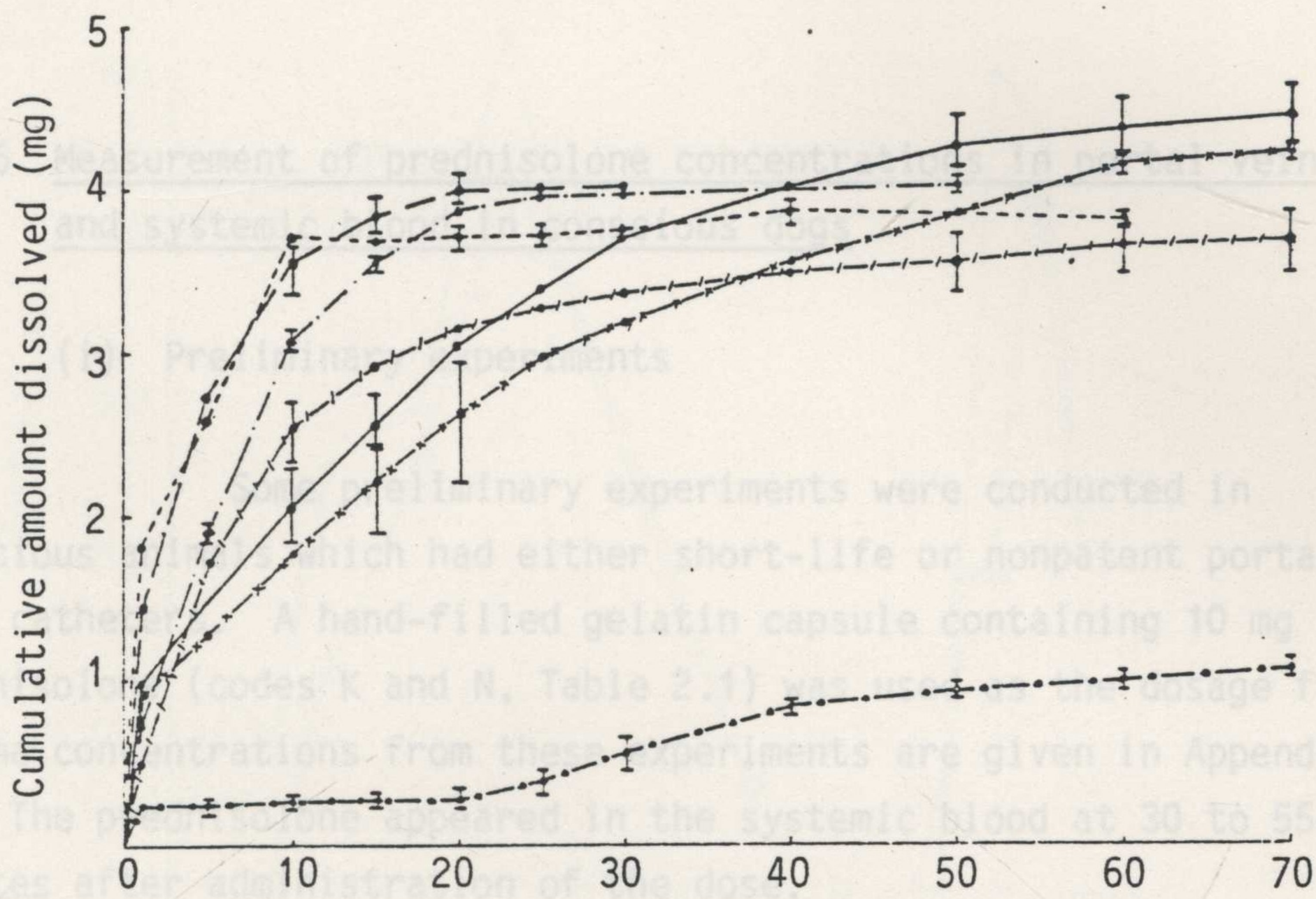


Figure 3.16. Cumulative amount of prednisolone or prednisone dissolved from dosage forms against time using a U.S.P. XVIII Dissolution Apparatus at 37^{o}. Speed of rotation of basket, 50 revs/min. Five examples of each preparation were evaluated and each preparation was stated to contain 5 mg. The range of the five values for selected points is shown by the vertical bars.

Key; ——— ,product A; — —,product B;-—-—-—,product C;
X—X—X,product D; /—/—,product E; •—•—,product F;
------,product G.

When the Knoll capsules were examined at the conclusion of the 70 minute run at 50 revs/min, it was found that very little of the gelatin capsule had dissolved but that a small hole was present which may have permitted some material to escape to the medium.

3.2.6 Measurement of prednisolone concentrations in portal vein and systemic blood in conscious dogs

(i) Preliminary experiments

Some preliminary experiments were conducted in conscious animals which had either short-life or nonpatent portal vein catheters. A hand-filled gelatin capsule containing 10 mg prednisolone (codes K and N, Table 2.1) was used as the dosage form. Plasma concentrations from these experiments are given in Appendix A2. The prednisolone appeared in the systemic blood at 30 to 55 minutes after administration of the dose.

The plasma concentrations of prednisolone from the portal vein, carotid artery and jugular vein obtained from experiments performed on two dogs when given one capsule (K), are shown in Figure 3.17. The arterial and jugular venous concentrations were very similar at all collection times. In Dogs P 5 and P 9, portal - arterial concentration differences (P - A) occurred between 0 and 2.5 hours, and 0.5 and 1.25 hours respectively. Peak plasma concentrations of 400 to 500 ng/ml occurred at 2 hours and 1 to 1.25 hours in P 5 and P 9, respectively.

Experiments conducted in dogs whose portal vein catheters had ceased to function are shown in Figures 3.18 and 3.19. Dog P 6 was dosed with 2 x 10 mg capsules (N) and Dog P 7

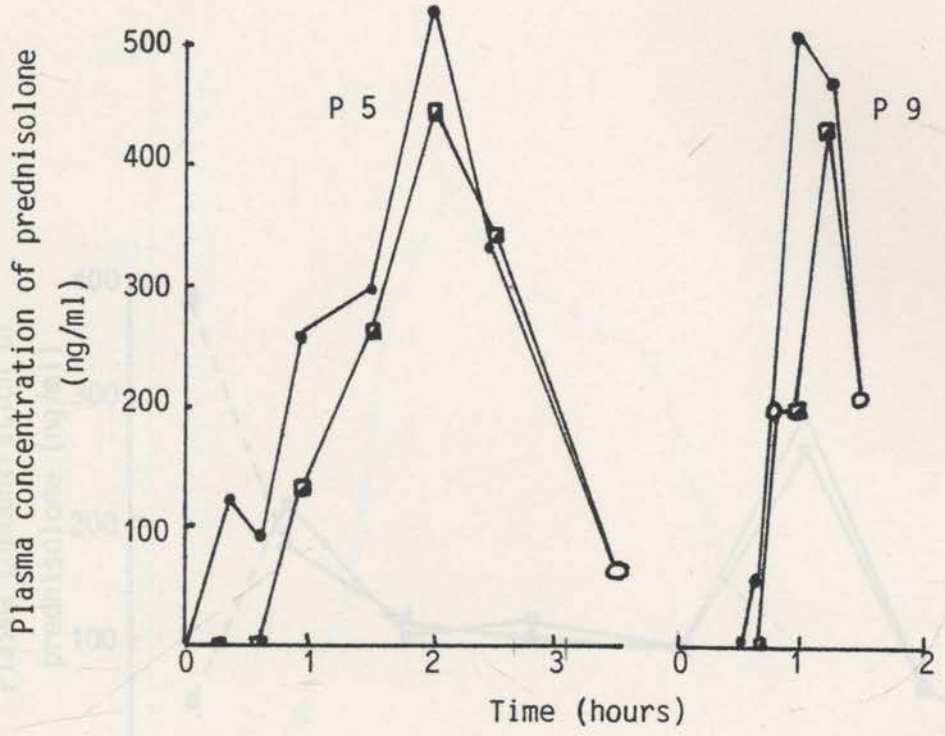


Figure 3.17. Plasma prednisolone concentrations after oral administration of a 10 mg hand-filled capsule (code K) to the preliminary dogs, P 5 and P 9. (● portal vein, ◢ carotid artery, □ jugular vein, O indicates all three values correspond)

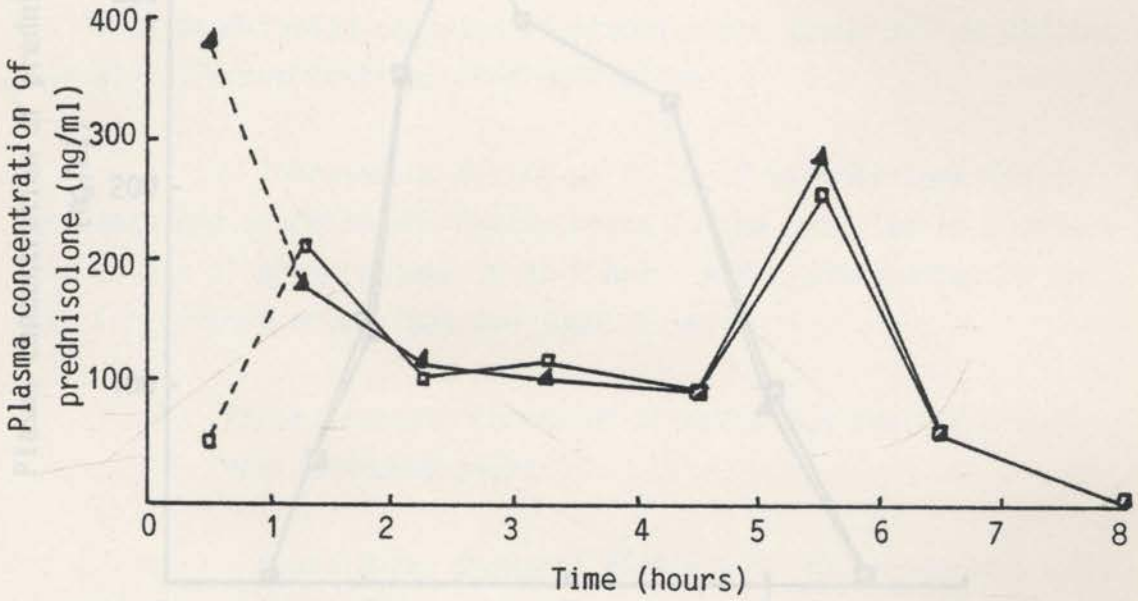


Figure 3.18. Plasma prednisolone concentrations after oral administration of 20 mg (2 x 10 mg hand-filled capsules, code N) to dog P 6. (◢ carotid artery, □ jugular vein)

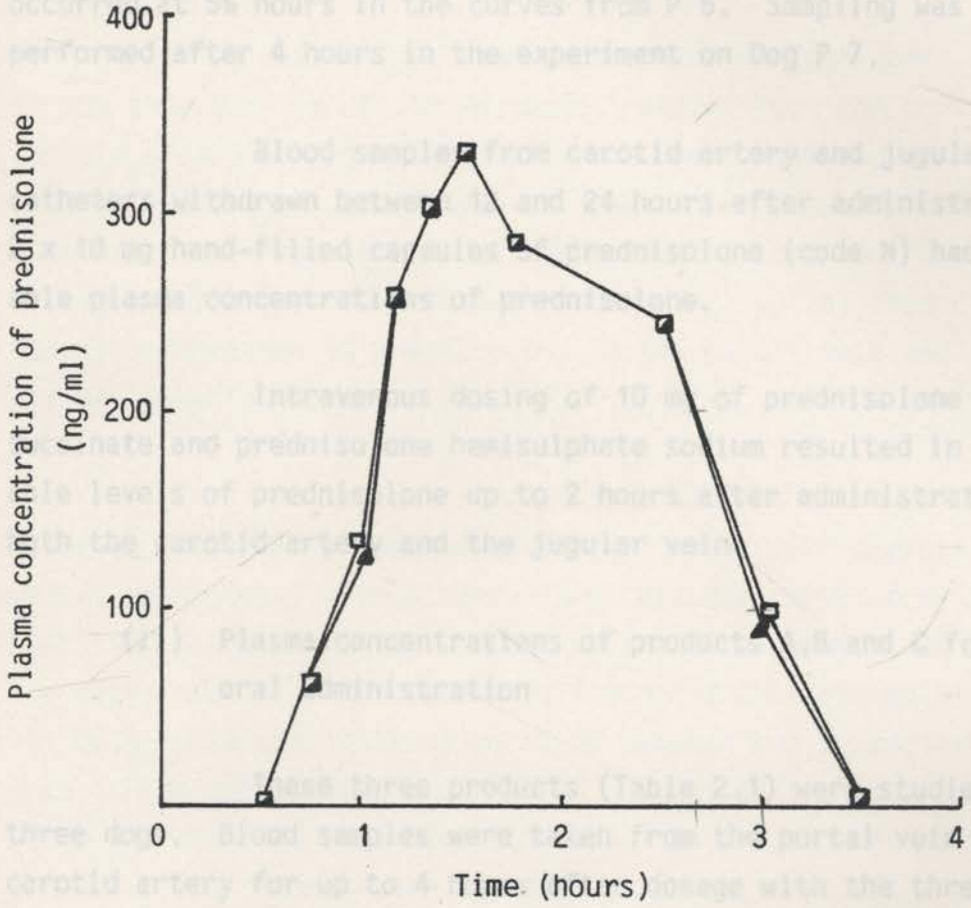


Figure 3.19. Plasma prednisolone concentrations after oral administration of a 10 mg hand-filled capsule (K) to dog P 7. (◢ carotid artery, □ jugular vein)

was dosed with one 10 mg capsule (K). The arterial and the jugular venous concentrations were again very similar. The low value for the jugular vein sample in P 6 at 0.5 hour was the only case of divergence of the arterial and jugular venous concentrations. A peak plasma concentration of approximately 300 ng/ml occurred in the range 0.5 to 1.5 hours in these two experiments. A second peak occurred at 5½ hours in the curves from P 6. Sampling was not performed after 4 hours in the experiment on Dog P 7.

Blood samples from carotid artery and jugular vein catheters withdrawn between 12 and 24 hours after administration of 2 x 10 mg hand-filled capsules of prednisolone (code N) had undetectable plasma concentrations of prednisolone.

Intravenous dosing of 10 mg of prednisolone sodium succinate and prednisolone hemisulphate sodium resulted in undetectable levels of prednisolone up to 2 hours after administration in both the carotid artery and the jugular vein.

(ii) Plasma concentrations of products A,B and C following oral administration

These three products (Table 2.1) were studied in all three dogs. Blood samples were taken from the portal vein and carotid artery for up to 4 hours after dosage with the three formulations of prednisolone. The experimental design is unbalanced (Table 2.1) because of the difficulties associated with the patency of the portal vein catheters. All three products were commercial tablets of prednisolone but product C had been claimed to be a therapeutic failure. The individual and mean portal vein and carotid artery plasma concentrations and the P - A concentration differences are found in Appendix A3.

Prednisolone appeared in the portal vein and arterial plasma simultaneously between 15 and 60 minutes after administration of the tablets in most experiments. Rises and falls in the concentrations of prednisolone in the portal vein and arterial plasma generally corresponded although the portal vein concentrations tended to be higher than the systemic concentrations for nearly all of the sampling period.

Two, and in some cases three or four peaks were seen in the time courses of plasma concentrations from the portal vein and carotid artery of Dogs II and III. In Dog I, the curves tended to have only a single peak. However, the times of sampling in Dog I were somewhat less frequent and did not extend as long and consequently multiple peaks may not have been detected. Typical time courses of the concentrations of prednisolone in the portal vein and carotid artery are shown in Figure 3.20.

Peak plasma concentrations and the times for prednisolone to appear in plasma were very variable. For example, for product A, peak portal vein plasma concentrations ranged from 283 to 833 ng/ml while peak arterial concentrations were between 126 to 621 ng/ml. The appearance of prednisolone following administration of product A was delayed in one instance until 90 minutes and in another the appearance in the portal vein occurred 30 minutes earlier than any prednisolone was detected in arterial plasma. In one experimental run with product B, no prednisolone appeared in portal vein and arterial plasma until 135 minutes after dosing and in one instance prednisolone was detected 15 minutes earlier in the portal vein than in the carotid artery although the appearance may have occurred less than 15 minutes earlier. In one case after product C, no prednisolone was detected in the portal vein and carotid artery until 90 minutes after dosing and in three experiments the appearance in the arterial plasma was delayed 30 minutes after that in the portal vein.

Individual experimental runs for products A and B show in some cases two separate P - A peaks at 0.5 to 1 hour and 2 to 3 hours while in other cases only one peak occurred. The mean

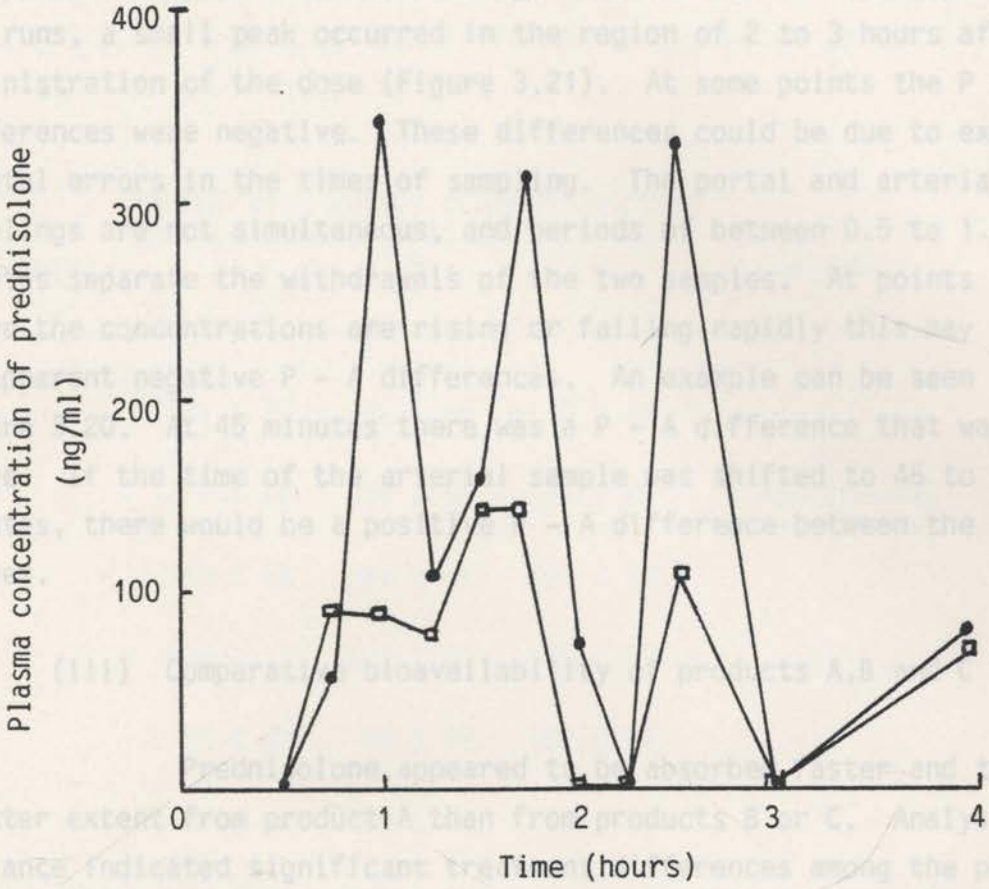


Figure 3.20. Typical time courses of the concentrations of prednisolone in the portal vein (●) and carotid artery (□) following the oral administration of a 5 mg commercial tablet to a greyhound dog (Product B in Dog II).

difference curves (Figure 3.21) reflect this pattern. The P - A concentration differences for product C showed only one major peak at approximately 60 minutes, although in about half of the experimental runs, a small peak occurred in the region of 2 to 3 hours after administration of the dose (Figure 3.21). At some points the P - A differences were negative. These differences could be due to experimental errors in the times of sampling. The portal and arterial samplings are not simultaneous, and periods of between 0.5 to 1.5 minutes separate the withdrawals of the two samples. At points where the concentrations are rising or falling rapidly this may lead to apparent negative P - A differences. An example can be seen in Figure 3.20. At 45 minutes there was a P - A difference that was negative. If the time of the arterial sample was shifted to 46 to 47 minutes, there would be a positive P - A difference between the two curves.

(iii) Comparative bioavailability of products A,B and C

Prednisolone appeared to be absorbed faster and to a greater extent from product A than from products B or C. Analysis of variance indicated significant treatment differences among the portal vein plasma concentrations at 30, 150, 180 and 240 minutes and the arterial plasma concentrations at 30, 180 and 240 minutes (Tables 3.6 and 3.7, Figure 3.21). Simple contrasts between the plasma concentrations showed significant differences between the plasma concentrations following dosage with products A and B at 30 minutes for both portal and arterial concentrations and the portal concentrations at 180 and 240 minutes.

There was a similar pattern of differences in the plasma concentrations of prednisolone after dosage with products A and C. Significant differences occurred in the portal vein plasma concentrations at 30, 150 and 180 minutes (Table 3.6, Figure 3.21), while the significant differences in the arterial plasma concentrations were at 30, 180 and 240 minutes (Table 3.7, Figure 3.21). No significant differences were found among the P - A differences at any sampling time (Table 3.8).

TABLE 3.6. Concentrations of prednisolone in portal vein plasma (mean ± S.E.M.) following administration of products A,B and C to three greyhound dogs (I to III).

Time (min)	Portal vein plasma concentration of prednisolone (ng/ml) after product A	B	C	Significant contrasts
15	71 ± 32	22 ± 40	0 ± 44	
30	231 ± 56	34 ± 59	19 ± 63	A>B (P<.03) A>C (P<.03)
45	243 ± 58	121 ± 66	163 ± 66	
60	189 ± 61	207 ± 69	155 ± 69	
75	67 ± 43	59 ± 47	100 ± 47	
90	141 ± 46	120 ± 52	114 ± 52	
105	194 ± 77	192 ± 77	78 ± 77	
120	113 ± 47	176 ± 53	99 ± 53	
135	166 ± 67	110 ± 67	79 ± 67	
150	180 ± 38	102 ± 43	52 ± 43	A>C (P<.05)
180	167 ± 44	22 ± 44	11 ± 44	A>B (P<.05) A>C (P<.04)
240	96 ± 25	16 ± 25	17 ± 25	A>B (P<.05) A>C (0.1>P>.05)

TABLE 3.7. Concentrations of prednisolone in arterial plasma (mean ± S.E.M.) following administration of products A,B and C to three greyhound dogs (I to III).

Time (min)	Arterial plasma concentration of prednisolone (ng/ml) after product A	B	C	Significant contrasts
15	51 ± 25	16 ± 32	0 ± 35	
30	154 ± 39	11 ± 44	8 ± 47	A>B (P<.03) A>C (P<.03)
45	155 ± 48	26 ± 54	38 ± 58	
60	98 ± 43	155 ± 48	76 ± 52	
75	85 ± 38	66 ± 42	68 ± 42	
90	113 ± 35	101 ± 42	141 ± 42	
105	138 ± 55	143 ± 55	76 ± 55	
120	94 ± 31	123 ± 37	81 ± 37	
135	28 ± 37	115 ± 37	82 ± 37	
150	105 ± 27	59 ± 32	52 ± 32	
180	183 ± 53	20 ± 53	0 ± 53	A>B (0.1>P>.05) A>C (P<.04)
240	134 ± 36	27 ± 36	14 ± 36	A>B (0.1>P>.05) A>C (P<.05)

TABLE 3.8. P - A concentration differences of prednisolone (mean ± S.E.M.) following administration of products A,B and C to three greyhound dogs (I to III).

Time (min)	P - A concentration differences of prednisolone (ng/ml) after product A	B	C	Significant contrasts
15	14 ± 6	7 ± 8	0 ± 8	
30	74 ± 23	15 ± 26	11 ± 26	
45	84 ± 55	91 ± 62	125 ± 62	
60	93 ± 36	57 ± 41	81 ± 41	
75	-18 ± 18	-7 ± 19	33 ± 19	
90	26 ± 34	19 ± 38	-27 ± 38	N.S. at any time
105	56 ± 34	48 ± 34	2 ± 34	
120	18 ± 27	53 ± 31	18 ± 31	
135	138 ± 69	-5 ± 69	-3 ± 69	
150	64 ± 42	43 ± 47	0 ± 47	
180	-16 ± 23	1 ± 23	11 ± 23	
240	-38 ± 34	-11 ± 34	3 ± 34	

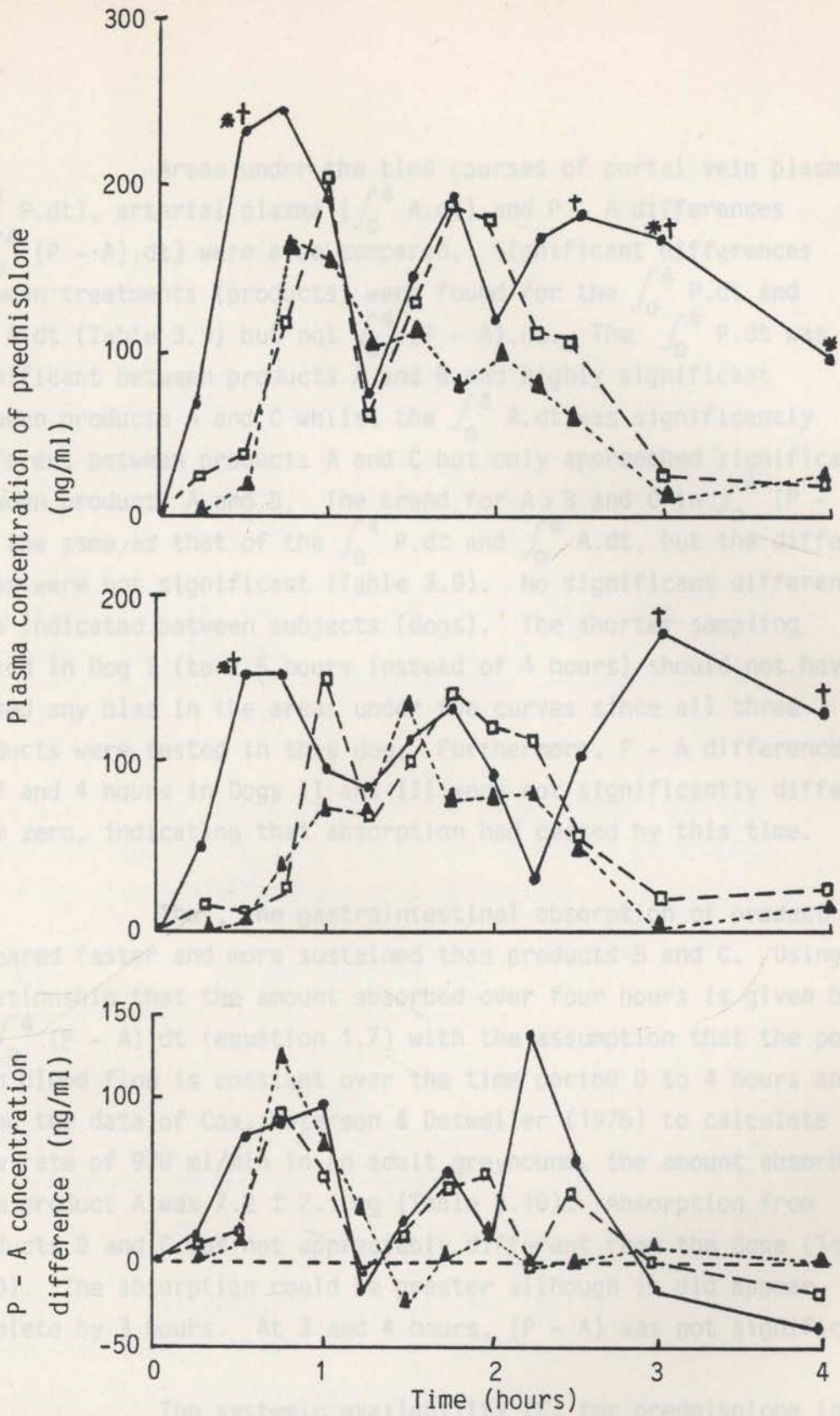


Figure 3.21. Time courses of mean plasma concentrations of prednisolone after dosage with products A (●——●), B (□— —□) and C (▲-----▲). Top, portal vein concentrations; middle, carotid artery concentrations and bottom, P - A concentration differences. ✱ indicates significant difference in the concentrations at that time between A and B. † indicates significant difference in the concentrations at that time between A and C.

Areas under the time courses of portal vein plasma ($\int_0^4$ P.dt), arterial plasma ($\int_0^4$ A.dt) and P - A differences [$\int_0^4$ (P - A).dt] were also compared. Significant differences between treatments (products) were found for the $\int_0^4$ P.dt and $\int_0^4$ A.dt (Table 3.9) but not $\int_0^4$ (P - A).dt. The $\int_0^4$ P.dt was significant between products A and B and highly significant between products A and C whilst the $\int_0^4$ A.dt was significantly different between products A and C but only approached significance between products A and B. The trend for A > B and C in $\int_0^4$ (P - A).dt was the same as that of the $\int_0^4$ P.dt and $\int_0^4$ A.dt, but the differences were not significant (Table 3.9). No significant differences were indicated between subjects (dogs). The shorter sampling period in Dog I (to 2.5 hours instead of 4 hours) should not have caused any bias in the areas under the curves since all three products were tested in this dog. Furthermore, P - A differences at 3 and 4 hours in Dogs II and III were not significantly different from zero, indicating that absorption had ceased by this time.

Thus, the gastrointestinal absorption of product A appeared faster and more sustained than products B and C. Using the relationship that the amount absorbed over four hours is given by $\dot{Q}. \int_0^4$ (P - A) dt (equation 1.7) with the assumption that the portal vein blood flow is constant over the time period 0 to 4 hours and using the data of Cox, Peterson & Detweiler (1976) to calculate a flow rate of 970 ml/min in an adult greyhound, the amount absorbed from product A was 7.2 ± 2.1 mg (Table 3.10). Absorption from products B and C was not appreciably different from the dose (Table 3.10). The absorption could be greater although it did appear complete by 3 hours. At 3 and 4 hours, (P - A) was not significant.

The systemic availability (F) for prednisolone in the greyhound was estimated from the equation

$$F = \frac{Q}{Q + \frac{D}{\int_0^\infty A.dt}}$$

(Perrier, Gibaldi & Boyes, 1973c)

TABLE 3.9. Areas under the time courses (AUC) of plasma concentrations in the portal vein and carotid artery and the P - A concentration differences of products A,B and C (± S.E.M.).

Product	$AUC_{portal\ vein}$ (ng/ml).min	$AUC_{arterial}$ (ng/ml).min	AUC_{P-A} (ng/ml).min
A	31071 ± 3540	22453 ± 2780	7417 ± 2197
B	19018 ± 4014	14082 ± 3322	4936 ± 2491
C	14731 ± 4014	10118 ± 3322	4613 ± 2491
Significant contrasts	A > B (P<.05) A > C (P<.01)	A > B (0.1>P>.05) A > C (P<.02)	N.S.

TABLE 3.10. The amount of prednisolone absorbed from the gastrointestinal tract into the portal system of greyhounds following oral administration of 5 mg tablets.

Product	Amount absorbed (mg)
A	7.2 ± 2.1
B	4.8 ± 2.4
C	4.5 ± 2.4

TABLE 3.11. Estimates of systemic availability (F) and plasma clearance of prednisolone in greyhounds calculated from the data of products A,B and C.

Data from product	F	clearance (ml/min)
A	0.75	241
B	0.74	252
C	0.69	307

where Q is the hepatic blood flow rate, D is the oral dose and $\int_0^\infty A.dt$ is the area under the time course of arterial plasma concentrations from zero to infinity and the drug is completely absorbed and is eliminated solely by hepatic metabolism. The actual amount absorbed into the portal system (Table 3.10) was used for D, portal blood flow rate was used for Q and $\int_0^4 A.dt$ was used (in most cases plasma concentrations at 4 hours were approaching zero and therefore the $\int_4^\infty A.dt$ should be small compared with $\int_0^4 A.dt$). Since the renal elimination of unchanged prednisolone was very low (Table 1.3), the assumption of complete hepatic metabolism held. Values of 0.69 to 0.75 were calculated for F from the data of the three products (Table 3.11) and estimates of the plasma clearance of prednisolone of 241 to 307 ml/min (Table 3.11) were obtained from the relationship

$$cl = \frac{F.D}{\int_0^4 A.dt}$$

Since the clearance of prednisolone was by hepatic elimination and was approximately ¼ to ⅓ of the portal blood flow (Table 3.11), the first-pass effect of prednisolone in the greyhound dog was of the order of 25 to 32 per cent.

(iv) Plasma concentrations of other formulations

Results obtained with formulations D to N were incomplete due to the difficulties associated with the patency of the portal vein catheter and consequently detailed statistical analysis was not carried out. The individual portal vein and carotid artery values together with the means and the P – A differences are given in Appendix A3. In general the time courses of the portal vein and arterial plasma concentrations and P - A concentration differences of the oral preparations showed multiple peaks similar to that from products A,B and C.

(a) Commercial prednisolone dosage forms (D and F)

Product D was studied five times in only one dog. Appearance of prednisolone in the plasma was between 30 and 60 minutes after dosage with the tablets although in one experiment it was delayed until 90 minutes. The appearance in the portal vein tended to be 15 minutes earlier than in the arterial blood. The plasma concentrations found from individual experiments were quite variable. They showed from one to three separate peaks at varying times, however the peak portal and arterial concentrations corresponded. Concentrations of the various peaks ranged from 76 to 579 ng/ml for the portal vein and 65 to 218 ng/ml for the arterial plasma. The time courses of P - A values showed several peaks also. The times at which these peaks were observed were not consistent. The amount absorbed was estimated from the relationship $\int_0^4 (P - A)\, dt \cdot Q$ (Table 3.12) to be 13.5 mg which was more than twice the dose administered.

The plasma concentrations of prednisolone after dosage with the capsules (product F) also differed markedly between experiments. Peak concentrations in both portal and arterial plasma occurred at approximately 15 and 105 minutes.

(b) Prednisone tablets (product E)

The prednisone tablets were studied in only one dog. The plasma concentrations of prednisolone following the administration of prednisone were of a similar range to those following administration of prednisolone tablets. However only a single peak was seen in each experiment. Prednisolone appeared in the plasma 30 minutes after the prednisone administration although in one experiment there was a delay until 90 minutes before the appearance of prednisolone. A single peak was also seen in the time courses of the P - A concentration differences.

(c) Extemporaneously prepared capsules (products G,J,K and N)

The plasma concentrations after dosage with the 5 mg hand-filled capsules (G) were in the same range as those of product A (Figure 3.22). The time courses of the plasma concentrations also had similar profiles with multiple peaks. The P - A concentration differences between 2 and 3 hours were much greater than those found from products A,B and C (Figure 3.23) and consequently $\int_0^4$ (P - A) dt was large, resulting in an estimate of absorption of 21 mg from the 5 mg dosage form (Table 3.12). The P - A differences from 1.25 to 2 hours were approximately zero giving rise to two distinct sections of the time course. It was of note that Q. $\int_0^{1¼}$ (P - A) dt was approximately equal to the administered dose.

The portal vein and arterial plasma concentrations from the 10 mg capsule (K) resembled those after dosage with capsule G although P - A values were significant only between 1.5 to 3 hours. This suggested delayed absorption from this capsule. Administration of 2 x 10 mg capsules (N = 2 x K) resulted in similar plasma concentrations to those from only one capsule.

Considerably higher portal vein plasma concentrations were seen following administration of 50 mg hand-filled capsules (J) (Figure 3.24), although the arterial concentrations did not appear to be markedly greater than those from 5 mg dosage forms. In the two experiments in Dog III, the maximal P - A concentration differences occurred at 15 minutes which indicated that absorption of prednisolone was very rapid. The area under the time course of the P - A differences [$\int_0^4$ (P - A) dt], was similar to that from 5 mg capsules (Table 3.12) and only 18.3 mg was estimated to have been absorbed from the 50 mg dose although it is possible that more frequent sampling both earlier than and later than 15 minutes may have indicated greater P - A differences and therefore given a higher estimate of the absorption.

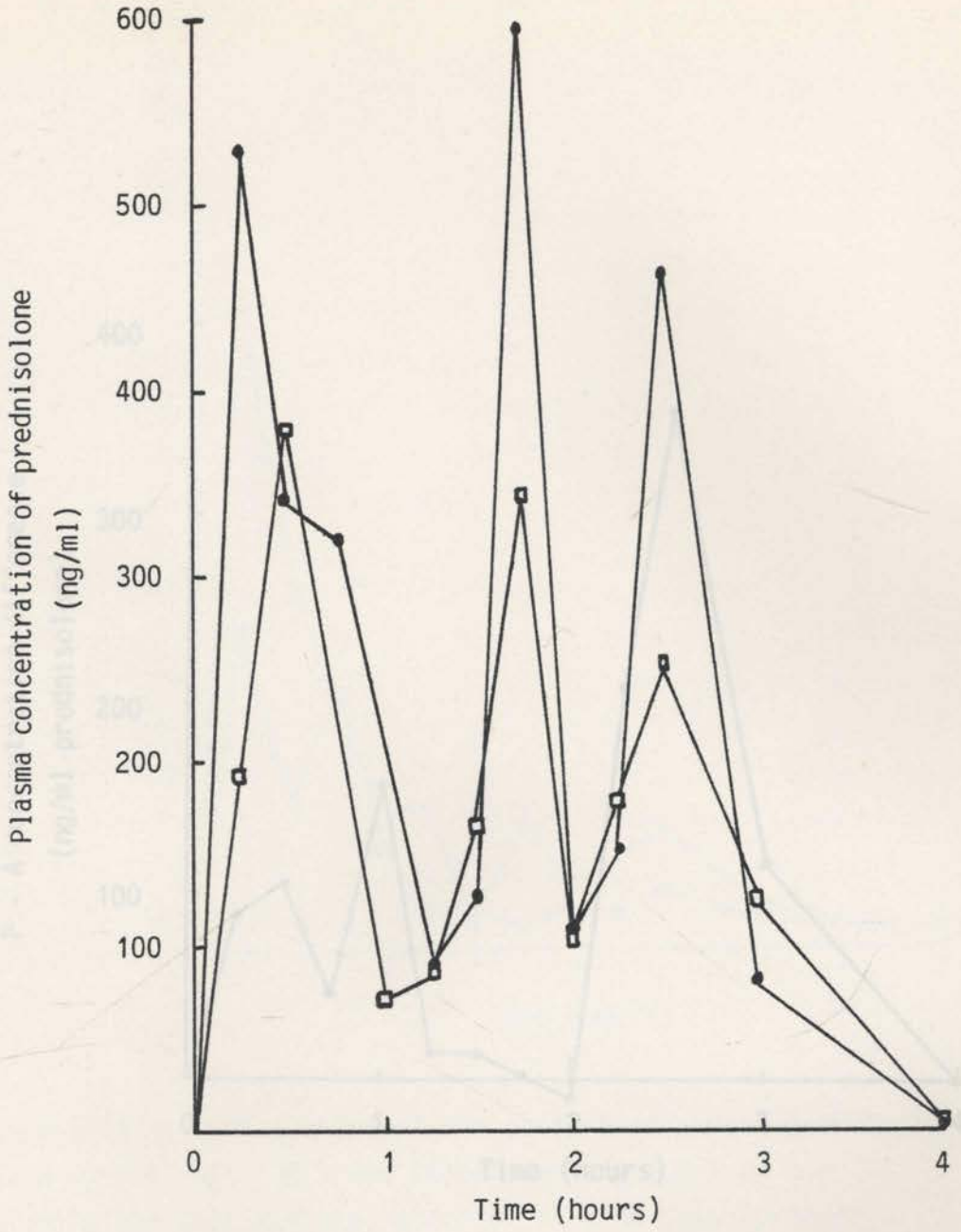


Figure 3.22. Time courses of the concentrations of prednisolone in the portal vein (●) and carotid artery (□) following the oral administration of 5 mg to Dog II (Product G, experiment 2).

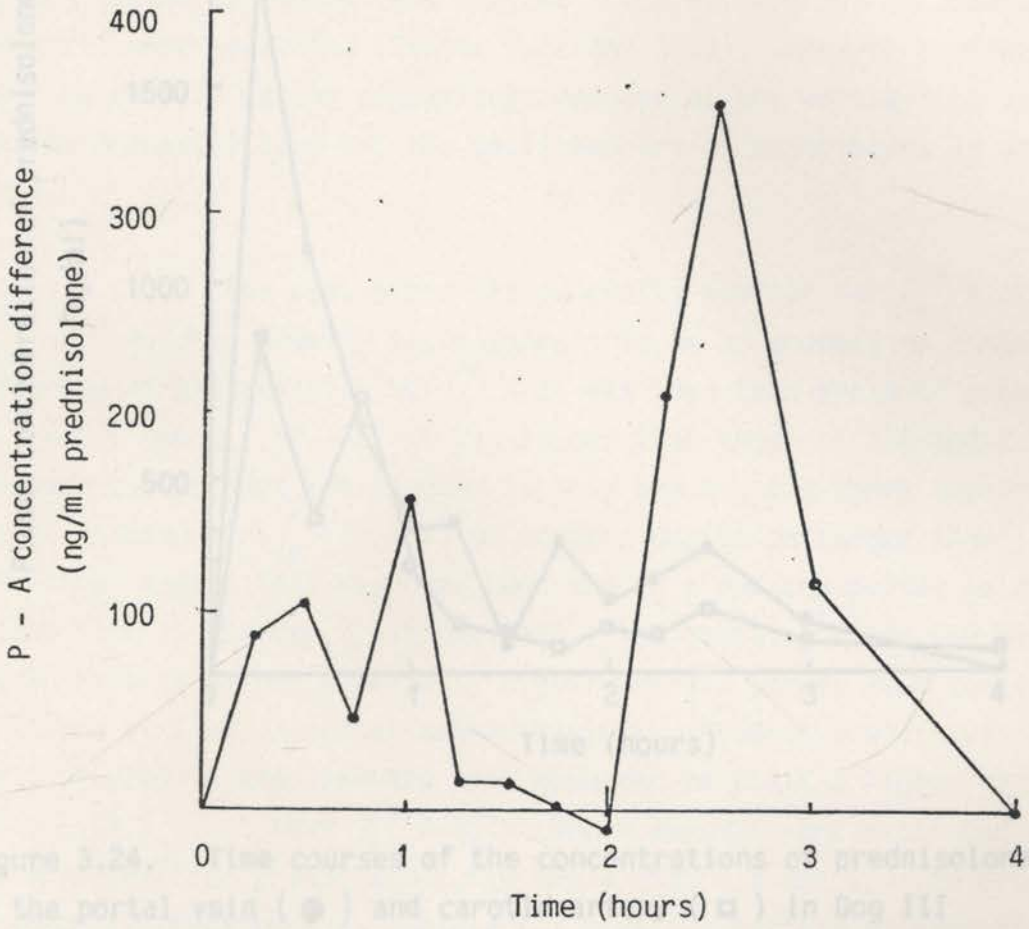


Figure 3.23. Mean time course of the portal - arterial (P - A) concentration differences of product G in Dogs II and III.

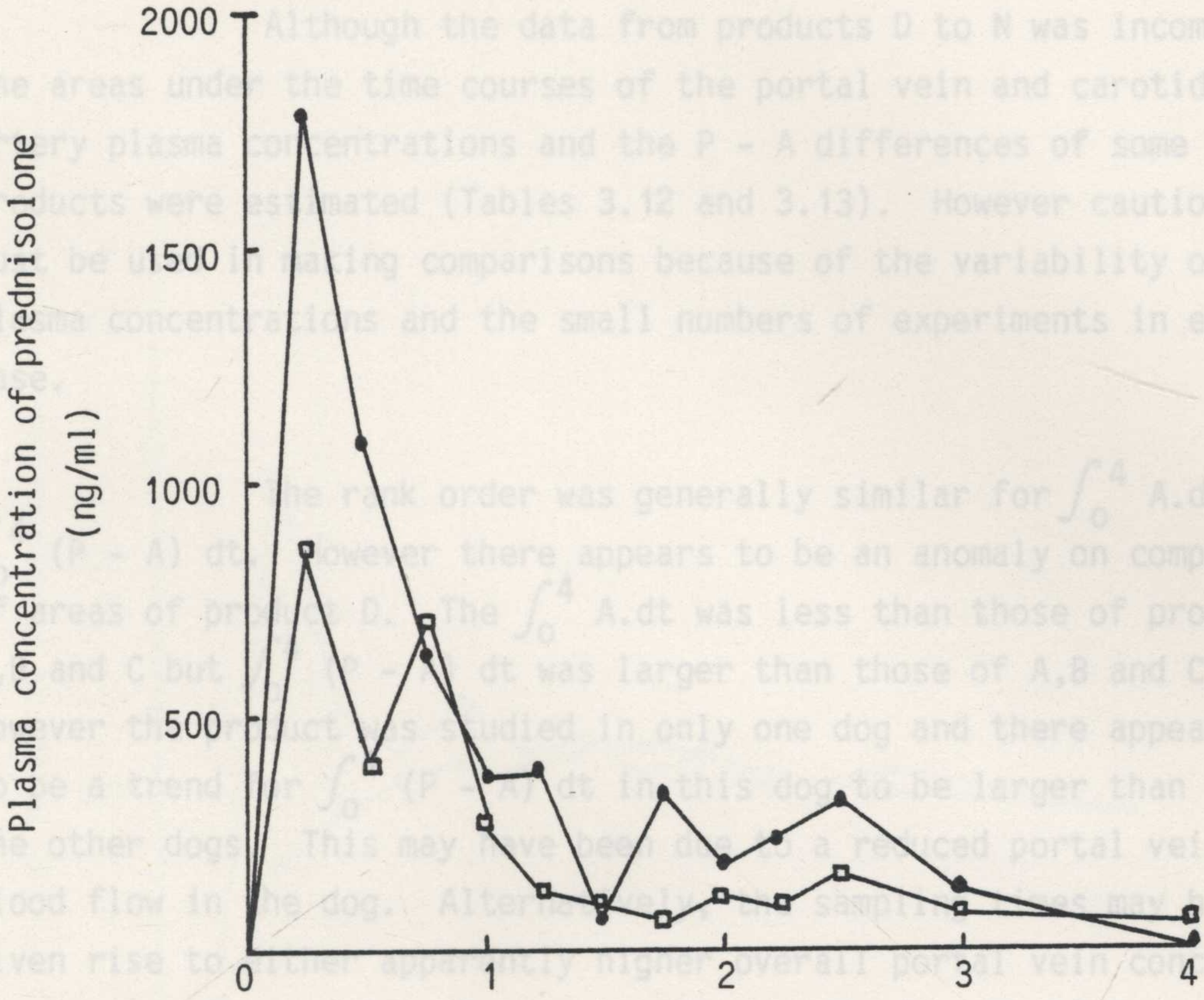


Figure 3.24. Time courses of the concentrations of prednisolone in the portal vein (●) and carotid artery (□) in Dog III following the oral administration of a 50 mg dose (product J, experiment 2).

(d) Comparison of areas under the time courses of products D,E,F,G and J and with A,B and C

Although the data from products D to N was incomplete, the areas under the time courses of the portal vein and carotid artery plasma concentrations and the P - A differences of some of the products were estimated (Tables 3.12 and 3.13). However caution must be used in making comparisons because of the variability of the plasma concentrations and the small numbers of experiments in each case.

The rank order was generally similar for $\int_0^4$ A.dt and $\int_0^4$ (P - A) dt. However there appears to be an anomaly on comparison of areas of product D. The $\int_0^4$ A.dt was less than those of products A,B and C but $\int_0^4$ (P - A) dt was larger than those of A,B and C. However the product was studied in only one dog and there appeared to be a trend for $\int_0^4$ (P - A) dt in this dog to be larger than in the other dogs. This may have been due to a reduced portal vein blood flow in the dog. Alternatively, the sampling times may have given rise to either apparently higher overall portal vein concentrations or lower arterial concentrations. Product E which was also only studied in the same dog also appeared to yield a higher ranking of $\int_0^4$ (P - A) dt than $\int_0^4$ A.dt. Larger amounts were estimated to be absorbed from products D,E,F,G and J than A,B and C. The extemporaneously prepared 5 mg capsule (G) gave the highest absorption (21 mg); however the 50 mg capsule (J) only gave 18.3 mg even though $\int_0^4$ P.dt was larger than those from the 5 mg formulations.

(e) ^{3}H labelled 5 mg capsules (H)

The pattern of the plasma radioactivity concentrations after ^{3}H-prednisolone differed from the prednisolone concentrations found following non-radioactive prednisolone. They appeared more sustained and had an absence of multiple peaking (Figure 3.25) although there were small fluctuations in the concentrations of radioactivity. No radioactivity was present in samples from arterial plasma 24 hours after dosing. P - A concentration differences occurred between 0 and

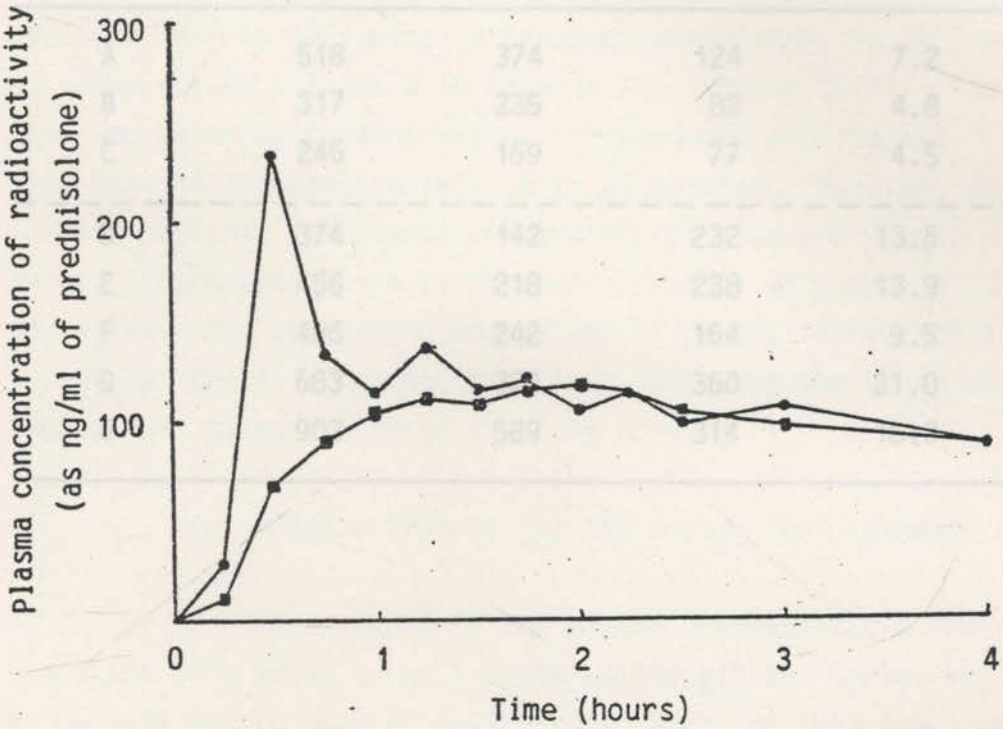


Figure 3.25. Mean portal vein (●) and carotid artery (■) plasma radioactivity concentrations after oral administration of ^{3}H labelled prednisolone (product H) to Dog III (n=2). The plasma concentrations were calculated assuming that total radioactivity represented prednisolone only.

TABLE 3.12. Areas under the time courses from 0 to 4 hours (AUC) of the plasma concentrations of the portal vein, carotid artery and the P - A differences.

Preparation	AUC (ng-hour/ml) (portal)	(arterial)	(P - A)	Estimated amount absorbed (mg)
A	518	374	124	7.2
B	317	235	82	4.8
C	246	169	77	4.5
D	374	142	232	13.5
E	456	218	238	13.9
F	406	242	164	9.5
G	683	323	360	21.0
J	903	589	314	18.3

TABLE 3.13. Rank order of the AUC values listed in Table 3.13.

P	A	(P - A)
J	J	G
G	A	J
A	G	E
E	F	D
F	E	F
D	B	A
B	C	B
C	D	C

60 minutes but were small after 60 minutes which indicated that absorption had occurred within that time. However, the radioactivity assay was non-specific and therefore metabolites may have been included in the determinations.

(f) Intravenous administration (I and L)

The portal vein and arterial plasma concentrations declined rapidly following intravenous administration of 5 mg doses of prednisolone (I) and a 10 mg dose (L) (Figure 3.26). The portal vein and arterial concentrations corresponded and reached near zero after approximately 15 to 30 minutes. However, after the initial decline, the plasma concentrations rose and showed one or two peaks. There were P - A differences for most of these latter plasma concentrations, suggesting absorption of prednisolone from the gastrointestinal tract. The concentrations following the 10 mg dose were higher than those following the 5 mg doses.

(g) Administration *via* the portal vein catheter (M)

The pattern of the plasma concentrations following injection of a 10 mg dose of prednisolone *via* the portal vein catheter was similar to that following administration into the jugular vein, although very little of the initial rapid decline was detected.

(h) Effect of food

In two experiments with hand-filled capsules (J and K), the dose was administered one hour after a substantial meal. In one experiment there appeared to be no effect but in the other the plasma concentrations were very low; in this case however the sampling times were less frequent than when the products were administered to fasting animals. In contrast to other experiments, the portal vein concentrations in the two with food were markedly lower than the arterial concentrations over significant time periods (Figure 3.27).

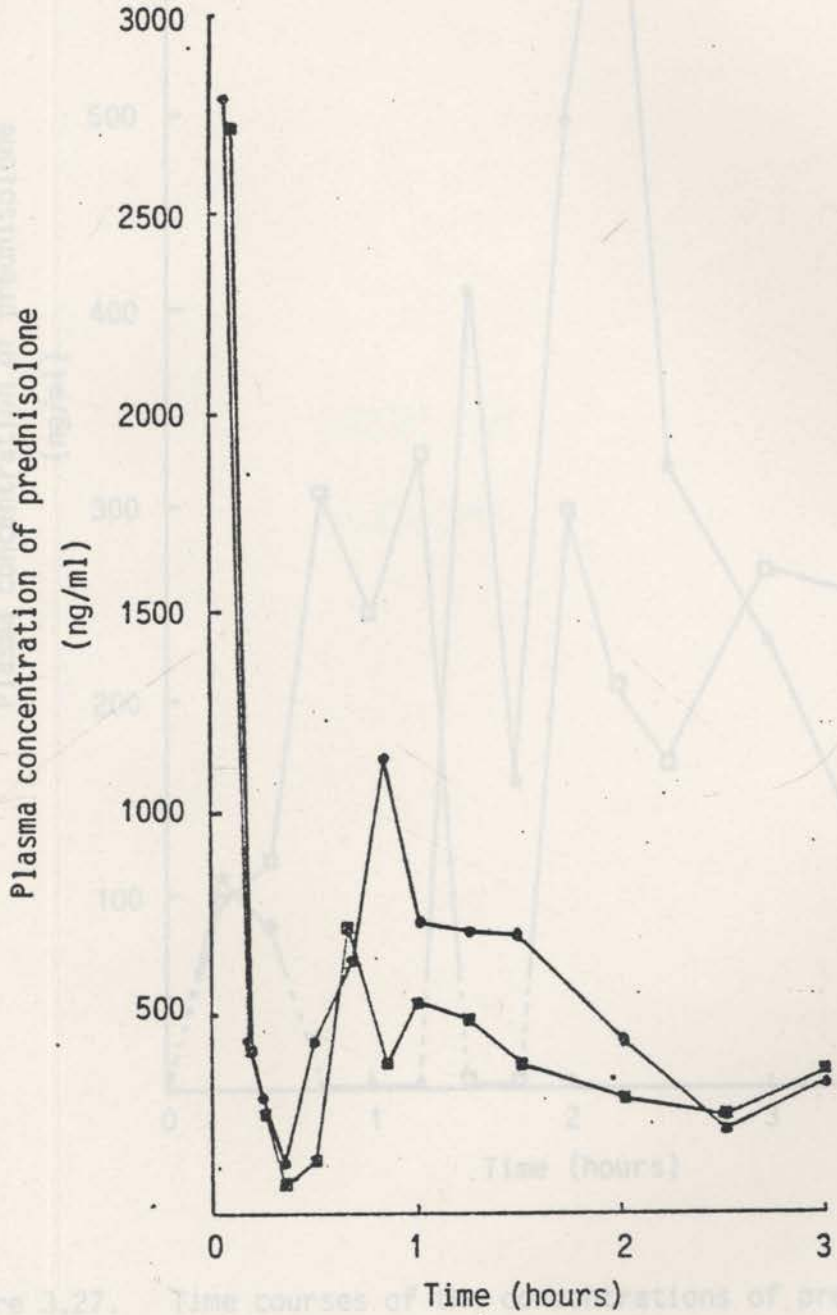


Figure 3.26. Portal vein (●) and carotid artery (■) plasma prednisolone concentrations after an intravenous bolus injection of 10 mg via the jugular vein catheter to Dog III (L).

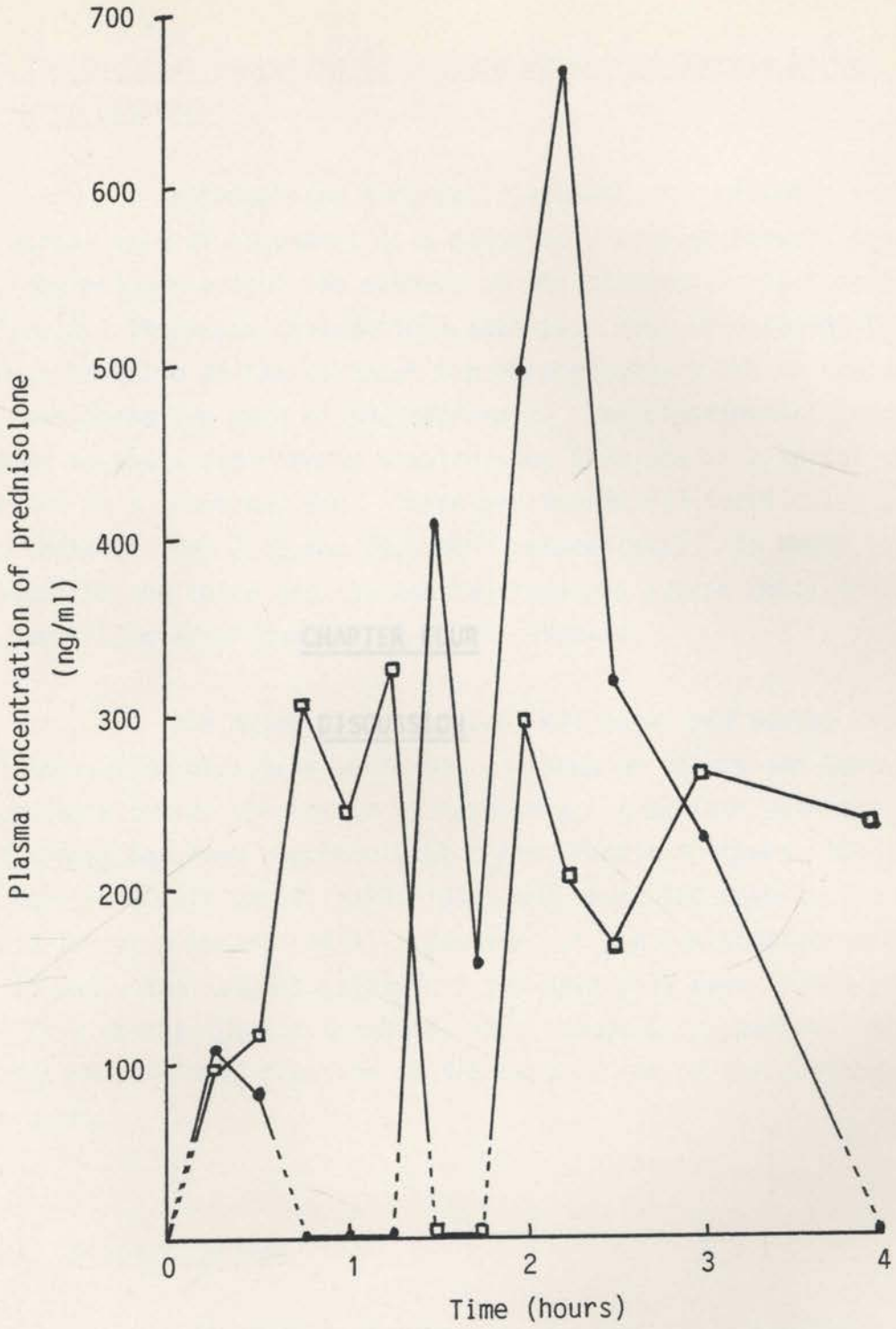


Figure 3.27. Time courses of the concentrations of prednisolone in the portal vein (●) and carotid artery (□) in Dog II from oral administration of product K one hour after a meal.

CHAPTER FOUR

DISCUSSION

4.1 The Surgical Technique to prepare Dogs with Chronic Portal Vein Catheters

Although the surgical placement of a catheter into the portal vein of an animal is a relatively straightforward procedure, the maintenance of the patency of the catheter is much more difficult. Therefore considerable attention must be directed to the correct location of the catheter tip during surgery and to the design and post-operative care of the catheters. The experimental procedure adopted in these experiments enabled long term use of a portal vein catheter in a conscious dog. Three greyhounds had functioning portal vein catheters for 2, 3 and 5½ months respectively. In fact, the catheter in the third dog (5½ months) remained patent until the dog was sacrificed after completion of the studies.

The majority of studies that have used portal vein catheterisation have been performed in sheep or calves and have been concerned with the absorption of nutrients. A maximum catheter life of 161 days has been reported with sheep (Moodie & others, 1963), 148 days in a calf (McGilliard, 1971) and up to 180 days in the rat (Gallo-Torres & Ludorf, 1974). However, in the few studies carried out in dogs, the longest catheter lives have only been in the range of 1 to 2 months (Denton & others, 1953; Hauge & Krippaehne, 1970) which contrasts with the life of 175 days in one of the greyhounds of this study.

4.1.1 Choice of animal

The experimental animal required (1) a similar gastrointestinal physiology to man, (2) an ability to be dosed with oral dosage forms such as tablets and capsules and (3) to be amenable to handling. The rabbit was rejected because its gastrointestinal physiology is significantly different from that of man (Chiou, Riegelman & Amberg, 1969). The rat and the guinea-pig were considered unsuitable because of their small size and inconvenience in handling was the reason for rejection of the cat, monkey, pig and

the "small" or slow growing pig. The sheep, in addition to difficulty in handling, has different gastrointestinal physiology to man.

The dog best fulfilled the 3 requirements. Dog and man are very similar in respect to metabolism and pharmacokinetics (Chodera & Feller, 1978). However a disadvantage in using the dog for studies of gastrointestinal absorption is that compounds and/or metabolites in the range of 350 to 550 daltons may undergo enterohepatic cycling in the dog although it would not occur in man (page 105).

The greyhound was successfully used in these experiments and it has been used in other pharmacokinetic studies as a model species for man (Bevan, Caldwell, D'Souza & Smith, 1978). There are no known major metabolic or enzymic differences between greyhounds and other breeds of dogs. The metabolism of oestradiol is identical in greyhounds and German shepherds (Beling, Gustafsson & Kasstrom, 1975). However some differences, connected with their athletic activity, occur: they have slightly higher levels of enzyme activity within the muscles and slightly higher red blood cell, haematocrit and haemoglobin levels (Porter & Canaday, 1971), the proportion of muscle is significantly greater and they have no body fat (Gunn, 1978). Greyhounds also are moderately hypertensive compared with mongrels (Fischer, Cox & Detweiler, 1975; Cox, Peterson & Detweiler, 1976) and exhibit some other cardiovascular differences which are related to and favour muscular activity and are a physiological adaptation of the breed (Detweiler & co-workers, 1974; Steel, Taylor, Davis, Stewart & Salmon, 1976). However no differences exist in splanchnic blood flow between mongrels and greyhounds (Fischer & others, 1975) and there are no deleterious effects on the greyhound arising from its higher blood pressure (Cox & others, 1976). No differences in metabolism of drugs have been observed in greyhounds, with the exception of barbiturates which is attributed to the lack of body fat in the greyhound (Bellenger, 1976; Gunn, 1978). The patterns of absorption of ampicillin and amoxycillin in the greyhound and man are identical (Watson & Egerton, 1977).

It has been questioned whether beagles, greyhounds or any other breed of dog is representative of the species in drug trials (Watson & Egerton, 1977). However it appears that the greyhound is a satisfactory breed of dog for use in model gastrointestinal absorption studies for man from both the practical aspects found in this work and physiological evidence.

4.1.2 Surgical approach

From the published work on chronic catheterisation of the portal vein, seven different routes of approach can be delineated (Table 4.1). The first route considered was *via* the umbilical vein. In humans it had long been assumed that the umbilical vein thrombosed after birth and became a white, solid cordlike structure of fibrous tissue called the *ligamentum teres*. However Bayly & Gonzales Carbalhaez (1964) reported that the vein could be reopened down to its junction with the left branch of the portal vein. The possibility of using this route in the dog was explored as it could be performed with only a local anaesthetic and would not involve major abdominal surgery and be relatively free from complications; less than one hour is required for the surgery.

Taher & Ibrahim (1969) claimed that the lumen of the umbilical vessels in puppies disappeared in the cranial section 20 days after birth. Similar claims had been made earlier about the umbilical vein in the human (Arey, 1956). Therefore, it was possible that since the patency of the umbilical vein in the human was unrealised until recently, the situation in the dog may have been similar.

Attempts have been made in other species to utilise the umbilical vein for portal vein catheterisation. McGilliard (1968) found it possible in 8 to 10 days old calves but impossible after they were 25 days old. It is probable that the umbilical vein in a calf degenerates in a similar manner to that found in the dog. Five-day old pigs have been catheterised successfully (Shimada & Zimmerman, 1973) although after 1 to 2 weeks the catheters were then of insuff-

TABLE 4.1. Summary of the routes of approach to the portal vein used by various workers.

Route	Animal used
via umbilical vein	Human, 8 to 10 day old calf, monkey, 5 day old pig
"London" cannula	Dog, sheep
Direct	Dog, cattle, sheep
T-tube	Dog, rat
via mesenteric vein	Calf, sheep, pig, monkey
via splenic vein	Dog
percutaneous transhepatic	Human, dog

Detailed references are given in the section on the "portal vein" (page 57).

icient length because of growth. Attempts using older pigs have not been made. It has been found possible, although difficult, to re-establish the patency of the umbilical vein in the monkey (Faulkner & others, 1976). It therefore appears that the vein remains intact in primates but probably not in other mammals.

Other methods or routes suffer from disadvantages compared to the splenic vein. The "London" cannula has been used with considerable success in sheep (Schambye, 1951). However it has not appeared to be successful in the dog because the increased general activity of the dog compared with the sheep has probably led to failures caused by the cannula slipping away from the vein wall. It also has the disadvantage of requiring two separate operations and it has not been used recently. The McGilliard hydraulic needle which is similar also appears to require a less active animal for success and the hydraulic apparatus itself is complex.

Direct insertion of a catheter and the T-tube method are more difficult in the dog than in other species due to the "narrow and deep" shape of the body trunk which restricts access and working space around the vein. In addition, adequate anchorage of the catheter is difficult and success is again favoured in less active animals such as sheep and cattle. Denton & others (1953) had only limited success with direct insertion in dogs.

The route <u>via</u> a mesenteric vein has the advantages of easy access to a suitable vein for insertion and good anchorage because of the mobility of the mesentery. A disadvantage is that the position of the catheter tip in the portal vein is known only indirectly and it may vary during the course of experiments because of intestinal mobility and could in the extreme regress to the superior mesenteric vein. Thus complete intestinal drainage will not be sampled because blood draining <u>via</u> the gastroduodenal vein will be missed.

The advantages of the splenic vein route are the good access to a suitable vein for catheter entry, elimination of anchor-

age problems, more uniform position of catheter entry than use of a mesenteric vein gives, avoidance of surgical trauma to the intestines compared with the mesenteric vein route and elimination of possible disturbance of mesenteric venous drainage. The spleen and the network of veins from it do not have the mobility of the intestines and the distance from the spleen to the portal vein is not variable as is the distance from a specific mesenteric vein.

The procedure adopted by Shoemaker & others (1959) was to insert the catheter into the main splenic vein, ligate the vein and remove the spleen. However splenectomy removes the reticuloendothelial system for up to 10 days until the functions are taken over by other organs in the body and an anaemia-causing rickettsial infection has been reported in splenectomised research dogs (Pryor & Bradbury, 1975). Harris & Riegelman (1969) used a branch of the splenic vein. However the use of a very small branch of the splenic vein close to the hilus of the spleen, as used in the present studies, minimised any effect of lack of venous drainage from the spleen. There is an abundance of small branches draining the spleen, which also has a richly supplied interconnected pulp and therefore the loss of drainage is negligible. No abnormalities of the spleen were found at autopsy in the chronic dogs. In contrast, dogs used for acute (anaesthetised) experiments where a major splenic branch was used for insertion of the catheter, had shrivelling of the area drained by the branch after approximately 30 minutes and enlargement of the remaining areas.

4.1.3 Post-operative recovery

To ensure complete recovery, a period of two weeks was allowed before beginning experiments. This was also approximately one week after the last administration of prophylactic antibiotics and potential interactions with these and other drugs used during the surgery were avoided.

A period of 4 to 5 days was left between individual experimental runs to ensure that elimination of prednisolone was

complete and the metabolic activity and blood volume of the dog had returned to normal. Prednisolone was not present in blood samples taken 24 hours after administration and together with the short half-life in the dog provides evidence that this time interval between experiments was adequate. Also suppression of plasma concentrations of hydrocortisone ceases 24 hours after administration of an equivalent single prednisolone dose in man (English & others, 1975).

4.1.4 Catheters

A combination of three factors resulted in the eventual success of keeping portal vein catheters patent. They were the flexibility of "Silastic" tubing, the V-notch tip and careful positioning of the tip.

The V-notch catheter tip was found to be more successful than the 45 degree bevel tip and although thrombus formation still occurred at the tips, the V-notch avoided a one-way valve action. Other investigators have had difficulties with one-way valve-like action of portal vein catheters when they attempted long-term use due to the formation of fibrin flaps around the catheter tips (Webster, Osuji, White & Ingram, 1975).

An alternative catheter tip that has been used by other workers (eg Faulkner & others, 1976) has holes (openings) cut into the side near the tip. However it is inferior to the V-notch and has most often resulted in shortening the catheter life. The catheters were found to be completely covered with thrombus from the tip to the most distal opening. The probable reason for this is that blood penetrates the holes which then become stagnant pools. Stasis of blood causes thrombus formation and the holes become blocked very quickly. The importance of geometric shape on the rate of clotting on foreign surfaces has been demonstrated by Fry, Eggleton, Kelly & Fry (1965).

4.1.5 Carotid artery and jugular vein catheters

The trouble-free nature of the carotid artery and jugular vein catheters was probably due to the environment of the catheter tips. In the aorta, intimal damage probably occurs, but the increased flow and the pulse probably result in the thrombi breaking free and not being able to embed a catheter tip in fibrous tissue. Angus (1973) found areas of necrosis in the kidneys of a dog with a carotid artery catheter that were caused by thrombi formed on the catheter tip that were readily broken off and swept away. It was possible that the thrombi formed on the walls as well, as a result of intimal damage. It was possible that the stroke that occurred in one dog was caused by a large thrombus breaking away from the carotid artery catheter. The jugular vein catheter tip environment at the junction of the superior and inferior vena cavas was very turbulent, which would also cause thrombi to break free.

4.1.6 Termination of the useful life of portal vein catheters

(i) Thrombus formation

Most other workers have experienced portal vein catheters acting as effective one way valves and fibrin formation on the tips (Webster, 1974). Although "Silastic" is claimed to be non-thrombogenic, it is probable that it still possesses some degree of thrombogenicity and together with intimal damage gives rise to thrombus formation. Angus (1973) found that thrombi formed very rapidly on the tips of "Silastic" catheters after insertion into blood vessels. This agrees with the finding that thrombus formation in the portal vein occurred within a short time after catheterisation with "Silastic".

The low thrombogenicity of "Silastic" and the presence of a flow of blood past the catheter in the portal vein probably does not cause direct thrombus build-up on the tip. Such thrombi would most likely be broken off as the free catheter tip came into contact

with the vessel wall. This would result in the thrombus travelling along the portal vein and into the branches in the liver until it became wedged. Necrosis of liver tissue may occur, however the liver has an alternate arterial blood supply together with venous anastomoses and necrosis therefore may not occur. Absence of areas of liver necrosis in the greyhounds supports the latter conclusion.

It is probable that the thrombus first occurred as a result of intimal damage to the vessel wall (the thrombi found were on the wall) caused by contact with the catheter. Intimal damage is a trigger for thrombus formation which results in the gradual build-up of the fibrous tissue papilla from the vein wall. The catheter tip eventually becomes partly entrapped in this fibrous tissue, leaving a sufficient area of the tip available for sample withdrawal. With time the thrombus covers the whole tip plugging it. However when fluid is forced down the catheter the newest area of the thrombus covering is probably not firmly attached and can be forced out as if it were a flap. This would correspond to the "half-blocked" situation described (page 146). This situation resembles a valve-like action. With further time the flap becomes firmly attached resulting in complete blockage.

Catheter material more flexible and softer than "Silastic" would probably be impracticable and would probably still cause intimal damage, although the rate of thrombus growth may be slower. Under the present conditions therefore it appears that thrombus formation is inevitable with a catheter in the portal vein. An alternative could be the use of a graphite-benzalkonium-heparin coating on the tubing exterior. These coatings have been claimed to be highly effective in preventing thrombus formation (Gott & co-workers, 1964) but their stability to physical contact, such as with a vessel wall, is doubtful (Indeglia & co-workers, 1966).

It is also probable that siting the tip where the portal vein is widest (the bifurcation) results in a decrease in intimal damage because it has a larger area of freedom and therefore minimum contact with a specific section of vessel wall.

Fibrous tissue growth explains why attempts at unblocking portal vein catheters were failures. The strongly attached catheter in Dog I would be resistant to any attempt to push from inside the catheter. An inner catheter would only be of use if it was pushed out of the larger catheter before complete covering of the tip and left in position past the thrombus. This could lead to thrombus formation occurring all over again but it may result in longer use of a single animal. However a disadvantage could be that the original thrombus would continue to increase in size and obstruct portal vein blood flow and give rise to portal hypertension.

(ii) Anticoagulants

The failure of urokinase can be explained by the fibrous tissue growth. Urokinase does not work directly on fibrin but works by activating plasminogen to plasmin which is the fibrinolytic agent. Reported uses of urokinase have all involved newly formed thrombi and it is possible that plasmin is not active against fibrous tissue of several months standing. Alternatively it could be that insufficient time was allowed for the urokinase solution to digest the fibrin or the amount of fibrin to be digested could have required more urokinase than that used. Another consideration is that urokinase is of human origin, and it may not be active in the dog. However routine intraportal infusion of urokinase or streptokinase may stop thrombus formation from becoming troublesome.

The dogs were dosed daily with heparin via the daily catheter flushing. This prevented clotting within catheters when reflux of blood up an arterial catheter occurred. Approximately 3,000 units of heparin were given daily in flushing but this dose may be insufficient for an intravascular effect. Possible interactions with the drugs to be studied should also be considered. Corticosteroids together with some other drugs have been implicated as having potential interactions with heparin anticoagulant activity (Colburn, 1976). Long term administration of heparin in dogs has been found to have no effects on haematologic and blood chemistry (Smith & Case, 1978). Conner & Fries (1960) used dicoumarol in an

attempt to prolong portal vein catheter life but had complications from its use. Whether routine anticoagulant or fibrinolytic dosage would be useful for increasing catheter patency and free of undesirable complications warrants further investigation, but for drug studies, possible interactions with oral anticoagulants would probably preclude their use.

4.1.7 Effect of catheter tip position in the portal vein on blood sampling

X-ray fluoroscopy confirmed the position from which the portal vein samples were drawn *in vivo*. For all of the intestinal drainage to be sampled, the catheter tip must reside after the gastroduodenal vein junction. However, although blood is sampled after all branches from the splanchnic area have united, there is doubt about its representation. This doubt arises from the fact that streamline flow in the portal vein could result in uneven concentration across the portal vein at any one time. Streams of higher or lower concentration could be present and if the blood sample withdrawn comes predominantly from one stream the results obtained could be of doubtful value. Streamline flow in the portal vein of dogs has been demonstrated (Hahn, Donald & Grier, 1945; Bianchi & Rossi, 1957). The blood from the splenic vein predominantly flows to the left lobe of the liver and that from the mesenteric vein to the right lobe. Exertion and positional changes alter both the degree and direction of streamlining. Gates & Dore (1973) demonstrated streamline flow in the portal vein of humans. The pattern was the same as found in the dog, blood from the superior mesenteric vein directed predominantly to the right lobe of the liver. However in conscious humans, the streamlining has been found to be a dynamic phenomenon (Erwald, Wiechel & Strandell, 1977). At high flow rates, turbulence caused thorough mixing. In conditions which reduce splanchnic blood flow such as upright position and physical exercise, possibilities for streamline flow increase. In ruminants, because the junction of the mesenteric vein and the truncus gastrolienalis is not at an acute angle, the flow in the portal vein is well mixed due to turbulence at the junction (Schambye, 1955).

The blood flow traces from the anaesthetised dogs provide evidence to conclude that portal vein samples are representative of the whole vein. Figure 3.14 shows that the sampling caused sufficient disturbance to the flow to provide a representative sample. However the flow in the anaesthetised animals was much reduced and whether the disturbance to flow in a conscious animal is of the same magnitude when compared with the increased flow is unknown and it remains to be explored whether streamline flow has any effect on the portal vein sampling method.

4.2 Gas Chromatographic Method of Assay for Prednisolone

At the time that the work reported in this thesis was commenced, the methods available for the estimation of prednisolone in plasma were limited. A nonspecific colorimetric method for 17-hydroxycorticosteroids commonly referred to as "Porter-Silber chromogens" (Porter & Silber, 1950; Silber & Porter, 1954) had been used (Jenkins & Sampson, 1967; Isbister & others, 1969) and fluorescence methods had also been reported (De Moor & co-workers, 1960; Silber, 1966). A difficult gas chromatographic method (Bailey, 1967) involving removal of the side chain from C-17 and formation of the 17-ketosteroid had also been attempted.

Prednisolone chromatographs well on OV-1; however on 3 per cent OV-1 it has a similar retention time to endogenous compounds and Bacon & Kokenakes (1969) found it necessary to use a preliminary column chromatographic procedure which was responsible for a significant loss of prednisolone. It was found that the use of a 5 per cent OV-1 column allows the separation of prednisolone from endogenous compounds. The higher liquid loading increases the separation due to increased retention times. Consequently, the preliminary step as well as derivatisation could be eliminated, and this simplified method was used for the analysis of prednisolone in this work. Tse & Welling (1977) have also modified the method of Bacon & Kokenakes (1969).

The lack of sensitivity below concentrations of 50 ng/ml as well as the long retention time required due to the presence of cholesterol are disadvantages of the glc method used. However the only metabolite of prednisolone with a similar retention time to prednisolone itself, hydrocortisone, is present at a concentration of 18 ng/ml in the dog (Chen, Kumar, Williard & Liao, 1978) and therefore was undetectable and caused negligible interference in the prednisolone assay. The lack of sensitivity of the assay may be a contributing factor to the variability of the data. Low concentrations of prednisolone probably were present but undetectable.

Since the major portion of the work reported herein was carried out, several methods have been developed which are extremely sensitive, require only small plasma volumes and can be performed rapidly enabling large numbers of samples to be processed. The two main methods are radioimmunoassay (RIA) and competitive protein binding (CPB).

The RIA method for prednisolone was introduced by Colburn & Buller (1973) and has been used by a number of investigators (Sullivan & others, 1974; Colburn & others, 1976; Schalm & others, 1976, 1977; Chakraborty, English, Marks, Dumasia & Chapman, 1976; Tembo & others, 1977b). It has been modified to overcome criticisms concerning the cross-reactivity of certain prednisolone metabolites. Extensive cross-reactivity with 20β-hydroxyprednisolone, prednisolone glucuronide as well as endogenous steroids has been found (Schalm & others, 1976). This cross-reactivity has been avoided by tedious paper chromatography prior to RIA (Meikle & others, 1975), by chromatography on "Sephadex" columns (Green & others, 1978) and by reaction with Girard Reagent T which results in derivatives that do not cross-react with the specific antibody for prednisolone (Loo & Jordan, 1978).

Leclercq & Copinschi (1974) used CPB as the assay method in their study. It has been used by several other investigators (English & others, 1975; Hulme & others, 1975; Wilson & others, 1975, 1977; Davis & others, 1978) and has the advantage over RIA that it

does not use a special antiserum which is expensive and time-consuming to produce. However a disadvantage is that a preliminary chromatographic separation procedure is usually required before CPB. The relative merits of CPB compared to RIA have been disputed (Colburn & Buller, 1975) but a study comparing both methods (Tembo, Ayres, Sakmar, Hallmark & Wagner, 1977a) concluded that CPB has considerably greater precision and sensitivity than the RIA method.

Other methods that have been reported include a quantitative thin layer chromatographic technique (Morrison & others, 1977), high-performance liquid chromatography (HPLC) (Loo, Butterfield, Moffatt & Jordan, 1977) and glc-mass spectrometry (Matin & Amos, 1978).

4.3 Pharmacokinetics of Prednisolone in the Dog

4.3.1 General

The values for $t_{½\beta}$ obtained from the femoral arterial plasma of the anaesthetised dogs dosed intravenously appear to fall into a similar pattern as the arterial half-lives following dosage *via* the portal vein. Excluding the ^{3}H-prednisolone tracer, the $t_{½\beta}$ ranged from 0.1 to 0.6 hour. This value is in agreement with the value of 0.3 hour obtained by Tse & Welling (1977) in conscious dogs following intravenous dosage of prednisolone. However, other workers have reported much longer half-lives in dogs, and that the half-lives obtained following oral dosage appear to be longer than those following intravenous dosage. Half-lives reported following intravenous dosage were in the range 1.0 to 1.2 hours whilst half-lives range from 1.8 to 2.9 hours after oral dosage (Table 1.5). The much longer $t_{½\beta}$ from the ^{3}H-prednisolone tracer dose may be due to inclusion of metabolites in the assay. The repeat dosage in one experiment also gave a long $t_{½\beta}$ and therefore these longer half-lives may be due to the longer sampling time and be a truer estimate of the terminal elimination half-life. The values of 1.2 and 2 hours obtained agree more closely with the half-lives found by other workers.

It is not possible to determine whether the pharmacokinetics of prednisolone in anaesthetised dogs differs from that in conscious dogs due to the variability of the data from the conscious animals.

It has been claimed that the kinetics of elimination of prednisolone are dose-dependent (Pickup & others, 1977; Tanner & others, 1979a; Rose & others, 1979). However there is disagreement (Green, 1979). Overall, examination suggests that the half-lives of prednisolone in both man and the dog (Tables 1.4 and 1.5) are not dose-dependent and the data from the anaesthetised dogs also indicates that prednisolone kinetics are independent of dose.

The kinetics of prednisolone in the anaesthetised dogs were biexponential and there were indications that there may have been an initial transient phase in which the plasma concentrations decreased very rapidly. Other kinetic data for prednisolone from dogs has been assumed to be monoexponential (Tse & Welling, 1977). However in man it is now generally accepted that the kinetics of prednisolone can be described by a two-compartment model (Pickup, 1979; Tanner & others, 1979a). The distribution phases of corticosteroid kinetics appear to be complex; Glantz & others (1976) have proposed a third shorter initial half-life for hydrocortisone due to an early distribution phase. It is probable that the kinetics in the dog are more appropriately represented by a two-compartment model, although in some cases a three-compartment model may be necessary.

4.3.2 Anomalous portal-arterial concentration differences following intraportal administration

The differences in the time courses of plasma concentrations from the portal vein and femoral artery following intraportal bolus injections of prednisolone to anaesthetised dogs were quite surprising. It would be expected that the portal and arterial curves would correspond although the concentrations may have been

lower than an identical dose administered intravenously. However the portal concentrations were considerably higher than the arterial concentrations for approximately 30 minutes after the administration of the dose. For example in one case the concentration of prednisolone in the portal vein was 157 µg/ml at the 5 minute sampling time whilst the arterial concentration was 40 µg/ml. This phenomenon was real since it occurred irrespective of dose, vehicle and dosage form. Furthermore, there was a difference in the blood pressure response between the intravenous and intraportal routes of administration. The most probable explanation for this effect is that osmoreceptors present in the portal system respond to the intraportal injection by bringing about the uptake of fluid in either spleen or splanchnic tissue. Haemoconcentration of the portal blood would result and there would appear to be a positive P - A difference.

Osmoreceptors (sensitive to changes in osmolality of blood) (Haberich, Aziz, Nowacki & Ohm, 1969; Adachi, Niijima & Jacobs, 1976) and sodium receptors (Rogers, Novin & Butcher, 1979) exist in the portal circulation and a different physiological effect has been demonstrated between intravenous and intraportal administration of hypertonic saline (Haberich, 1968). Although the solutions used for injection were diluted with isotonic sodium chloride, the final solutions were not necessarily isotonic. The low dose of ^{3}H-prednisolone would be expected to be very close to an isotonic solution, however it is claimed that the osmoreceptors and sodium receptors are very sensitive (Rogers & others, 1979). When absorption of water occurs, the osmolality of the portal blood decreases and storage of minerals and water then follows in the liver. Storage may also occur in the spleen. The spleen of the dog is capable of storing blood and can liberate a volume of blood equivalent to 16 to 20 per cent of the normal blood volume (Kramer & Luft, 1951), as well as sequester up to one third of the erythrocytes (Steffey, Gillespie, Berry, Eger & Schalm, 1976; Kraan, Huisman & Velthuizen, 1978). Prednisolone is distributed into red blood cells (see page 226) and effective uptake of prednisolone into the spleen may occur.

It appears that after the initial uptake, fluid and erythrocytes are released and create P - A differences similar to those seen after intestinal absorption. The P - A differences continue to approach zero until all stored fluid is released. It is also possible that continuing haemoconcentration occurred from a continuous but decreasing rate of uptake of fluid.

Other possible causes that could have been responsible for higher concentrations of prednisolone in the portal vein are: (1) precipitation of prednisolone in the vein, and (2) adsorption to the catheter tubing. Since a high dose was given, there may have been precipitation of the prednisolone within the portal vein followed by a slow dissolution. However, because of the velocity of injection, precipitation should occur some distance away from the catheter tip and subsequent sampling through the catheter tip should not include any material from a precipitate in the vein downstream from the sampling point. The effect was independent of the vehicle used in administering the dose. Administration of a water-soluble ester of prednisolone and tracer doses (approximately 130 ng) from which precipitation would not be expected to occur, gave higher portal vein concentrations and excluded the possibility of precipitation. The sampling in the experiments was through the same catheter that was used for administration of the doses. There was the possibility that adsorption of material to the catheter sides occurred. However this seems unlikely as doses via the venous catheters that were subsequently used for venous sampling did not show higher concentrations from this site.

The data have shown that the response and kinetics may vary from different routes of administration. Therefore plasma concentration data from intraportal infusion of drugs must be carefully analysed for possible effects in the portal system.

4.3.3 Entero-hepatic recycling of prednisolone

It appears that entero-hepatic circulation of prednisolone occurs in the dog. This makes studies on absorption and bioavailability of prednisolone in the dog difficult. The entero-hepatic circulation is indicated by several findings. The molecular weight of prednisolone and its glucuronide conjugate is above the threshold value necessary in the dog for extensive biliary elimination. From published data on corticosteroid excretion in various species, it appears that appreciable biliary excretion of prednisolone and/or its metabolites may occur (page 86 , Table 1.3) and entero-hepatic circulation of other steroids (including testosterone metabolites and oestrogens) has been reported in the dog. Entero-hepatic circulation of hydrocortisone has been reported in the rat which has a similar molecular weight threshold to that of the dog and exhibits similar excretory patterns (Hyde & Williams, 1957; Taylor, 1971) and the bile is the major pathway of elimination of prednisolone in the rat (Ware & Combes, 1973). Prednisolone does not undergo significant entero-hepatic circulation in man (Table 1.3) whose molecular weight threshold for biliary excretion is much higher than that of the dog (Smith, 1974).

The presence of secondary peaks in the time courses of plasma concentrations following intravenous dosage is commonly taken to indicate biliary excretion and subsequent re-absorption (Jusko & Levy, 1967). Secondary peaks were seen in the time courses following both oral and intravenous administration of prednisolone. The simultaneous rises and falls in both the portal vein and carotid artery plasma concentrations strongly suggests that these peaks are not artifacts. Furthermore, calculation of the amounts absorbed from the 5 mg oral preparations revealed that up to 21 mg was absorbed from some of them indicating considerable entero-hepatic cycling. It is extremely unlikely that a fourfold overestimate of portal vein blood flow was made for the greyhound dogs used. It is interesting to note that Andersson & others (1977) found similar correspondence of portal and peripheral venous rises and falls following oral administration of proscillaridin to patients catheterised through the

umbilical vein. Proscillaridin undergoes entero-hepatic circulation in man and multiple peaks were present in the time courses of plasma concentrations.

Experiments in the anaesthetised dogs showed extensive biliary excretion of prednisolone and/or metabolites and is further evidence for entero-hepatic recycling. Almost 50 per cent of the radioactivity from a tritium labelled dose was excreted in 4 hours. This radioactivity presumably contains glucuronide and sulphate conjugates of prednisolone because steroids are usually excreted in the bile as these forms. The biliary excretion of prednisolone has not been previously investigated in the dog, and the faster elimination half-lives reported in the dog compared with man are possibly due to rapid biliary excretion.

The time courses of the plasma concentrations following the intravascular dosage to anaesthetised dogs do not appear to show signs of recycling. However irregularities in the form of a small peak occurred 40 minutes after dosing and this may correspond to an early entero-hepatic recycling of some of the drug. It is possible that sampling was not performed for a sufficient length of time to detect further recycling and that anaesthesia may affect the rate of bile flow and/or gallbladder release. No irregularities were seen in the curves resulting from intravenous dosage during complete biliary collection. Entero-hepatic circulation could possibly be the reason why the reported half-lives of prednisolone in the dog (Table 1.5) appear different for intravenous and oral dosage; in man where entero-hepatic circulation is not significant no such difference exists (Table 1.4).

For entero-hepatic circulation to occur it is assumed that conjugates of unchanged prednisolone (presumably glucuronides and sulphates) are excreted into the bile and are hydrolysed in the small intestine. The free prednisolone liberated is re-absorbed and gives rise to the secondary peaks seen in the time courses of the plasma concentrations. Free prednisolone and prednisolone-21-acetate

have been found in human bile (Scheiffarth, Zicha, Funck & Engelhardt, 1963). However, the nature of the biliary metabolites of prednisolone in the dog still remains to be elucidated.

The second peak following intravenous prednisolone dosage occurred after approximately 1 to 2 hours whereas after oral dosage, the times of the secondary peaks due to entero-hepatic circulation occurred later and P - A differences were observed at the times of the secondary peaks following intravenous administration. The faster recycling following intravenous dosage probably occurs because intravenous administration presents a greater amount of drug to the liver more rapidly than oral administration.

Secondary peaks have not been extensively reported in the literature for any compounds undergoing entero-hepatic circulation. Belz, Stauch & Rudofsky (1974) reported that the time course of the systemic plasma concentration of proscillaridin when administered orally to man had a large second peak after 3 hours. Andersson & others (1977) found both portal vein and systemic plasma concentrations were similarly increased between 4 and 10 hours after dosage. However as sampling was infrequent during this period it may be that more than one peak occurred. Irregularities in the time course of plasma concentrations have also been found 6 to 12 hours after oral administration of norethisterone in the human (Odlind, Weiner, Victor & Johansson, 1979) although the sampling was again too infrequent to determine the exact extent of this phenomenon. A second peak has been observed at 6 hours after oral administration of morphine to the rat (Dahlstrom & Paalzow, 1978). Morphine-3-glucuronide was eliminated in the bile, hydrolysed in the small intestine to morphine and re-absorbed in the caecum.

The time courses of plasma concentrations of prednisolone in the greyhounds showed considerable fluctuation. The absorption of testosterone in the dog has been shown to appear as a series of sharp distinct peaks over a period of 1.5 hours (Martin & others, 1965) indicating erratic absorption of the steroid. Therefore erratic absorption may be a second mechanism contributing to the fluctuations

in the time course of plasma concentrations of prednisolone.

Irregular gallbladder emptying may also have contributed to the fluctuations in the plasma concentrations seen. During an overnight fast, bile is stored in the gallbladder (Van Berge Henegouwen & Hofmann, 1978) and can be released without the presence of food in the duodenum (Read, Cooper & Fordtran, 1978). Normal delivery of bile to the duodenum occurs in waves (Ashkin, Lyon, Shull, Wagner & Soloway, 1978), hence fluctuations in the re-absorption pattern may be seen. Repeated measurements in a single subject for the absorption of prednisolone have not been reported and no comparison of the variability of the time courses described in this work can thus be made.

Other explanations for the rise in the plasma concentrations following intravenous dosage are unlikely. They are (1) precipitation of prednisolone in the vein after the injection and subsequent re-dissolution and (2) intestinal uptake followed by secretion of prednisolone from the intestines and subsequent absorption. The first explanation does not seem probable because the catheter tip was at the junction of the superior and inferior vena cavas and the turbulence and flow rate at that point would not be expected to allow for deposition. A precipitated mass of prednisolone would continuously dissolve and therefore should not yield sharp peaks in the time course of plasma concentrations.

Recycling of prednisolone after intestinal secretion is even less likely to occur. Lien, McCormick, Davies & Egdahl (1970) found that extrahepatic elimination of hydrocortisone occurred in anaesthetised dogs and monkeys. Twenty-nine per cent was removed by the gastrointestinal tract in dogs and 20 per cent in monkeys. They suggested that either free hydrocortisone diffused through the gut wall and into the lumen or that it was metabolised by the cells of the gut. Paterson & Harrison (1972) showed that 12 per cent of ^{3}H-hydrocortisone was taken up in the intestinal area of sheep during a constant perfusion and Zierler (1961) has shown theoretically that storage of a compound in the splanchnic area leads to P - A differences

when it is released. However the magnitude of the P - A differences in the secondary peaks appears too large to be accounted for as intestinal secretion or storage.

It therefore appears from consideration of the comparative data from other workers (page 105) and from the indications arising in the present work that in the dog prednisolone and/or its conjugates are excreted in appreciable amounts in the bile and an entero-hepatic circulation of prednisolone exists. Since prednisolone appeared to be very rapidly absorbed as shown by the high portal vein concentration at 15 minutes in Figure 3.24, the peaks at 2 to 4 hours in most of the oral prednisolone curves are probably due to recycling.

While entero-hepatic circulation of prednisolone is indicated, this could be confirmed by carrying out two experiments. In the first, total collection of bile should eliminate secondary peaks and alter the elimination kinetics. In the second, administration of bile collected from a previously dosed animal into the duodenum of a second animal should result in absorption of prednisolone into the portal vein of the second animal. Both these types of experiments are discussed in the Introduction (page 107).

In contrast to the data obtained in the present studies, the time courses of plasma concentrations obtained on prednisolone by other workers in beagle dogs (Colburn & Buller, 1973; Colburn & others, 1976; Tse & Welling, 1977) do not exhibit multiple peaks. Tse & Welling (1977) showed smooth computer curves of mean data only and did not give the raw data. The assay method used by Tse & Welling (1977) was similar to that of the present study and if a linear relationship between dose and area under the time course of the systemic plasma concentrations is assumed, the areas found from their study correspond with those found in this study. However Tse & Welling (1977) found an unexplicable discrepency in the half-lives that they obtained. The half-life following intravenous administration of a 30 mg dose was much shorter than an equivalent oral dose while 60 mg intravenous and oral doses gave the same half-life

as the 30 mg oral dose. This discrepency may also be explained by the effect of entero-hepatic circulation; the sampling times after the 30 mg intravenous dose apparently ceased after one hour in contrast to 6 hours in the other cases where terminal elimination would appear to be prolonged.

The range of the plasma concentrations found by Colburn & Buller (1973) and Colburn & others (1976) was similar to that found in the present work (see Figure 4.1). Two reasons for the difference in the appearance of the time courses found by them may be infrequent sampling times and lack of assay specificity. Infrequent sampling may have missed fluctuations in the time courses of plasma concentrations and given apparent smooth curves. Colburn & Buller (1973) and Colburn & others (1976) used a radioimmunoassay which has been criticised for lack of specificity in regard to certain metabolites of prednisolone including glucuronides (Chakraborty & others, 1976; Schalm & others, 1976). The finding that smooth curves were obtained following administration of ^{3}H-prednisolone can be compared with the smooth curves obtained by Colburn & Buller (1973) and Colburn & others (1976). The plasma radioactivity concentrations almost certainly contain measurements of metabolite concentrations which presumably vary in such a manner that the time courses are smoothed out. The time course of the plasma concentrations was very sustained from 1 to 4 hours after dosing where a decline in plasma concentrations was noted using the specific gas chromatographic assay. The calculated areas under the time courses of the systemic plasma concentrations from 0 to 4 hours of the 5 mg dosage forms of prednisolone used by Colburn & Buller (1973) are approximately twice those found in the greyhound suggesting that metabolites may have contributed to the larger areas they found. Further study of the absorption, pharmacokinetics and metabolism of prednisolone is required in order to answer the question of what is the cause of the differences between this and other studies.

Pharmacokinetic studies of prednisolone in man have generally not shown fluctuations in the time course of plasma concentrations. However few blood samples have been taken in most studies.

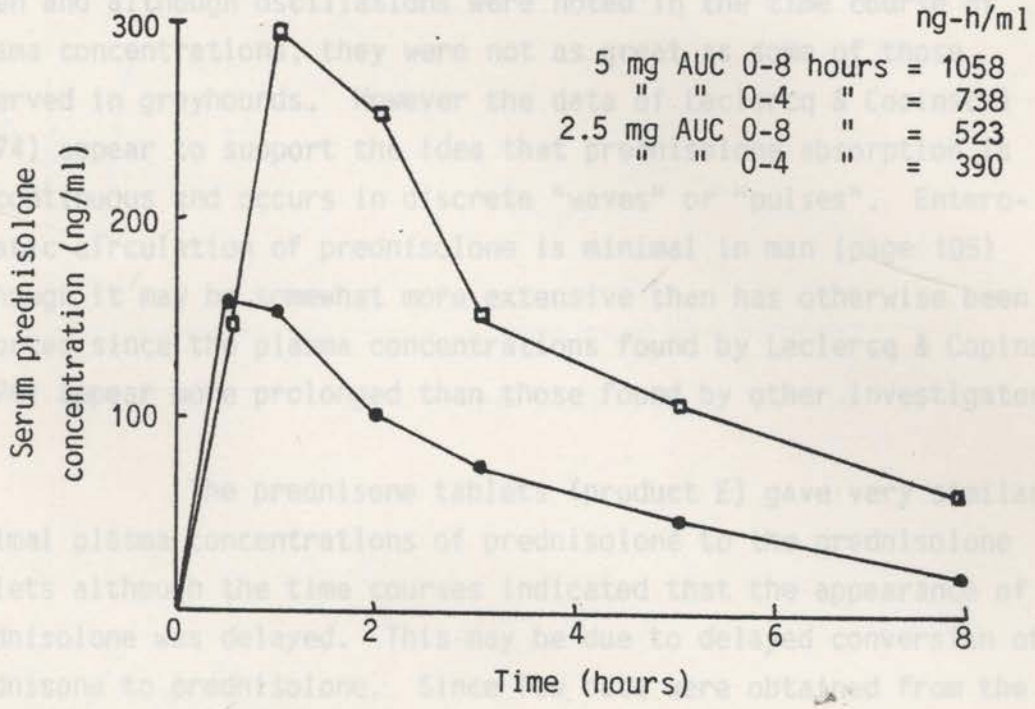


Figure 4.1. Serum prednisolone levels in beagle dogs following oral administration of a 2.5 mg prednisolone tablet (Delta-Cortef, Upjohn), ● , and a 5 mg tablet (Delta-Cortef), □ . From the data of Colburn & Buller (1973).

Leclercq & Copinschi (1974) used a competitive protein binding assay which Tembo & others (1977a) found to be more desirable than the radioimmunoassay; they found plasma concentrations of prednisolone that had some variability (see Figure 4.2). Frequent samples were taken and although oscillations were noted in the time course of plasma concentrations, they were not as great as some of those observed in greyhounds. However the data of Leclercq & Copinschi (1974) appear to support the idea that prednisolone absorption is discontinuous and occurs in discrete "waves" or "pulses". Entero-hepatic circulation of prednisolone is minimal in man (page 105) although it may be somewhat more extensive than has otherwise been reported since the plasma concentrations found by Leclercq & Copinschi (1974) appear more prolonged than those found by other investigators.

The prednisone tablets (product E) gave very similar maximal plasma concentrations of prednisolone to the prednisolone tablets although the time courses indicated that the appearance of prednisolone was delayed. This may be due to delayed conversion of prednisone to prednisolone. Since few data were obtained from the prednisone formulation it is not possible to determine whether its bioavailability was significantly different from prednisolone formulations in the greyhound. Colburn & others (1976) found statistically significant differences in the areas under the time course of plasma concentrations between prednisone and prednisolone.

However there appears to be a discrepency with prednisone. Prednisone is converted to prednisolone in the liver (page 84) and therefore positive P - A differences for prednisolone should not be found following prednisone dosage. However P - A differences for prednisolone were found. Since the conversion of prednisone to prednisolone has been claimed to be unaltered in the presence of severe impairment of liver function (Schalm & others, 1977) it is possible that other sites within the body may be capable of the conversion; to account for P - A differences of prednisolone it is possible that the conversion may occur in the intestinal tissue during the absorption. Steroids have been shown to be metabolised in intestinal tissue during absorption (Diczfalusy & others, 1961; Nienstedt & Harri, 1979).

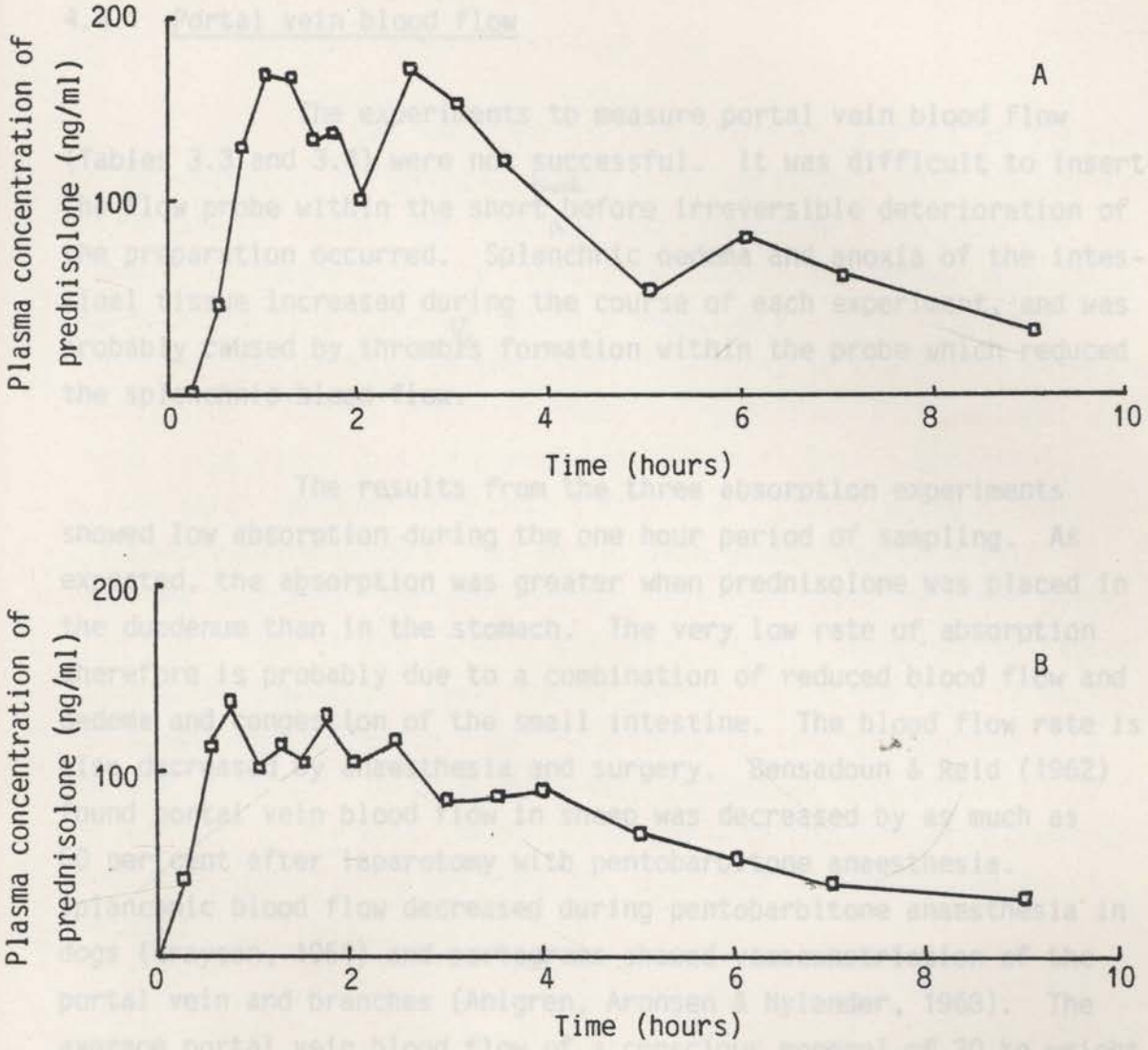


Figure 4.2. Plasma prednisolone levels in two human subjects after oral administration of a 20 mg dose: A, does not show a smooth curve but instead three peaks can be observed, resembling the data obtained from greyhound dogs; B, shows small oscillations superimposed on a basic single peak. From the data of Leclercq & Copinschi (1974).

4.4 Absorption and Bioavailability of Prednisolone

4.4.1 Portal vein blood flow

The experiments to measure portal vein blood flow (Tables 3.3 and 3.4) were not successful. It was difficult to insert the flow probe within the short time before irreversible deterioration of the preparation occurred. Splanchnic oedema and anoxia of the intestinal tissue increased during the course of each experiment, and was probably caused by thrombus formation within the probe which reduced the splanchnic blood flow.

The results from the three absorption experiments showed low absorption during the one hour period of sampling. As expected, the absorption was greater when prednisolone was placed in the duodenum than in the stomach. The very low rate of absorption therefore is probably due to a combination of reduced blood flow and oedema and congestion of the small intestine. The blood flow rate is also decreased by anaesthesia and surgery. Bensadoun & Reid (1962) found portal vein blood flow in sheep was decreased by as much as 60 per cent after laparotomy with pentobarbitone anaesthesia. Splanchnic blood flow decreased during pentobarbitone anaesthesia in dogs (Grayson, 1954) and portograms showed vasoconstriction of the portal vein and branches (Ahlgren, Aronsen & Nylander, 1968). The average portal vein blood flow of a conscious mongrel of 20 kg weight is about 700 ml/min (Cox & others, 1976) whereas the average flow in the acute flow experiments after commencing recording was only 100 ml/min. Grisafe & Hayton (1978) found that the absorption of prednisolone was not blood flow rate dependent but severely reduced blood flow must effect the rate of absorption.

The practical problems of measuring portal vein blood flow with an electromagnetic flowmeter could be overcome by use of a non-invasive measuring system such as a Doppler flowmeter (Prewitt, Jacobson, Hemken & Hatton, 1975). A peg type electromagnetic probe was tried, but the requirement of a tight fit to the vessel together with spasm of the vein made it impractical. A catheter-tip electro-

magnetic probe has been successfully used in the conscious human after being introduced via the reopened umbilical vein (Strandell & co-workers, 1973) and this could possibly be used successfully in both the anaesthetised and conscious dog.

It has been demonstrated (Zierler, 1961) that the use of concentration differences across a tissue to determine the uptake or release of a substance requires the blood flow to be constant and known. Furthermore, if the blood flow rate in the portal vein is not constant it is possible to have negative P - A differences (Zierler, 1961). Data on the blood flow rates in the portal vein are conflicting. Carr & Jacobson (1968) using Doppler shift telemetry found large variations in portal vein blood flow in a conscious, moving calf. However Wangsness & McGilliard (1972) using a dye-dilution technique found that portal vein blood flow in a calf remained constant. Postural changes have been found to alter portal vein blood flow in man (Erwald & others, 1977). Therefore if random changes in flow occur, knowledge of portal vein blood flow is essential for measuring absorption from P - A concentration differences. However it appears to be a reasonable assumption that portal blood flow is constant in the experiments conducted with the conscious greyhounds since they were fasted and were not exercised during the period of sampling.

4.4.2 Comparative bioavailability study

The bioavailability of prednisolone tablets has been of concern for some time (page 100), and the present studies provide the first evidence of low bioavailability from a conventional tablet which was suspected of being clinically ineffective. The only reported comparison of a clinically ineffective conventional prednisolone tablet with an effective preparation showed no differences in the plasma concentrations (Isbister & others, 1969).

Detailed studies on products A, B and C were carried out. Product C had been claimed by a physician to be a therapeutic failure. The areas under the time courses of the portal and arterial

plasma concentrations for product A were significantly greater than those for products B and C indicating greater total absorption of prednisolone from product A. In addition, comparison of the portal vein and arterial plasma concentrations showed that product A was also absorbed significantly faster and had more sustained plasma concentrations than products B and C. It is significant that the total absorption of product C, as indicated by areas under the time courses of the portal and arterial concentrations, was significantly less than product A which was another tablet from the same manufacturer. Product A was presumably clinically effective. This would seem to indicate that the claim of clinical inefficacy of product C was justified. However, no difference was found between products B and C although product B was also presumably therapeutically active.

The in vitro dissolution tests carried out on products A, B and C showed only marginal differences in the rate of release, even at the slower speed recommended by Kendall (1972). This slower speed of basket rotation was claimed to discriminate more effectively between the rates of dissolution of prednisolone tablets. It is interesting to note that product A showed the slowest rate of release. The lack of in vitro - in vivo correlation confirms a similar finding by Tembo & others (1977b). They found differences in dissolution but did not observe differences in vivo. Products A and C, which as previously noted, were two different batches from the same manufacturer, showed no differences in vitro although differences were seen in vivo. In contrast, Tembo & others (1977b) found that the rate of dissolution of two tablets also from different batches by the same manufacturer were markedly different although no differences were seen in vivo. Therefore it appears that a better in vitro test for prednisolone tablets needs to be developed for routine quality control. In addition caution should be exercised in deciding whether different brands of prednisolone tablets may be bioequivalent.

The absorption of products D to G was studied in less detail than products A, B and C. Although the data are limited, the total absorption of prednisolone from several of these products appeared to be much greater than the total absorption of prednisolone

from products A, B and C. Despite this, no difference in *in vitro* rates of release were seen and provided further evidence of lack of *in vitro* - *in vivo* correlation.

However there was an anomaly with product F, which was a capsule. Its *in vitro* release was very poor, but its *in vivo* absorption was similar to that of products A, B and C. The extemporaneously prepared capsules (product G) had a release rate *in vitro* similar to the tablets and therefore the poor release of product F does not appear to be a result of the dissolution test being unsuitable for capsules. This result is consistent with that of Tembo & others (1977b), who found that formulations with a markedly different release *in vitro* did not show differences *in vivo*.

The relative bioavailability of the different products was determined from the time course of plasma concentrations over four hours. This is a relatively short period. Lovering, McGilveray, McMillan & Tostowaryk (1975) have shown that comparative bioavailabilities can be obtained from truncated blood level curves. They found that the ratios of areas under the plasma curve changed little between the end of the absorption period and the time when blood sampling was terminated. The bioavailability comparison appears valid because P - A differences were negligible between 3 and 4 hours indicating that absorption had ceased.

The calculation of absorption by the portal-arterial concentration difference method only requires samples to be taken until the P - A differences are zero. Consequently it was not necessary to take blood samples for several half-lives or to extrapolate to infinity in order to obtain the bioavailability estimates and sampling was not carried out past 4 hours.

Although significant differences were found between the portal vein concentrations, arterial concentrations and the areas under the time courses of the portal and arterial concentrations, no significant differences were found between the P - A concentration differences and $\int_0^4 (P - A)\, dt$. However a similar trend was seen and

the differences between some P - A values were approaching statistical significance. The coefficient of variation of the P - A values was greater than the corresponding coefficients of variation of the concentrations of prednisolone in the portal vein and carotid artery. Three factors may contribute. They are (1) individual errors from the portal and arterial concentrations, (2) the inability to remove the two samples exactly simultaneously (page 176) and (3) variable blood flow. Experimental errors occur in both portal and arterial concentration values, and the percentage error in the P - A values will clearly be greater than in either the portal or arterial values alone. The coefficients of variation tended to be large where the concentrations were rising and falling rapidly. A similar situation can be seen in the data from Sullivan & others (1976a) and Tembo & others (1977b). A high coefficient of variation occurred at 15 minutes when the plasma concentrations of prednisolone were rising rapidly but they were considerably lower for the later concentrations when the plasma concentrations were more stable. Increased blood flow will reduce P - A difference values. Since food increases portal vein blood flow (Webster & others, 1975; Bond, Prentiss & Levitt, 1979), it is possible that the negative P - A differences seen after prednisolone tablets were administered following food arose from blood flow variation. It is apparent that the precision of the determination of P - A is critical. Webster (1974) has also mentioned this aspect of using portal vein - arterial concentration differences.

The investigation of the absorption and bioavailability of prednisolone in greyhounds has been complicated by the presence of entero-hepatic circulation (page 212). It is difficult to calculate the actual amount absorbed because the presence of entero-hepatic circulation will result in an overestimate. However the total biliary excretion should be proportional to total absorption and therefore should not interfere with comparisons of relative availability. It also appears that the P - A difference peaks are very sharp peaks and that in some cases these peaks may have been missed due to an insufficient number of blood samples being taken.

Negative values were found for some determinations of P - A following prednisolone administration. Six factors that could contribute are (1) timing errors in simultaneous sampling of the portal vein and carotid artery (see page 176), (2) errors in the assay of the portal and arterial plasma (see page 206), (3) sampling errors as a result of streamline flow (see page 205), (4) uptake of drug from the blood by intestinal tissue and secretion into the gut lumen (page 215), (5) variable portal vein blood flow (page 222) and (6) simultaneous absorption of water from the lumen resulting in dilution of the portal vein blood.

There are conflicting data concerning the absorption or secretion of water during concurrent absorption of other substances. Dencker & others (1973), who also found negative P - A differences after administration of sugars in humans, found no changes in haemoglobin concentrations in arterial and portal blood at the times when P - A differences were negative. This indicated no net transfer of water. However Fries & Conner (1961) found haemodilution of the portal blood in calves, and Moodie & Walker (1963) found random haemodilution and haemoconcentration of blood passing through the splanchnic area in sheep. The data of Moodie & Walker (1963) showed negative P - A differences for Ca^{2+} until corrected for water absorption. In contrast, Wangsness & McGilliard (1972) found that portal-arterial haemoglobin concentration differences were small and concluded that no correction was necessary. It is also possible that water secretion into the intestinal lumen could enlarge positive P - A differences. Further investigation of the effects of water absorption or secretion on P - A values in portal vein catheterised dogs is required.

In calculating the amounts of prednisolone absorbed, it is necessary to know the red blood cell/plasma concentration distribution ratio. If no prednisolone was associated with the red blood cells, then the plasma flow should be used instead of the total blood flow. However the red blood cell/plasma concentration distribution ratio of the related corticosteroids methylprednisolone and triamcinolone has been found to be approximately 1 (Slaunwhite & Sandberg, 1961; Garg & others, 1978; Florini & others, 1961a,b) and

therefore it has been assumed that the concentration in the plasma equals the concentration in the blood.

The first-pass effect of 25 to 32 per cent estimated from the plasma clearance and systemic availability appears to be the first approximation of this value for prednisolone in the dog. It is in reasonable agreement with the value of 50 per cent obtained for hydrocortisone by McCormick & others (1974). A significant first-pass effect has also been suggested for prednisolone in man (Pickup, 1979).

There are conflicting data concerning the in vivo hydrolysis of water-soluble salts of prednisolone. Some investigators claim that they are hydrolysed rapidly and quantitatively to prednisolone (Pickup & others, 1977; Hsueh, Paz-Guevara & Bledsoe, 1979). Kitagawa & others (1972) found that the concentrations of prednisolone 5 minutes after intravenous administration of prednisolone-21-phosphate were approximately half those following the equivalent dosage of free prednisolone. Methylprednisolone sodium phosphate has been found to give plasma concentrations of methylprednisolone 20 per cent higher than after methylprednisolone sodium succinate although no pharmacological difference was evident (Novak, DiSanto, Seckman, Elliott, Lee & Stubbs, 1977). Data obtained in the present study indicate that the hydrolysis of prednisolone sodium succinate and prednisolone hemisulphate sodium is quite slow. If indeed the hydrolysis is slow, the contradictory information concerning water-soluble salts of prednisolone may arise from specificity of analytical methods. Novak & others (1977) also suggested that hydrolysis of the succinate ester may be slow. Alternatively there may be considerable tissue uptake or biliary excretion of the prednisolone esters. The esters are large molecular weight anions which could be secreted readily into bile.

4.4.3 Gastric absorption of prednisolone

The human intubation experiments were carried out in an attempt to correlate aspects of prednisolone absorption in humans with the absorption in the dog. No other studies on the absorption of prednisolone from the human stomach have been reported and the lack of prednisolone absorption from the stomach in man was as expected for a neutral steroid. Prednisolone has been implicated in causing peptic ulceration by disrupting the gastric mucus layer (Swartz & Dluhy, 1978). However lack of absorption from the stomach tends to support the view that prednisolone does not cause peptic ulceration (Conn & Blitzer, 1976) and therefore the use of enteric-coated tablets with their consequent unreliable bioavailability (Lee & others, 1979a) may be avoided.

The results from the dog appeared to indicate that absorption of prednisolone from the stomach was greater than in man. However, the conditions used and the method of measurement in the two experiments were different. Absorption in the dog was surprisingly high considering blood flow to the splanchnic area was reduced under the experimental conditions. Other studies in man and the dog suggest that prednisolone absorption takes place from the upper jejunum (Hulme & others, 1975; Tse & Welling, 1977) and it is therefore hard to postulate reasons for the apparent high absorption of prednisolone which was indicated from the portal vein plasma concentrations of radioactivity in the dogs.

4.5 Summary and Conclusions

An *in vivo* model system for measuring gastrointestinal absorption into the portal system and studying the first-pass effect of drugs and their relationship to bioavailability has been developed. The experimental animal used was the greyhound dog and catheters were implanted in the portal vein, carotid artery and jugular vein enabling chronic sampling for periods of over 5 months. The product of the area under the time course of the P - A concentration differ-

ences and the estimated portal vein blood flow yields the amount of drug absorbed into the portal vein. This determination is direct and is not influenced by metabolism, distribution or elimination of the drug and is obtained from animals in a near-normal physiological condition.

However, the investigation has shown that further refinements in the use of the portal vein catheterised dog are necessary; two of them are, more intensive sampling to obtain the true absorption peaks and minimisation of all errors in determining P - A values including provision for exact simultaneous blood sampling and sensitive, rapid assay methods. In addition, the provision of portal vein blood flow rate measurement in the dog itself is required for accurate determination of amounts absorbed and rates of absorption. The greyhound dog appears to be an ideal animal for use in any absorption or bioavailability study that cannot be performed in man. The model as described can yield worthwhile data on comparative rates of absorption if repeated measurements in a single animal are used under conditions which promote constant portal vein blood flow. In this way the difficulties associated with flowmeters may sometimes be avoided.

The pharmacokinetics of prednisolone in the human are slowly becoming understood although there are still areas where research is required. However, very little is known about prednisolone pharmacokinetics in the dog. The kinetic findings from the use of prednisolone to investigate the portal vein sampling model are not clear; they appear to indicate that the pharmacokinetics in the dog are more complex than previously assumed. The major finding was the possibility that prednisolone undergoes entero-hepatic circulation in the dog. The entero-hepatic circulation severely complicated the study of the gastrointestinal absorption and in retrospect made prednisolone an unsuitable drug to investigate in the model. The potential of this method has been clearly demonstrated and its use for further study of drugs without entero-hepatic circulation is needed.

No correlation was found between *in vitro* dissolution of prednisolone tablets and *in vivo* absorption. This confirms findings of others concerning prednisolone bioavailability correlations. It may be that a more appropriate *in vitro* test is required. However further work on the *in vivo* absorption of prednisolone in man and a suitable animal model is indicated. Since the excretion patterns of prednisolone in the dog differ from those in man, care must be taken if the dog is used as the model animal species.

BIBLIOGRAPHY

ABBOTT, W.O., KARR, W.G. & MILLER, T.G. (1938). Intubation Studies of the Human Small Intestine. VII. Factors Concerned in Absorption of Glucose from the Jejunum and Ileum. Am. J. dig. Dis., 4, 742-752.

ABBOTT, W.O. & MILLER, T.G. (1936). Intubation Studies of the Human Small Intestine III. A Technic for the Collection of Pure Intestinal Secretion and for the Study of Intestinal Absorption. J. Am. med. Ass., 106, 16-18.

ABSHAGEN, U., GRODZICKI, V. von., HIRSCHBERGER, U. & RENNEKAMP, H. (1977). Effect of Enterohepatic Circulation on the Pharmacokinetics of Spironolactone in Man. Naunyn-Schmiedeberg's Arch. Pharmac., 300, 281-287.

ADACHI, A., NIIJIMA, A. & JACOBS, H.L. (1976). An Hepatic Osmoreceptor Mechanism in the Rat: Electrophysiological and Behavioral Studies. Am. J. Physiol., 213, 1043-1049.

ADMANS, M., COBCROFT, A.J., FINNIN, B.C., NOLAN, J. & REED, B.L. (1976). Effect of Dioctyl Sodium Sulphosuccinate on the Absorption of Phenol Red from the Small Intestine of the Rat. Aust. J. pharm. Sci., NS5, 51-54.

AGAR, W.T., HIRD, F.J.R. & SIDHU, G.S. (1954). The Uptake of Amino Acids by the Intestine. Biochim. biophys. Acta, 14, 80-84.

AHLGREN, I., ARONSEN, K.F. & NYLANDER, G. (1968). Splanchnic and Portal Angiographic Patterns during Increasing Pento-Barbital Sodium Intoxication. Acta anaesth. Scand., 12, 103-113.

AMIROV, N.S. (1966). A Method for Repeated Sampling of Blood from the Portal Vein (in Russian). Fiziel. Zh. (Mosk.), 52, 312-313. Cited by MOUZAS, G.L. & SMITH, G. (1969).

ANDERSSON, K.-E., BERGDAHL, B., DENCKER, H. & WETTRELL, G. (1977). Proscillaridin Activity in Portal and Peripheral Venous Blood After Oral Administration to Man. Eur. J. clin. Pharmac., 11, 277-281.

ANDERSSON, K.-E., NYBERG, L., DENCKER, H. & GÖTHLIN, J. (1975). Absorption of Digoxin in Man After Oral and Intrasigmoid Administration Studied by Portal Vein Catheterization. Eur. J. clin. Pharmac., 9, 39-47.

ANGELI, A., FRAJRIA, R., DE PAOLI, R., FONZO, D. & CERESA, F. (1978). Diurnal Variation of Prednisolone Binding to Serum Corticosteroid-Binding Globulin in Man. Clin. Pharmac. Ther., 23, 47-53.

ANGUS, J.A. (1973). An Investigation of the Cardiovascular Effects of Some Cardiac Stimulants and Depressants with particular reference to Myocardial Contractability. Ph.D. thesis, The University of Sydney.

ANNISON, E.F., HILL, K.J. & LEWIS, D. (1955). Portal Blood Analysis in the Study of the Absorption of Ruminal Fermentation Products. Biochem. J., 60, xix.

ARAKI, Y., YOKOTA, O., KATO, T., KASHIMA, M. & MIYAZAKI, T. (1966). Dynamics of Synthetic Corticosteroids in Man. In Steroid Dynamics, ed. Pincus, G., Nakao, T. & Tait, J.F. pp. 463-480. New York and London: Academic Press.

AREY, L.B. (1956). Developmental Anatomy, 6th Ed., p. 389. Philadelphia and London: W.B. Saunders Company.

ASHKIN, J.R., LYON, D.T., SCHULL, S.D., WAGNER, C.I. & SOLOWAY, R.D. (1978). Factors Affecting Delivery of Bile to the Duodenum in Man. Gastroenterology, 74, 560-565.

ATKINSON, R.M., PARSONS, B.J. & SMYTH, D.H. (1957). The Intestinal Absorption of Glucose. J. Physiol., 135, 581-589.

AUDETAT, V. & BIRCHER, J. (1976). Bioavailability of Prednisolone during Simultaneous Treatment with Cholestyramine. Gastroenterology, 71, 110-111.

AUDETAT, V., PAUMGARTNER, G. & BIRCHER, J. (1977). Beeinträchtigt Cholestyramin die biologische Verfügbarkeit von Prednisolon? Schweizerische Medizinische Wochenschrift, 107, 527-528.

BACK, D.J., BRECKENRIDGE, A.M., CHALLINER, M., CRAWFORD, F.E., ORME, M. L'E., ROWE, P.H. & SMITH, E. (1978). The Effect of Antibiotics on the Enterohepatic Circulation of Ethinyl-estradiol and Norethisterone in the Rat. J. steroid Biochem., 9, 527-531.

BACON, G.E. & KOKENAKES, S. (1969). Measurement of Plasma Prednisolone by Gas Chromatography. J. Lab. clin. Med., 73, 1030-1035.

BAILEY, E. (1967). The Estimation of Cortisol, Prednisolone and Some of Their Unconjugated Metabolites in Biological Fluids Using Gas Liquid Chromatography. In Mem. Soc. Endocrinol. No.16, The Gas Liquid Chromatography of Steroids, ed. Grant, J.K. pp. 183-197. Cambridge: Cambridge University Press.

BAMFORD, D.R. (1966). Studies in vitro of the Passage of Serum Proteins across the Intestinal Wall of Young Rats. Proc. R. Soc. B., 166, 30-45.

BARLOW, O.W. & CLIMENKO, D.R. (1941). Studies on the Pharmacology of Sulfapyridine and Sulfathiazole. The Absorption of the Free Acid, the Sodium Salt and the Glucoside from the Alimentary Tract. J. Am. med. Ass., 116, 282-286.

BARNETT, G., HUI, S. & BENET, L.Z. (1978). Effects of Theophylline on Salicylate Transport in Isolated Rat Jejunum. Biochim. biophys. Acta, 507, 517-523.

BARR, W.H. & RIEGELMAN, S. (1970). Intestinal Drug Absorption and Metabolism I: Comparison of Methods and Models to Study Physiological Factors of in vitro and in vivo Intestinal Absorption. J. pharm. Sci., 59, 154-163.

BARRY, R.J.C., DIKSTEIN, S., MATTHEWS, J., SMYTH, D.H. & WRIGHT, E.M. (1964). Electrical Potentials Associated with Intestinal Sugar Transfer. J. Physiol., 171, 316-338.

BATES, T.R. & GIBALDI, M. (1970). Gastrointestinal Absorption of Drugs. In Current Concepts in the Pharmaceutical Sciences. Biopharmaceutics. ed. Swarbrick, J. pp. 57-99. Philadelphia: Lea and Febiger.

BAYLY, J.H. & GONZALES CARBALHAEZ, O. (1964). The Umbilical Vein in the Adult: Diagnosis, Treatment and Research. Am. Surgeon, 30, 56-60.

BEAN, W.B., FRANKLIN, M., EMBICK, J.F. & DAUM, K. (1951). Absorption Studies Using Portal Anastomotic Veins. J. clin. Invest., 30, 263-273.

BECKETT, A.H. & ROWLAND, M. (1965). Urinary Excretion Kinetics of Amphetamine in Man. J. Pharm. Pharmac., 17, 628-639.

BELING, C.G., GUSTAFSSON, P.O. & KASSTROM, H. (1975). Metabolism of Estradiol in Greyhounds and German Shepherd Dogs. An Investigation with Special Reference to Hip Dysplasia. Acta Radiol. Suppl., 344, 109-120.

BELL, G.H. & PARSONS, D.S. (1976). Edward Waymouth Reid: A Pioneer Investigator of Epithelial Transport. J. Physiol., 263, 75P-78P.

BELLENGER, C.R. (1976). University of Sydney Veterinary Clinic. Personal communication.

BELZ, G.G., STAUCH, M. & RUDOFSKY, G. (1974). Plasma Levels after a Single Oral Dose of Proscillaridin. Eur. J. clin. Pharmac., 7, 95-97.

BENCE JONES, H. & DUPRE, A. (1867). On a Fluorescent Substance, resembling Quinine in Animals and the Rate of Passage of Quinine into the Vascular and Non-vascular Textures of the Body. Proc. R. Soc., 15, 73-93.

BENET, L.Z., ORR, J.M., TURNER, R.H. & WEBB, H.S. (1971). Effect of Buffer Constituents and Time on Drug Transfer Across in vitro Rat Intestine. J. pharm. Sci., 60, 234-238.

BENSADOUN, A. & REID, J.T. (1962). Estimation of Rate of Portal Blood Flow in Ruminants: Effect of Feeding, Fasting, and Anesthesia. J. Dairy Sci., 45, 540-543.

BERGER, E.Y., KANZAKI, G., HOMER, M.A. & STEELE, J.M. (1959). Simultaneous Flux of Sodium into and out of the Dog Intestine. Am. J. Physiol., 196, 74-82.

BEVAN, D.R., CALDWELL, J., D'SOUZA, J. & SMITH, R.L. (1978). Influence of Impaired Renal Function on the Disposition of [^{14}C] Fazadinium in the Anaesthetized Greyhound. Br. J. Pharmac., 64, 402P-403P.

BIANCHI, R. & ROSSI, R. (1957). Rilievi Anatomo-funzionali sul Circolo Portoepatico e Polmonare Indacati con Au - 198 Adsorbito su Particelle di Carbone. Poll. Soc. Med. Chir., Pisa, 25, 1-4.

BIERMAN, H.R., BYRON, R.L., KELLY, K.H., GILFILLAN, R.S., WHITE, L.P., FREEMAN, N.E. & PETRAKIS, N.L. (1953). The Characteristics of Thoracic Duct Lymph in Man. J. clin. Invest., 32, 637-649.

BLANKENHORN, D.H., HIRSCH, J. & AHRENS, E.H., JR. (1955). Trans Intestinal Intubation: Technic for Measurement of Gut Length and Physiologic Sampling at known Loci. Proc. Soc. exp. Biol. Med., 88, 356-362.

BOASS, A. & WILSON, T.H. (1962). Development of Mechanisms for Intestinal Absorption of Vitamin B_{12} in the Growing Rat. Fedn Proc., 21, 469.

BOLLMAN, J.L., CAIN, J.C. & GRINDLAY, J.H. (1948). Techniques for the Collection of Lymph from the Liver, Small Intestine or Thoracic Duct of the Rat. J. Lab. clin. Med., 33, 1349-1352.

BOND, J.H., PRENTISS, R.A. & LEVITT, M.D. (1979). The Effects of Feeding on Blood Flow to the Stomach, Small Bowel, and Colon of the Conscious Dog. J. Lab. clin. Med., 93, 594-599.

BORGSTROM, B., DAHLQVIST, A., LUNDH, G. & SJOVALL, J. (1957). Studies of Intestinal Digestion and Absorption in the Human. J. clin. Invest., 36, 1521-1536.

BORLE, A.B., KEUTMANN, H.T. & NEUMAN, W.F. (1963). Role of Parathyroid Hormone in Phosphate Transport Across Rat Duodenum. Am. J. Physiol., 204, 705-709.

BRAASTAD, F.W., CONDON, R.E. & GYORKEY, F. (1967). The Umbilical Vein: Surgical Anatomy in the Normal Adult. Archs Surg., Chicago, 95, 948-955.

BREWSTER, D., JONES, R.S. & SYMONS, A.M. (1977). Effects of Neomycin on the Biliary Excretion and Enterohepatic Circulation of Mestranol and 17β-Oestradiol. Biochem. Pharmac., 26, 943-946.

BROOKS, S.M., WERK, E.E., ACKERMAN, S.J., SULLIVAN, I. & THRASHER, K. (1972). Adverse Effects of Phenobarbital on Corticosteroid Metabolism in Patients with Bronchial Asthma. New England J. Med., 286, 1125-1128.

BUCHER, G.R., ANDERSON, C.E. & ROBINSON, C.S. (1950). Chemical Changes Produced in Isotonic Solutions of Sodium Sulfate and Sodium Chloride by the Small Intestine of the Dog. Am. J. Physiol., 163, 1-13.

BUHLER, D.R., THOMAS, R.C. & SCHLAGEL, C.A. (1965). Absorption, Metabolism and Excretion of 6α-Methyl-prednisolone-^{3}H,21-Acetate Following Oral and Intramuscular Administrations in the Dog. Endocrinology, 76, 852-864.

BURCHARTH, F. (1979). Percutaneous Transhepatic Catheterization Technique of the Portal Venous System. Scand. J. Gastroent., 14, Suppl.53, 133-139.

BURGENER, F.A., GOTHLIN, J.H. & GUTIERREZ, O.H. (1978). Percutaneous Transhepatic Portography in the Dog. Investigative Radiology, 13, 26-27.

BUTLER, J. & GRAY, C.H. (1970). The Metabolism of Betamethasone. J. Endocr., 46, 379-390.

CAMPAGNA, F.A., CURETON, G., MIRIGIAN, R.A. & NELSON, E. (1963). Inactive Prednisone Tablets U.S.P. XVI. J. pharm. Sci., 52, 605-606.

CARR, S.B. & JACOBSON, D.R. (1968). Method for Measurement of Gastrointestinal Absorption in Normal Animals, Combining Portal-Carotid Differences and Telemetered Portal Flow by Doppler Shift. J. Dairy Sci., 51, 721-729.

CHAKRABORTY, J., ENGLISH, J., MARKS, V., DUMASIA, M.C. & CHAPMAN, D.J. (1976). A Radioimmunoassay Method for Prednisolone: Comparison with the Competitive Protein Binding Method. Br. J. clin. Pharmac., 3, 903-906.

CHAPMAN, D.G., CRISAFIO, R. & CAMPBELL, J.A. (1954). The Relation Between in vitro Disintegration Time of Sugar-Coated Tablets and Physiological Availability of Riboflavin. J. Am. pharm. Ass., Sci. Ed., 43, 297-304.

CHAPMAN, D.G., CRISAFIO, R. & CAMPBELL, J.A. (1956). The Relation Between in vitro Disintegration Time of Sugar-Coated Tablets and Physiological Availability of Sodium p-Aminosalicylate. J. Am. pharm. Ass., Sci. Ed., 45, 374-378.

CHEN, C.L., KUMAR, M.S.A., WILLIARD, M.D. & LIAO, T.F. (1978). Serum Hydrocortisone (Cortisol) Values in Normal and Adrenopathic Dogs as Determined by Radioimmunoassay. Am. J. vet. Res., 39, 179-181.

CHERRY, I.S. & CRANDALL, L.A. (1939). The Absence of a Significant Glucose-Lactic Acid Cycle (involving the liver) in Normal Unanesthetized Dogs. Am. J. Physiol., 125, 41-47.

CHIOU, W.L., RIEGELMAN, S. & AMBERG, J.R. (1969). Complications in Using Rabbits for the Study of Oral Drug Absorption. Chem. Pharm. Bull., 17, 2170-2173.

CHODERA, A. & FELLER, K. (1978). Some Aspects of Pharmacokinetic and Biotransformation Differences in Humans and Mammal Animals. Int. J. clin. Pharmac. Biopharm., 16, 357-360.

CHOWHAN, Z.T. & AMARO, A.A. (1977). Everted Rat Intestinal Sacs as an in vitro Model for Assessing Absorptivity of New Drugs. J. pharm. Sci., 66, 1249-1253.

CHRISTOPHERSEN, E.B. & JACKSON, F.C. (1967). A Technique of Transumbilical Portal Vein Catheterization in Adults. Archs Surg., Chicago, 95, 960-963.

COLBURN, W.A. (1976). Pharmacologic Implications of Heparin Interactions with Other Drugs. Drug Metabolism Reviews, 5, 281-293.

COLBURN, W.A. & BULLER, R.H. (1973). Radioimmunoassay for Prednisolone. Steroids, 21, 833-846.

COLBURN, W.A. & BULLER, R.H. (1975). RIA vs CPB for Prednisolone. J. Pharmacokinet. Biopharm., 3, 329-332.

COLBURN, W.A., SIBLEY, C.R. & BULLER, R.H. (1976). Comparative Serum Prednisone and Prednisolone Concentrations following Prednisone or Prednisolone Administration to Beagle Dogs. J. pharm. Sci., 65, 997-1001.

COMMONWEALTH DEPARTMENT OF HEALTH, CANBERRA, A.C.T. (1976). Guidelines for Bioavailability Studies. NDF 4.

CONN, H.O. & BLITZER, B.L. (1976). Nonassociation of Adrenocorticosteroid Therapy and Peptic Ulcer. New England J. Med., 294, 473-479.

CONNER, G.H. & FRIES, G.F. (1960). Studies of Bovine Portal Blood. I. Establishment and Maintenance of Portal and Mesenteric Vein Catheters. Am. J. vet. Res., 21, 1028-1031.

COOPER, H., LEVITAN, R., FORDTRAN, J.S. & INGELFINGER, F.J. (1966). A Method for Studying Absorption of Water and Solute from the Human Small Intestine. Gastroenterology, 50, 1-7.

CORI, C.F. (1925). The Fate of Sugar in the Animal Body. I. The Rate of Absorption of Hexoses and Pentoses from the Intestinal Tract. J. biol. Chem., 66, 691-715.

COX, R.H., PETERSON, L.H. & DETWEILER, D.K. (1976). Comparison of Arterial Hemodynamics in the Mongrel Dog and the Racing Greyhound. Am. J. Physiol., 230, 211-218.

CRANDALL, L.A. & CHERRY, I.S. (1936). The Effect of Dextrose on Hepatic Gluconeogenesis. Am. J. Physiol., 116, 32.

CRANE, R.K. & MANDELSTAM, P. (1960). The Active Transport of Sugars by Various Preparations of Hamster Intestine. Biochim. biophys. Acta, 45, 460-476.

CRANE, R.K. & WILSON, T.H. (1958). In vitro Method for the Study of the Rate of Intestinal Absorption of Sugars. J. appl. Physiol., 12, 145-146.

CRONE, H.D. & KEEN, T.E.B. (1969). An in vitro Study of the Intestinal Absorption of Pyridinium Aldoximes. Br. J. Pharmac., 35, 304-312.

CROUTHAMEL, W.G., DIAMOND, L., DITTERT, L.W. & DOLUISIO, J.T. (1975). Drug Absorption VII: Influence of Mesenteric Blood Flow on Intestinal Drug Absorption in Dogs. J. pharm. Sci., 64, 664-671.

CROUTHAMEL, W.G., DOLUISIO, J.T., JOHNSON, R.E. & DIAMOND, L. (1970). Effect of Mesenteric Blood Flow on Intestinal Drug Absorption. J. pharm. Sci., 59, 878-879.

CURRY, S.H., D'MELLO, A. & MOULD, G.P. (1970). Destruction of Chlorpromazine During Absorption by Rat Intestine in vitro. Br. J. Pharmac., 40, 538p-539p.

CURRY, S.H., D'MELLO, A. & MOULD, G.P. (1971). Destruction of Chlorpromazine During Absorption in the Rat in vivo and in vitro. Br. J. Pharmac., 42, 403-411.

DAHLSTROM, B.E. & PAALZOW, L.K. (1978). Pharmacokinetic Interpretation of the Enterohepatic Recirculation and First-Pass Elimination of Morphine in the Rat. J. Pharmacokinet. Biopharm., 6, 505-519.

D'ARCY, P.F., GRIFFIN, J.P., JENKINS, J.S., KIRK, W.F. & PEACOCK, A.W.C. (1971). Sustained-Release Formulation of Prednisolone Administered Orally to Man. J. pharm. Sci., 60, 1028-1033.

DAVENPORT, H.W. (1964). Gastric Mucosal Injury by Fatty and Acetylsalicylic Acids. Gastroenterology, 46, 245-253.

DAVENPORT, H.W. (1967). Absorption of Taurocholate-24-^{14}C through the Canine Gastric Mucosa. Proc. Soc. exp. Biol. Med., 125, 670-673.

DAVIS, M., WILLIAMS, R., CHAKRABORTY, J., ENGLISH, J., MARKS, V., IDEO, G. & TEMPINI, S. (1978). Prednisone or Prednisolone for the Treatment of Chronic Active Hepatitis? A Comparison of Plasma Availability. Br. J. clin. Pharmac., 5, 501-505.

DE MARCO, T.J. & LEVINE, R.R. (1969). Role of the Lymphatics in the Intestinal Absorption and Distribution of Drugs. J. Pharmac. exp. Ther., 169, 142-151.

DE MOOR, P., STEENO, O., RASKIN, M. & HENDRIKX, A. (1960). Fluorimetric Determination of Free Plasma 11-Hydroxycorticosteroids in Man. Acta endocr., Copenh., 33, 297-307.

DENCKER, H., GOTHLIN, J., MEEUWISSE, G. & TRANBERG, K.-G. (1972a). Human Intestinal Absorption of Glucose Measured by Portal Catheterization. Scand. J. Gastroent., 7, 451-453.

DENCKER, H., JOHANNESSON, E., MEEUWISSE, G., NORRYD, C. & TRANBERG, K. -G. (1972b). Absorption of Maltose as Measured by Portal Catheterization. Scand. J. Gastroent., 7, 707-710.

DENCKER, H., LUNDERQUIST, A., MEEUWISSE, G., NORRYD, C. & TRANBERG, K. -G. (1972c). Absorption of Fructose as Measured by Portal Catheterization. Scand. J. Gastroent., 7, 701-705.

DENCKER, H., MEEUWISSE, G., NORRYD, C., OLIN, T. & TRANBERG, K. -G. (1973). Intestinal Transport of Carbohydrates as Measured by Portal Catheterization in Man. Digestion, 9, 514-524.

DENT, C.E. & SCHILLING, J.A. (1949). Studies on the Absorption of Proteins: The Amino-Acid Pattern in the Portal Blood. Biochem. J., 44, 318-333.

DENTON, A.E., GERSHOFF, S.N. & ELVEHJEM, C.A. (1953). A New Method for Cannulating the Portal Vein of Dogs. J. biol. Chem., 204, 731-735.

DETWEILER, D.K., COX, R.H., ALONSO, R., KNIGHT, D.H., BAGSHAW, R., JONES, A. & PETERSON, L.H. (1974). Hemodynamic Characteristics of the Young Adult Greyhound. Fedn Proc., 33, 360.

DICZFALUSY, E., FRANKSSON, C. & MARTINSEN, B. (1961). Oestrogen Conjugation by the Human Intestinal Tract. Acta endocr., Copenh., 38, 59-72.

DIETSCHY, J.M., SALOMON, H.S. & SIPERSTEIN, M.D. (1966). Bile Acid Metabolism. I. Studies on the Mechanisms of Intestinal Transport. J. clin. Invest., 45, 832-846.

DiSANTO, A.R. & DeSANTE, K.A. (1975). Bioavailability and Pharmacokinetics of Prednisone in Humans. J. pharm. Sci., 64, 109-112.

DITTERT, L.W., ADAMS, H.J., ALEXANDER, F., CHONG, C.W., ELLISON, T. & SWINTOSKY, J.V. (1968). 4-Acetamidophenyl-2,2,2-Trichloroethyl Carbonate Particle Size Studies in Animals and Man. J. pharm. Sci., 57, 1146-1151.

DOLUISIO, J.T., BILLUPS, N.F., DITTERT, L.W., SUGITA, E.T. & SWINTOSKY, J.V. (1969). Drug Absorption I: An in situ Rat Gut Technique Yielding Realistic Absorption Rates. J. pharm. Sci., 58, 1196-1200.

DOMINGUEZ, R. & POMERENE, E. (1945). Calculation of the Rate of Absorption of Exogenous Creatinine. Proc. Soc. exp. Biol. Med., 60, 173-181.

EL DAREER, S.M., STRUCK, R.F., WHITE, V.M., MELLET, L.B. & HILL, D.L. (1977). Distribution and Metabolism of Prednisone in Mice, Dogs and Monkeys. Cancer Treatment Reports, 61, 1279-1289.

ELLIOTT, M.H. & FURNEAUX, R.W. (1971). Surgical Preparation of the Pig for in vivo Hepatic Function Studies. Res. vet. Sci., 12, 594-596.

ENGEL, G. (1972). National Biological Standards Laboratory, Canberra, A.C.T. Personal communication to J.S.Robertson.

ENGLISH, J., CHAKRABORTY, J. & MARKS, V. (1974). A Competitive Protein Binding Method for Plasma Prednisolone Assay. Ann. clin. Biochem., 11, 11-14.

ENGLISH, J., CHAKRABORTY, J., MARKS, V., TRIGGER, D.J. & THOMSON, A.G. (1975). Prednisolone Levels in the Plasma and Urine: A Study of Two Preparations in Man. Br. J. clin. Pharmac., 2, 327-332.

ERWALD, R., WIECHEL, K.-L. & STRANDELL, T. (1977). Effect of Vasopressin on the Mixing of Portal Venous Blood in Awake Man. Am. J. Physiol., 233, H50-H56.

FABRI, P.J., SCOTT, B.C. & NELSON, E.W. (1977). An in vitro Preparation (Thiry-Vella Fistula) Suitable for the Measurement of Intestinal Absorption in the Guinea Pig. Lab. Anim. Sci., 27, 687-689.

FAULKNER, R.T., CZAJKOWSKI, W.P., RAYFIELD, E.J. & HICKMAN, R.L. (1976). Technique for Portal Catheterization in Rhesus Monkeys (Macaca mulatta). Am. J. vet. Res., 37, 473-475.

FELDMAN, S., RICHARDSON, J., FREEMAN, C., POLLOCK, S., BERGER, J., KAPLAN, F. & RHOA, G. (1974). In vitro Assessment of in vivo Absorption of Drug Complexes. J. pharm. Sci., 63, 454-456.

FELDMAN, S., SALVINO, M. & GIBALDI, M. (1970). Physiologic Surface-Active Agents and Drug Absorption VII: Effect of Sodium Deoxycholate on Phenol Red Absorption in the Rat. J. pharm. Sci., 59, 705-707.

FELDMAN, S., WYNN, R.J. & GIBALDI, M. (1968). Effect of Sodium Deoxycholate on Gastric Emptying in the Rat. J. pharm. Sci., 57, 1493-1496.

FERNANDO, O.N. & MOORHEAD, J. (1979). Absorption of Enteric-Coated Prednisolone. Br. med. J., 1, 1795.

FICK, A. (1870). Ueber die Messung des Blutquantums in den Herzventrikeln. S.-B. phys.-med. Ges. Würzburg, p. 16. Cited by ZIERLER, K.L. (1961).

FISCHER, G.M., COX, R.H. & DETWEILER, D.K. (1975). Altered Arterial Connective Tissue in Racing Greyhound Dogs. Experientia, 31, 1426-1427.

FISHER, R.B. & PARSONS, D.S. (1949). A Preparation of Surviving Rat Small Intestine for the Study of Absorption. J. Physiol., 110, 36-46.

FLORINI, J.R., PEETS, E.A. & BUYSKE, D.A. (1961a). Plasma Half-Life, Tissue Distribution, and Excretion of Triamcinolone-H^3. J. Pharmac. exp. Ther., 131, 287-293.

FLORINI, J.R., SMITH, L.L. & BUYSKE, D.A. (1961b). Metabolic Fate of a Synthetic Corticosteroid (Triamcinolone) in the Dog. J. biol. Chem., 236, 1038-1042.

FORTH, W., FURUKAWA, E. & RUMMEL, W. (1969). Die Bestimmung der Intestinalen Resorption von Herzglykosiden durch Messung der ^{3}H-markierten Glykoside im Portalvenenblut und in der Darmlymphe bei Katzen. Arch. Pharmak., 264, 406-419.

FOTHERBY, K. & JAMES, F. (1972). Metabolism of Synthetic Steroids. Advances in Steroid Biochemistry and Pharmacology, 3, 67-165. ed. Briggs, M.H. & Christie, G.A. London and New York: Academic Press.

FOX, J., CARE, A.D. & SWAMINATHAN, R. (1978). The Use of a Thiry-Vella Loop of Jejunum to Study the Intestinal Absorption of Calcium and Inorganic Sulphate in the Conscious Pig. Br. J. Nutr., 39, 431-439.

FRENCH, A.B., BROWN, I.F., GOOD, C.J. & McLEOD, G.M. (1968). Comparison of Phenol Red and Polyethyleneglycol as Nonabsorbable Markers for the Study of Intestinal Absorption in Humans. Am. J. dig. Dis., 13, 558-564.

FRIES, G.F. & CONNER, G.H. (1961). Studies on Bovine Portal Blood. II. Blood Flow Determinations with Observations on Hemodilution in the Portal Vein. Am. J. vet. Res., 22, 487-491.

FRY, F.J., EGGLETON, R.C., KELLY, E. & FRY W.J. (1965). Properties of the Blood Interface Essential to Successful Artificial Heart Function. Trans. Am. Soc. artif. internal Organs, 11, 307-313.

GALLO-TORRES, H.E. & LUDORF, J. (1974). Techniques for the in vivo Catheterization of the Portal Vein in the Rat. Proc. Soc. exp. Biol. Med., 145, 249-254.

GARG, D.C., NG, P., WEIDLER, D.J., SAKMAR, E. & WAGNER, J.G. (1978). Preliminary in vitro and in vivo Investigations on Methylprednisolone and its Acetate. Res. Comm. chem. Path. Pharmac., 22, 37-48.

GATES, G.F. & DORE, E.K. (1973). Streamline Flow in the Human Portal Vein. J. nucl. Med., 14, 79-83.

GIBALDI, M. & GRUNDHOFER, B. (1972a). Drug Transport VI: Functional Integrity of the Rat Everted Small Intestine with Respect to Passive Transfer. J. pharm. Sci., 61, 116-119.

GIBALDI, M. & GRUNDHOFER, B. (1972b). Rate-Limiting Barriers in Intestinal Absorption. Proc. Soc. exp. Biol. Med., 141, 564-568.

GIBALDI, M. & PERRIER, D. (1975). Pharmacokinetics. New York: Marcel Dekker, Inc.

GLANTZ, S.A., LUETSCHER, J.A., DAY, R.P. & PERLOFF, M. (1976). A Compartment Description for Cortisol Secretion, Distribution, Binding and Metabolism in Man. IEEE Trans. biomed. Eng., BME-23, 36-44.

GOLD, N.I. (1961a). Partial Characterization of the Metabolites of Cortisol-4-C^{14} in the Dog I. The Intact Dog. J. biol. Chem., 236, 1924-1929.

GOLD, N.I. (1961b). Partial Characterization of the Metabolites of Cortisol-4-C^{14} in the Dog II. The Totally Hepatectomized Dog. J. biol. Chem., 236, 1930-1933.

GOTT, V.L., WHIFFEN, J.D., KOEPKE, D.E., DAGGETT, R.L., BOAKE, W.C. & YOUNG, W.P. (1964). Techniques of Applying a Graphite-Benzalkonium-Heparin Coating to Various Plastics and Metals. Trans. Am. Soc. artif. internal Organs, 10, 213-217.

GRAHAM, G.G. (1979). University of New South Wales, School of Physiology and Pharmacology. Personal communication.

GRALLA, E.J., CAPPIELLO, F.M. & JONAS, A.M. (1973). Technique for Chronic Cannulation of the Canine Thoracic Duct. Am. J. vet. Res., 34, 285-287.

GRAY, C.H., GREEN, M.A.S., HOLNESS, N.J. & LUNNON, J.B. (1956). Urinary Metabolic Products of Prednisone and Prednisolone. J. Endocr., 14, 146-154.

GRAYSON, J. (1954). The Intrinsic Factors in the Regulation of Liver Blood Flow. Gastroenterologia, 81, 81. Cited by Ahlgren, I., Aronsen, K.F. & Nylander, G. (1968).

GREEN, O.C. (1979). Prednisolone pharmacokinetics in Relation to Dose. J. Pediatrics, 94, 1015.

GREEN, O.C., WINTER, R.J., KAWAHARA, F.S., PHILLIPS, L.S., LEWY, P.R., HART, R.L. & PACHMAN, L.M. (1978). Pharmacokinetic Studies of Prednisolone in Children. Plasma Levels, Half-Life Values, and Correlation with Physiologic Assays for Growth and Immunity. J. Pediatrics, 93, 299-303.

GRINDLAY, J.H., CAIN, J.C., BOLLMAN, J.L. & MANN, F.C. (1950). Lymph Fistulas in Trained Dogs. An Experimental Technique. Surgery, 27, 152-158.

GRISAFE, J.A. & HAYTON, W.L. (1978). Effects of Short and Medium Chain Fatty Acids on Absorption of Lipophilic Drugs from Perfused Rat Intestine. J. pharm. Sci., 67, 1211-1215.

GRUNDY, R.U., MCAINSH, J. & TAYLOR, D.C. (1974). The Effect of Food on the in vivo Release of Propranolol from a PVC Matrix Tablet in the Dog. J. Pharm. Pharmac., 26, Suppl., 65P-66P.

GUNN, H.M. (1978). The Proportions of Muscle, Bone and Fat in Two Different Types of Dog. Res. vet. Sci., 24, 277-282.

HAASS, A., LULLMANN, H. & PETERS, Th. (1972). Absorption Rates of Some Cardiac Glycosides and Portal Blood Flow. Eur. J. Pharmac., 19, 366-370.

HABERICH, F.J. (1968). Osmoreception in the Portal Circulation. Fedn Proc., 27, 1137-1141.

HABERICH, F.J., AZIZ, O., NOWACKI, P.E. & OHM, W. (1969). Zur Spezifitat der Osmoreceptoren in der Leber. Pflugers Arch. ges. Physiol., 313, 289-299.

HAHN, P.F., DONALD, W.D. & GRIER, R.C. (1945). The Physiological Bilaterality of Portal Circulation; Streamline Flow of Blood into Liver as Shown by Radioactive Phosphorus. Am. J. Physiol., 143, 105-107.

HAKIM, A., LESTER, R.G. & LIFSON, N. (1963). Absorption by an in vitro Preparation of Dog Intestinal Mucosa. J. appl. Physiol., 18, 409-413.

HANNGREN, A., HANSSON, E., SJOSTRAND, S.E. & ULLBERG, S. (1964). Autoradiographic Distribution Studies with ^{14}C-Cortisone and ^{14}C-Cortisol. Acta endocr., Copenh., 47, 95-104.

HANZLIK, P.J. & COLLINS, R.J. (1913). Quantitative Studies on the Gastro-Intestinal Absorption of Drugs III. The Absorption of Alcohol. J. Pharmac. exp. Ther., 5, 185-213.

HARRIS, P.A. & RIEGELMAN, S. (1969). Influence of the Route of Administration on the Area Under the Plasma Concentration-Time Curve. J. pharm. Sci., 58, 71-75.

HARRISON, F.A. (1969). The Introduction and Maintenance of Permanently Indwelling Catheters in the Portal and Hepatic Veins of the Sheep. J. Physiol., 200, 28p-30p.

HARTIALA, K.J.V., LEIKKOLA, P. & SAVOLA, P. (1958). Further Studies on the Intestinal Glucuronide Synthesis. Acta physiol. scand., 42, 36-40.

HAUGE, C.W. & KRIPPAEHNE, W.W. (1970). Experimental Studies of Absorption of Methionine in Intestinal Loops of Dogs. Am. J. Surg., 119, 67-69.

HAYTON, W.L. (1975). Effects of Normal Alcohols on Intestinal Absorption of Salicylic Acid, Sulfapyridine and Prednisolone in Rats. J. pharm. Sci., 64, 1450-1456.

HAYTON, W.L. & LEVY, G. (1972a). Effect of Complex Formation on Drug Absorption XII: Enhancement of Intestinal Absorption of Prednisone and Prednisolone by Dialkylpropionamides in Rats. J. pharm. Sci., 61, 362-366.

HAYTON, W.L. & LEVY, G. (1972b). Effect of Complex Formation on Drug Absorption XIII: Effect of Constant Concentrations of N,N-di-n-propylpropionamide on Prednisolone Absorption from the Rat Small Intestine. J. pharm. Sci., 61, 367-371.

HENDERSON, R.G., WHEATLEY, T., ENGLISH, J., CHAKRABORTY, J. & MARKS, V. (1979). Variation in Plasma Prednisolone Concentrations in Renal Transplant Recipients Given Enteric-Coated Prednisolone. Br. med. J., 1, 1534-1536.

HEVESY, G. & HOFER, E. (1934). Elimination of Water from the Human Body. Nature, Lond., 134, 879.

HILEY, C.R., YATES, M.S. & BACK, D.J. (1978). The Effect of Urethane and Pentobarbital Anaesthesia and Hepatic Portal Vein Catheterization on Liver Blood Flow in the Rat. Experientia, 34, 1061-1062.

HIROM, P.C, MILLBURN, P. & SMITH, R.L. (1976). Bile and Urine as Complementary Pathways for the Excretion of Foreign Organic Compounds. Xenobiotica, 6, 55-64.

HIROM, P.C., MILLBURN, P., SMITH, R.L. & WILLIAMS, R.T. (1972). Species Variation in the Threshold Molecular-Weight Factor for Biliary Excretion of Organic Anions. Biochem. J., 129, 1071-1077.

HOGBEN, C.A.M., SCHANKER, L.S., TOCCO, D.J. & BRODIE, B.B. (1957). Absorption of Drugs from the Stomach II. The Human. J. Pharmac. exp. Ther., 120, 540-545.

HOGBEN, C.A.M., TOCCO, D.J., BRODIE, B.B. & SCHANKER, L.S. (1959). On the Mechanism of the Intestinal Absorption of Drugs. J. Pharmac. exp. Ther., 125, 275-282.

HOOVER, T.R., WOLFROM, G.W. & PADDLEFORD, R.R. (1976). Twelfth Rib Resection as an Approach for Portal Vein Cannulation in Sheep. Can. J. comp. Med., 40, 416-420.

HORNING, E.C., VANDENHEUVAL, W.J.A. & CREECH, B.G. (1963). Separation and Determination of Steroids by Gas Chromatography. In Methods of Biochemical Analysis, Volume 11, ed. Glick, D. pp. 69-147. New York and London: Interscience Publishers.

HOUSTON, J.B. & LEVY, G. (1975). Effect of Various Alcohols on Intestinal Net Water Flux and Theophylline Absorption in Rats. J. pharm. Sci., 64, 607-610.

HSUEH, W.A., PAZ-GUEVARA, A. & BLEDSOE, T. (1979). Studies Comparing the Metabolic Clearance Rate of 11β,17,21-Trihydroxypregn-1,4-diene-3,20-dione (Prednisolone) After Oral 17,21-Dihydroxy-pregn-1,4-diene-3,11,20-trione and Intravenous Prednisolone. J. clin. Endocr. Metab., 48, 748-752.

HULME, B., JAMES, V.H.T. & RAULT, R. (1975). Absorption of Enteric and Non-Enteric Coated Prednisolone Tablets. Br. J. clin. Pharmac., 2, 317-320.

HUNT, J.N. (1951). The Secretory Pattern of the Stomach of Man. J. Physiol., 113, 169-184.

HURWITZ, A. (1979). Drug Uptake into Everted Intestinal Sacs II. Inhibition of Secretion by Hypertonicity. Am. J. Physiol., 236, E57-E62.

HURWITZ, A. & GUTMAN, Y. (1979). Drug Uptake into Everted Intestinal Sacs I. Enhancement by Hypertonicity. Am. J. Physiol., 236, E52-E56.

HWANG, S.W. & SCHANKER, L.S (1973). Absorption of Organic Arsenical Compounds from the Rat Small Intestine. Xenobiotica, 3, 351-355.

HYDE, P.M. & WILLIAMS, R.H. (1957). Absorption and Metabolism of Hydrocortisone-4-C^{14}. J. biol. Chem., 227, 1063-1081.

INDEGLIA, R.A., DORMAN, F.D., CASTANEDA, A.R., VARCO, R.L. & BERNSTEIN, E.F. (1966). Use of GBH-Coated Tygon Tubing for Experimental Prolonged Perfusions Without Systemic Heparinization. Trans. Am. Soc. artif. internal Organs, 12, 166-173.

IRWIN, G.M., KOSTENBAUDER, H.B., DITTERT, L.W., STAPLES, R., MISHER, A. & SWINTOSKY, J.V. (1969). Enhancement of Gastrointestinal Absorption of a Quaternary Ammonium Compound by Trichloroacetate. J. pharm. Sci., 58, 313-315.

ISBISTER, J.P., SPEROS, J. & STEINBECK, A.W. (1969). The Absorption of Prednisolone B.P. as 5 mg and 25 mg Tablets. Med. J. Aust., 1, 1135-1136.

ISSELBACHER, K.J. (1956). Enzymatic Mechanisms of Hormone Metabolism II. Mechanism of Hormonal Glucuronide Formation. Recent Progr. Horm. Res., 12, 134-146.

IVEY, K.J., MORRISON, S. & GRAY, C. (1972). Effect of Salicylates on the Gastric Mucosal Barrier in Man. J. appl. Physiol., 33, 81-85.

IVEY, K.J., PARSONS, C. & WEATHERBY, R. (1975). Effect of Prednisolone and Salicylic Acid on Ionic Fluxes Across the Human Stomach. Aust. N.Z. J. Med., 5, 408-412.

IVEY, K.J. & SCHEDL, H.P. (1970). Gastric Nonabsorbable Indicators for Studies in Man. Gastroenterology, 59, 234-239.

IWAMOTO, K. & KLAASSEN, C.D. (1977). First-Pass Effect of Morphine in Rats. J. Pharm. exp. Ther., 200, 236-244.

JACKSON, J.B., RADELEFF, R.D. & BUCK, W.B. (1960). Multicatheterization of Blood Vessels of Sheep. J. Am. vet. med. Ass., 136, 440-443.

JENKINS, J.S. (1966). The Metabolism of Cortisol by Human Extra-Hepatic Tissues. J. Endocr., 34, 51-56.

JENKINS, J.S. & SAMPSON, P.A. (1967). Conversion of Cortisone to Cortisol and Prednisone to Prednisolone. Br. med. J., 2, 205-207.

JONES, R.S., TREDGER, J.M., CHAKRABORTY, J. & PARKE, D.V. (1975). The Biliary Excretion of Betamethasone in the Rat. Biochem. Soc. Trans., 3, 408-410.

JORDANA, R. & PONZ, F. (1969). Effects of the Pre-Incubation in a Na^+-free Medium on the O_2 Uptake and Glucose Utilization by the Intestine. Revta esp. Fisiol., 25, 225-232.

JORGENSEN, C.R., LANDAU, B.R. & WILSON, T.H. (1961). A Common Pathway for Sugar Transport in Hamster Intestine. Am. J. Physiol., 200, 111-116.

JUSKO, W.J. & LEVY, G. (1967). Absorption, Metabolism, and Excretion of Riboflavin-5'-Phosphate in Man. J. pharm. Sci., 56, 58-62.

KAKEMI, K., ARITA, T., HORI, R. & KONISHI, R. (1967). Absorption and Excretion of Drugs XXXII. Absorption of Barbituric Acid Derivatives from Rat Small Intestine. Chem. Pharm. Bull., 15, 1883-1887.

KAKEMI, K., ARITA, T. & MURANISHI, S. (1965). Absorption and Excretion of Drugs XXV. On the Mechanism of Rectal Absorption of Sulfonamides. Chem. Pharm. Bull., 13, 861-869.

KAKEMI,K., SEZAKI, H., KONISHI, R., KIMURA, T. & OKITA, A. (1970). Effect of Bile Salts on the Gastrointestinal Absorption of Drugs. II. Mechanism of the Enhancement of the Intestinal Absorption of Sulfaguanidine by Bile Salts. Chem. Pharm. Bull., 18, 1034-1039.

KAPLAN, S.A. & COTLER, S. (1972). Use of Cannulated Everted Intestinal Sac for Serial Sampling as a Drug Absorbability (Permeability) Screen. J. pharm. Sci., 61, 1361-1365.

KATZ, M.L. & BERGMAN, E.N. (1969). A Method for Simultaneous Cannulation of the Major Splanchnic Blood Vessels of the Sheep. Am. J. vet. Res., 30, 655-661.

KENDALL, C.E. (1972). National Biological Standards Laboratory, Fyshwick, A.C.T. Personal communication.

KITAGAWA, H., MOHRI, T. & KITAGAWA, M. (1972). Comparative Studies on Anti-Inflammatory Effect and Biological Fates of 21-Phosphates and -Sulfates of Dexamethasone and Prednisolone. Arzneim.-Forsch., 22, 402-410.

KITAZAWA, S. & JOHNO, I. (1977). Effects of Blood Glucose and Plasma Osmolality on Transmucosal Fluid Movement and Intestinal Drug Absorption. Chem. Pharm. Bull., 25, 115-121.

KLAASSEN, C.D. (1975). Biliary Excretion of Xenobiotics. CRC Critical Reviews in Toxicology, 4, 1-29.

KOIZUMI, T., ARITA, T. & KAKEMI, K. (1964a). Absorption and Excretion of Drugs XIX. Some Pharmacokinetic Aspects of Absorption and Excretion of Sulfonamides. (1). Absorption from Rat Stomach. Chem. Pharm. Bull., 12, 413-420.

KOIZUMI, T., ARITA, T. & KAKEMI, K. (1964b). Absorption and Excretion of Drugs XX. Some Pharmacokinetic Aspects of Absorption and Excretion of Sulfonamides. (2). Absorption from Rat Small Intestine. Chem. Pharm. Bull., 12, 421-427.

KOJIMA, S., SMITH, R.B., CROUTHAMEL, W.G. & DOLUISIO, J.T. (1972). Drug Absorption VI: Water Flux and Drug Absorption in an in situ Rat Gut Preparation. J. pharm. Sci., 61, 1061-1064.

KORNEL, L. & SAITO, Z. (1975). Studies on Steroid Conjugates - VIII: Isolation and Characterization of Glucuronide-Conjugated Metabolites of Cortisol in Human Urine. J. steroid Biochem., 6, 1267-1284.

KOZOWER, M., VEATCH, L. & KAPLAN, M.M. (1974). Decreased Clearance of Prednisolone, a Factor in the Development of Cortico-steroid Side Effects. J. clin. Endocr. Metab., 38, 407-412.

KRAAN, W.J., HUISMAN, G.H. & VELTHUIZEN, J. (1978). Splenic Storage Volume in the Unanesthetized Resting Beagle. Eur. J. appl. Physiol., 38, 197-206.

KRAMER, K. & LUFT, U.C. (1951). Mobilization of Red Cells and Oxygen From the Spleen in Severe Hypoxia. Am. J. Physiol., 165, 215-228.

KUIPERS, F., ELY, R.S., HUGHES, E.R. & KELLEY, V.C. (1957). Metabolism of Steroids II. Half-Life of Various Steroids in Dogs. Proc. Soc. exp. Biol. Med., 95, 187-189.

LACKMANN, L., BARRETT, W., RINEHART, R.K. & SHEPPARD, H. (1964). The in vivo Effectiveness of Enteric-Film Coatings Applied to Hydrochlorothiazide-potassium chloride Tablets by a Programmed Automated Coating Process. Curr. ther. Res., 6, 491-504.

LECLERCQ, R. & COPINSCHI, G. (1974). Patterns of Plasma Levels of Prednisolone After Oral Administration in Man. J. Pharmacokinet. Biopharm., 2, 175-187.

LEE, D.A.H., TAYLOR, G.M., WALKER, J.G. & JAMES, V.H.T. (1979a). The Effect of Food and Tablet Formulation on Plasma Prednisolone Levels Following Administration of Enteric-Coated Tablets. Br. J. clin. Pharmac., 7, 523-528.

LEE, D.A.H., TAYLOR, G.M., WALKER, J.G. & JAMES, V.H.T. (1979b). The Effect of Concurrent Administration of Antacids on Prednisolone Absorption. Br. J. clin. Pharmac., 8, 92-94.

LEPPER, K. & MAILMAN, D.S. (1977). An Ultrastructural Examination of Everted Rat Jejunum. Life Sci., Oxford, 20, 1697-1704.

LEVINE, R.R., BLAIR, M.R. & CLARK, B.B. (1955). Factors Influencing the Intestinal Absorption of Certain Monoquaternary Anticholinergic Compounds with Special Reference to Benzomethamine [N-diethylaminoethyl-N^1-methylbenzilamide methobromide (MC-3199)]. J. Pharmac. exp. Ther., 114, 78-86.

LEVINE, R.R., McNARY, W.F., KORNGUTH, P.J. & LeBLANC, R. (1970). Histological Reevaluation of Everted Gut Technique for Studying Intestinal Absorption. Eur. J. Pharmac., 9, 211-219.

LEVINE, R.R. & PELIKAN, E.W. (1961). The Influence of Experimental Procedures and Dose on the Intestinal Absorption of an Onium Compound, Benzomethamine. J. Pharmac. exp. Ther., 131, 319-327.

LEVINE, W.G. (1978). Biliary Excretion of Drugs and Other Xenobiotics. Annual Reviews in Pharmacology and Toxicology, 18, 81-96.

LEVY, G., HALL, N.A. & NELSON, E. (1964). Studies on Inactive Prednisone Tablets U.S.P. XVI. Am. J. hosp. Pharm., 21, 402.

LEWIS, D., HILL, K.J. & ANNISON, E.F. (1957). Studies on the Portal Blood of Sheep. 1. Absorption of Ammonia from the Rumen of the Sheep. Biochem. J., 66, 587-592.

LEWIS, G.P., JICK, H., SLONE, D. & SHAPIRO, S. (1971a). The Role of Genetic Factors and Serum Protein Binding in Determining Drug Response as Revealed by Comprehensive Drug Surveillance. Ann. N.Y. Acad. Sci., 179, 729-738.

LEWIS, G.P., JUSKO, W.J., BURKE, C.W. & GRAVES, L. (1971b). Prednisone Side-Effects and Serum Protein Levels. Lancet, 2, 778-781.

LIEN, W.M., McCORMICK, J.R., DAVIES, M.W. & EGDAHL, R.H. (1970). Corticosteroid Clearance by the Gastrointestinal Tract in Dogs and Monkeys. Endocrinology, 87, 206-208.

LONDON, E.S. (1928). Experimental Fistulae of Blood Vessels. Harvey Lect., Ser. 23, 208-221.

LONDON, E.S. & KOTSCHNEFF, N.P. (1934). In Welcher Form Wird Nahrungseiweiss Resorbiert? I. Mitteilung. Hoppe-Seyler's Z. physiol. Chem., 228, 235-242.

LOO, J.C.K., BUTTERFIELD, A.G., MOFFATT, J. & JORDAN, N. (1977). Analysis of Prednisolone in Plasma by High-Performance Liquid Chromatography. J. Chromatog., 143, 275-280.

LOO, J.C.K. & JORDAN, N. (1978). A Specific Radio-Immunological Assay for Prednisolone in Plasma. Res. Comm. chem. Path. Pharmac., 21, 523-529.

LOO, J.C.K., McGILVERAY, I.J., JORDAN, N., MOFFAT, J. & BRIEN, R. (1978). Dose-Dependent Pharmacokinetics of Prednisone and Prednisolone in Man. J. Pharm. Pharmac., 30, 736.

LOO, J.C.K. & RIEGELMAN, S. (1968). New Method for Calculating the Intrinsic Absorption Rate of Drugs. J. pharm. Sci., 57, 918-928.

LOVERING, E.G. & BLACK, D.B. (1974). Drug Permeation through Membranes III. Effect of pH and Various Substances on the Permeation of Phenylbutazone through Everted Rat Intestine and Polydimethylsiloxane. J. pharm. Sci., 63, 671-676.

LOVERING, E.G., McGILVERAY, I.J., McMILLAN, I. & TOSTOWARYK, W. (1975). Comparative Bioavailabilities from Truncated Blood Level Curves. J. pharm. Sci., 64, 1521-1524.

MACAROL, V., MORRIS, T.Q., BAKER, K.J. & BRADLEY, S.E. (1970). Hydrocortisone Choleresis in the Dog. J. clin. Invest., 49, 1714-1723.

McCANCE, R.A. & MADDERS, K. (1930). The Comparative Rates of Absorption of Sugars from the Human Intestine. Biochem. J., 24, 795-804.

McCORMICK, J.R., HERMAN, A.H., LIEN, W.M. & EGDAHL, R.H. (1974). Hydrocortisone Metabolism in the Adrenalectomized Dog. The Quantitative Significance of Each Organ System in the Total Metabolic Clearance of Hydrocortisone. Endocrinology, 94, 17-26.

McGILLIARD, A.D. (1968). Catheterization of the Thoracic Aorta and Portal Vein via the Umbilical Arteries and Umbilical Vein. J. Anim. Sci., 27, 1194.

McGILLIARD, A.D. (1971). An Implantable Hydraulic Needle for Sampling Blood or Infusion of the Portal Vein in Calves. J. appl. Physiol., 30, 297-301.

McGILLIARD, A.D. & THORP, J.W. (1971). Catheterization of the Mesenteric and Portal Vein in Calves. J. Dairy Sci., 54, 129-132.

MAGEE, H.E. & REID, E. (1931). The Absorption of Glucose from the Alimentary Canal. J. Physiol., 73, 163-183.

MALT, R.A., CORRY, R.J. & CHAVEZ-PEON, F. (1968). Umbilical Vein Cannulation in Portal-System Disease. New Engl. J. Med., 279, 930-932.

MANT, T.G.K. (1979). Investigation of a Case of Prednisolone Malabsorption. Postgrad. med. J., 55, 421-422.

MARKOWITZ, J., ARCHIBALD, J. & DOWNIE, H.G. (1964). Experimental Surgery, 5th ed. p. 143. Baltimore: Williams and Wilkins.

MARTIN, F., BHARGAVA, A.S. & ADLERCREUTZ, H. (1977). Androgen Metabolism in the Beagle: Endogenous $C_{19}O_2$ Metabolites in Bile and Faeces and the Effect of Ampicillin Administration. J. steroid Biochem., 8, 753-760.

MARTIN, R.P., LORIAUX, D.L. & FARNHAM, G.S. (1965). Enterohepatic Cycling of Metabolized Testosterone in the Male Dog. Steroids, Suppl., 2, 149-163.

MATIN, S.B. & AMOS, B. (1978). Quantitative Determination of Prednisone and Prednisolone in Human Plasma Using GLC and Chemical-Ionization Mass Spectrometry. J. pharm. Sci., 67, 923-926.

MATTHEWS, D.M. & SMYTH, D.H. (1954). The Intestinal Absorption of Amino-Acid Enantiomorphs. J. Physiol., 126, 96-100.

MAY, C.S., CAFFIN, J.A., HALLIDAY, J.W. & BOCHNER, F. (1980). Prednisolone Pharmacokinetics in Asthmatic Patients. Br. J. Dis. Chest, 74, 91-92.

MAYERSOHN, M. & GIBALDI, M. (1970). Drug Transport II. The Effect of Various Cations on the Passive Transfer of Drugs Across the Everted Rat Intestine. Biochim. biophys. Acta, 196, 296-304.

MAYERSOHN, M., GIBALDI, M. & GRUNDHOFER, B. (1971). Drug Transport V: Mechanism of Potassium-Ion Inhibition of Passive Transfer of Solutes Across Everted Rat Intestine. J. pharm. Sci., 60, 1813-1817.

MAYERSOHN, M. & SURYASAPUTRA, K. (1973). Transport Cell for Examining Solute Transfer Across Biological Membranes. J. pharm. Sci., 62, 681-683.

MEIKLE, A.W., WEED, J.A. & TYLER, F.H. (1975). Kinetics and Interconversion of Prednisolone and Prednisone Studied with New Radioimmunoassays. J. clin. Endocr. Metab., 41, 717-721.

MELNICK, D., HOCHBERG, M. & OSER, B.L. (1945). Physiological Availability of the Vitamins I. The Human Bioassay Technique. J. Nutr., 30, 67-79.

MERING, J. von (1877). Ueber die Abzugswege des Zuckers aus der Darmhohle. Arch. Anat. Physiol., Physiol. Abtheil., 379-415.

MILLER, D.L. & SCHEDL, H.P. (1970). Total Recovery Studies of Nonabsorbable Indicators in the Rat Small Intestine. Gastroenterology, 58, 40-46.

MILLER, M.E., CHRISTENSEN, G.C. & EVANS, H.E. (1964). Anatomy of the Dog. p. 704. Philadelphia and London: W.B. Saunders Company.

MILLER, T.G. (1944). Intubation Studies of the Human Small Intestine. XXIV. A Review of a Ten Year Experience. Gastroenterology, 3, 141-154.

MILLER, T.G. & ABBOTT, W.O. (1934). Intestinal Intubation: A Practical Technique. Am. J. med. Sci., 187, 595-599.

MIYABO, S., KISHIDA, S. & HISADA, T. (1976). Cortisol Metabolites in Dog Plasma. J. steroid Biochem., 7, 61-64.

MIYABO, S. & KORNEL, L. (1974). Corticosteroids in Human Blood. VI. Isolation, Characterization and Quantitation of Sulphate Conjugated Metabolites of Cortisol in Human Plasma. J. steroid Biochem., 5, 233-247.

MIYACHI, Y., YOTSUMOTO, H., KANO, T., MIZUCHI, A., MUTO, T. & YANAGIBASHI, K. (1979). Blood Levels of Synthetic Glucocorticoids After Administration by Various Routes. J. Endocr., 82, 149-157.

MODIGLIANI, R., RAMBAUD, J.C. & BERNIER, J.J (1978). Validation of the Use of a Tube with a Proximal Occlusive Balloon for Measurement of Intestinal Absorption in Man. Am. J. dig. Dis., 23, 720-722.

MOODIE, E.W. & WALKER, A.I.T. (1963). Arterial and Portal Venous Blood for Estimating Mineral Absorption in the Conscious Sheep. Q. Jl exp. Physiol., 48, 388-401.

MOODIE, E.W., WALKER, A.I.T. & HUTTON, P.H. (1963). The Collection of Portal and Hepatic Venous Blood in Conscious Sheep. Q. Jl exp. Physiol., 48, 379-387.

MORRISON, P.J., BRADBROOK, I.D. & ROGERS, H.J. (1977). Plasma Prednisolone Levels from Enteric and Non-Enteric Coated Tablets Estimated by an Original Technique. Br. J. clin. Pharmac., 4, 597-603.

MOSS, M.S. & RYLANCE, H.J. (1967). A Thin-Layer Chromatography Study on the Metabolism of Prednisolone in the Horse. J. Endocr., 37, 129-137.

MOUZAS, G.L. & SMITH, G. (1969). A Method for Withdrawing Blood from the Portal Vein in Dogs Using Silastic Tubing. Int. Surg., 51, 495-498.

NAYAK, R.K. & BENET, L.Z. (1971). Drug Transfer Across Rat Intestinal Musculature After Edetic Acid Treatment. J. pharm. Sci., 60, 1508-1511.

NAYAK, R.K. & BENET, L.Z. (1974). The Use of the Unanesthetized Rhesus Monkey as a Model for Studying the Gastrointestinal Absorption of Drugs. J. Pharmacokinet. Biopharm., 2, 417-431.

NEWSOME, H.H., TURLEY, G.T. & KENNERLY, M. (1977). Bioavailability of Synthetic Steroids for Canine Adrenal Suppression. J. surg. Res., 23, 315-320.

NICHOLSON, J.T.L. & CHORNOCK, F.W. (1942). Intubation Studies of the Human Small Intestine. XXII. An Improved Technic for the Study of Absorption; its Application to Ascorbic Acid. J. clin. Invest., 21, 505-509.

NIENSTEDT, W. & HARRI, M.P. (1979). Intestinal Absorption and Metabolism of 17-Hydroxyprogesterone, Deoxycorticosterone and Cortisol in the Dog. J. steroid Biochem., 11, 1209-1215.

NIGHTINGALE, C.H., AXELSON, J.E. & GIBALDI, M. (1971). Physiologic Surface-Active Agents and Drug Absorption VIII: Effect of Bile Flow on Sulfadiazine Absorption in the Rat. J. pharm. Sci., 60, 145-147.

NOGAMI, H., HANANO, M., AWAZU, S. & IGA, T. (1970). Pharmacokinetic Aspects of Biliary Excretion. Dose Dependency of Riboflavin in Rat. Chem. Pharm. Bull., 18, 228-234.

NOGAMI, H. & MATSUZAWA, T. (1961). Studies on Absorption and Excretion of Drugs. I. Kinetics of Penetration of Acidic Drug, Salicylic Acid through the Intestinal Barrier in vitro. Chem. Pharm. Bull., 9, 532-540.

NOTARI, R.E. (1975). Biopharmaceutics and Pharmacokinetics: An Introduction. 2nd ed. New York: Marcel Dekker, Inc.

NOVAK, E., DiSANTO, A.R., SECKMAN, C.E., ELLIOTT, G., LEE, J.G. & STUBBS, S.S. (1977). The Clinical Pharmacology of Methylprednisolone Sodium Phosphate. I. Intramuscular Route of Administration. J. clin. Pharmac., 17, 324-333.

NUGENT, C.A., WARNER, H.R., ESTERGREEN, V.L. & EIK-NES, K.B. (1965). The Distribution and Disposal of Cortisol in Humans. In Proceedings of the Second International Congress of Endocrinology, London, 1964, ed. Taylor, S. pp. 257-261.

OCHSENFAHRT, H. (1974). The Viability of the Vascularly Perfused Jejunal Loop of the Rat and its Significance for the Drug Absorption. Arch. Pharmak., 282, Suppl., R71.

OCHSENFAHRT, H. (1979). The Relevance of Blood Flow for the Absorption of Drugs in the Vascularly Perfused, Isolated Intestine of the Rat. Naunyn-Schmiedeberg's Arch. Pharmac., 306, 105-112.

OCHSENFAHRT, H. & WINNE, D. (1968). Intestinal Blood Flow and Drug Absorption from the Rat Jejunum. Life Sci., Oxford, 7, 493-498.

OCHSENFAHRT, H. & WINNE, D. (1969). Der EinfluB der Durchblutung auf die Resorption von Arzneimitteln aus dem Jejunum der Ratte. Arch. Pharmak., 264, 55-75.

ODLIND, V., WEINER, E., VICTOR, A. & JOHANSSON, E.D.B. (1979). Plasma Levels of Norethindrone After Single Oral Dose Administration of Norethindrone and Lynestrenol. Clinical Endocrinology, 10, 29-38.

ORTEN, A.U. (1961). Absorption of Amino Acids and Sugars from a Thiry Loop in Man. Fedn Proc., 20, 2.

OVERHOLT, B.F. & POLLARD, H.M. (1968). Acetylsalicylic Acid and Ionic Fluxes Across the Gastric Mucosa of Man. Gastroenterology, 54, 538-542.

PARSONS, D.S. (1968). Methods for Investigation of Intestinal Absorption. In Handbook of Physiology, Section 6, Alimentary Canal, Volume III, Intestinal Absorption, ed. Code, C.F. & Heidel, W. pp. 1177-1216. Washington, D.C.: American Physiological Society.

PARSONS, D.S. & PATERSON, C.R. (1965). Fluid and Solute Transport Across Rat Colonic Mucosa. Q. Jl exp. Physiol., 50, 220-231.

PARSONS, D.S. & PRICHARD, J.S. (1965). Hydrolysis of Disaccharides During Absorption by the Perfused Small Intestine of Amphibia. Nature, Lond., 208, 1097-1098.

PARSONS, D.S. & PRICHARD, J.S. (1968). A Preparation of the Perfused Small Intestine for the Study of Absorption in Amphibia. J. Physiol., 198, 405-434.

PATERSON, J.Y.F. & HARRISON, F.A. (1972). The Splanchnic and Hepatic Uptake of Cortisol in Conscious and Anaesthetized Sheep. J. Endocr., 55, 335-350.

PELZMANN, K.S. (1973). Absorption of Chlormadinone Acetate and Norethindrone from in situ Rat Gut. J. pharm. Sci., 62, 1609-1614.

PELZMANN, K.S. & HAVEMEYER, R.N. (1971). Portal Vein Blood Sampling in Intestinal Drug Absorption Studies. J. pharm. Sci., 60, 331-332.

PELZMANN, K.S. & HAVEMEYER, R.N. (1972). Concurrent Water and Drug Absorption in the Rat Intestine. J. pharm. Sci., 61, 110-112.

PENZOTTI, S.C. & POOLE, J.W. (1974). Transport Mechanisms of -Lactam Antibiotics Across Everted Rat Gut. J. pharm. Sci., 63, 1803-1806.

PERRIER, D. & GIBALDI, M. (1973a). Calculation of Absorption Rate Constants for Drugs with Incomplete Availability. J. pharm. Sci., 62, 225-228.

PERRIER, D. & GIBALDI, M. (1973b). Animal Models for Investigating Intestinal Drug Absorption: Various Antibiotics. J. pharm. Sci., 62, 1486-1490.

PERRIER, D., GIBALDI, M. & BOYES, R.N. (1973c). Prediction of Systemic Availability from Plasma-Level Data After Oral Drug Administration. J. Pharm. Pharmac., 25, 256-257.

PETERSON, R.E. (1959). The Miscible Pool and Turnover Rate of Adrenocortical Steroids in Man. Recent Progr. Horm. Res., 15, 231-261.

PETERSON, R.E., WYNGAARDEN, J.B., GUERRA, S.L., BRODIE, B.B. & BUNIM, J.J. (1955). The Physiological Disposition and Metabolic Fate of Hydrocortisone in Man. J. clin. Invest., 34, 1779-1794.

PHILLIPS, S.F. & SUMMERSKILL, W.H.J. (1966). Occlusion of the Jejunum for Intestinal Perfusion in Man. Mayo Clinic Proceedings, 41, 224-231.

PHILLIPS, W.A., WEBB, K.E. & FONTENOT, J.P. (1978). Isolation of Segments of the Jejunum and Ileum for Absorption Studies Using Double Reentrant Cannulae in Sheep. J. Anim. Sci., 46, 726-731.

PICKUP, M.E. (1979). Clinical Pharmacokinetics of Prednisone and Prednisolone. Clinical Pharmacokinetics, 4, 111-128.

PICKUP, M.E., LOWE, J.R., LEATHAM, P.A., RHIND, V.M., WRIGHT, V. & DOWNIE, W.W. (1977). Dose Dependent Pharmacokinetics of Prednisolone. Eur. J. clin. Pharmac., 12, 213-219.

PINDELL, M.H., CULL, K.M., DORAN, K.M. & DICKISON, H.L. (1959). Absorption and Excretion Studies on Tetracycline. J. Pharmac. exp. Ther., 125, 287-294.

PLAA, G.L. (1975). The Enterohepatic Circulation. In Handb. exp. Pharmac., ed. Gillette, J.R. & Mitchell, J.R., Part 3, pp. 130-149. New York: Springer-Verlag.

PORTER, C.C. & SILBER, R.H. (1950). A Quantitative Color Reaction for Cortisone and Related 17,21-Dihydroxy-20-ketosteroids. J. biol. Chem., 185, 201-207.

PORTER, J.A. & CANADAY, W.R. (1971). Hematologic Values in Mongrel and Greyhound Dogs being Screened for Research Use. J. Am. vet. med. Ass., 159, 1603-1606.

PORTMANN, G.A., MINATOYA, H. & LANDS, A.M. (1965). Absorption and Elimination Profile of Isoproterenol II. Parenteral, Oral and Rectal Administration to Unanesthetized Dogs. J. pharm. Sci., 54, 973-978.

POWELL, L.W. & AXELSEN, E. (1972). Corticosteroids in Liver Disease: Studies on the Biological Conversion of Prednisone to Prednisolone and Plasma Protein Binding. Gut, 13, 690-696.

PRATT, E.B., BURDICK, F.D. & HOLMES, J.H. (1952). Measurement of Liver Blood Flow in Unanesthetized Dog Using the Bromsulfalein Dye Method. Am. J. Physiol., 171, 471-478.

PREWITT, L.R., JACOBSEN, D.R., HEMKEN, R.W. & HATTON, R.H. (1975). Portal Blood Flow as Measured by the Doppler Shift Technique in Sheep Fed Forages. J. Anim. Sci., 41, 596-600.

PRYOR, W.H. & BRADBURY, R.P. (1975). Haemobartonella canis Infection in Research Dogs. Lab. Anim. Sci., 25, 566-569.

READ, N.W., COOPER, K. & FORDTRAN, J.S. (1978). Effect of Modified Sham Feeding on Jejunal Transport and Pancreatic and Biliary Secretion in Man. Am. J. Physiol., 234, E417-E420.

REID, E. WAYMOUTH (1892). Report on Experiments Upon "Absorption Without Osmosis". Br. med. J., 1, 323-326.

REID, E. WAYMOUTH (1900). On Intestinal Absorption, Especially on the Absorption of Serum, Peptone, and Glucose. Proc. R. Soc. B., 102, 211-297.

REID, E. WAYMOUTH (1901a). Intestinal Absorption of Maltose. J. Physiol., 26, 427-435.

REID, E. WAYMOUTH (1901b). Transport of Fluid by Certain Epithelia. J. Physiol., 26, 436-444.

REYNELL, P.C. & SPRAY, G.H. (1956a). The Simultaneous Measurement of Absorption and Transit in the Gastrointestinal Tract of the Rat. J. Physiol., 131, 452-462.

REYNELL, P.C. & SPRAY, G.H. (1956b). Absorption of Glucose by the Intact Rat. J. Physiol., 134, 531-537.

ROBINSON, J.W.L. & FELBER, J.P. (1966). The Effect of Tissue Ageing on Intestinal Uptake of Amino-Acids in vitro. Gastro-enterologia, 105, 17-26.

ROE, W.E., BERGMAN, E.N. & KON, K. (1966). Absorption of Ketone Bodies and Other Metabolites via the Portal Blood of Sheep. Am. J. vet. Res., 27, 729-736.

ROGERS, R.C., NOVIN, D. & BUTCHER, L.L. (1979). Electrophysiological and Neuroanatomical Studies of Hepatic Portal Osmo- and Sodium-Receptive Afferent Projections Within the Brain. J. Autonomic Nervous System, 1, 183-202.

ROLLINS, D.E. & KLAASSEN, C.D. (1979). Biliary Excretion of Drugs in Man. Clin. Pharmacokinet., 4, 368-379.

ROSE, J.Q., JUSKO, W.J. & NICKELSEN, J.A. (1979). Prednisolone Pharmacokinetics in Relation to Dose. J. Pediatrics, 94, 1014-1015.

SABLE-AMPLIS, R. & ABADIE, D. (1975). Permanent Cannulation of the Hepatic Portal Vein in Rats. J. appl. Physiol., 38, 358-359.

SALVIOLI, G. (1880). Eine neue Methode für die Untersuchung der Functionen des Dunndarms. Arch. Anat. Physiol., Physiol. Abtheil., Suppl. Band., 95-112.

SAMPLE, R.G., ROSSI, G.V. & PACKMAN, E.W. (1968). Thiry-Vella Dog as a Biologic Model for Evaluation of Drug Absorption from the Intestinal Mucosa. J. pharm. Sci., 57, 795-798.

SAMUELS, L.T. (1966). The Dynamics of Steroid Hormone Distribution in the Body, Particularly the Distribution of Cortisol. In Steroid Dynamics, ed. Pincus, G., Nakao, T. & Tait, J.F. pp. 385-391. New York and London: Academic Press.

SANDBERG, A.A. & SLAUNWHITE, W.R. (1957). Differences in Metabolism of Prednisolone-C^{14} and Cortisol-C^{14}. J. clin. Endocr. Metab., 17, 1040-1050.

SANDBERG, D.H., BACALLAO, C.Z. & CLEVELAND, W.W. (1970). Measurement of Plasma Prednisolone by a Competitive Protein-Binding Assay. Biochem. Med., 4, 383-390.

SCHALM, S.W., SUMMERSKILL, W.H.J. & GO, V.L.W. (1976). Development of Radioimmunoassays for Prednisone and Prednisolone - Application to Studies of Hepatic Metabolism of Prednisone. Mayo Clinic Proceedings, 51, 761-766.

SCHALM, S.W., SUMMERSKILL, W.H.J. & GO, V.L.W. (1977). Prednisone for Chronic Active Liver Disease: Pharmacokinetics, Including Conversion to Prednisolone. Gastroenterology, 72, 910-913.

SCHAMBYE, P. (1951). Volatile Acids and Glucose in Portal Blood of Sheep. 3. The Influence of Orally Administered Glucose. Nord. vet.-Med., 3, 1003-1014.

SCHAMBYE, P. (1955). Experimental Estimation of the Portal Vein Blood Flow in Sheep. 2. Chronic Experiments in Cannulated Sheep Applying Infusion and Injection Methods. Nord. vet. -Med., 7, 1001-1016.

SCHANKER, L.S. (1959). Absorption of Drugs from the Rat Colon. J. Pharmac. exp. Ther., 126, 283-290.

SCHANKER, L.S., SHORE, P.A., BRODIE, B.B. & HOGBEN, C.A.M. (1957). Absorption of Drugs from the Stomach I. The Rat. J. Pharmac. exp. Ther., 120, 528-539.

SCHANKER, L.S., TOCCO, D.J., BRODIE, B.B. & HOGBEN, C.A.M. (1958). Absorption of Drugs from the Rat Small Intestine. J. Pharmac. exp. Ther., 123, 81-88.

SCHEDL, H.P. (1965). Absorption of Steroid Hormones from the Human Small Intestine. J. clin. Endocr., 25, 1309-1316.

SCHEDL, H.P. & CLIFTON, J.A. (1961). Small Intestinal Absorption of Steroids. Gastroenterology, 41, 491-499.

SCHEDL, H.P. & CLIFTON, J.A. (1963). Cortisol Absorption in Man. Gastroenterology, 44, 134-145.

SCHEDL, H.P., CLIFTON, J.A. & NOKES, G. (1964). Relations of Rate of Absorption and Plasma Cortisol Concentration. J. clin. Endocr. Metab., 24, 224-226.

SCHEDL, H.P., MILLER, D. & WHITE, D. (1966). Use of Polyethylene Glycol and Phenol Red as Unabsorbed Indicators for Intestinal Absorption Studies in Man. Gut, 7, 159-163.

SCHEIFFARTH, F., ZICHA, L., FUNCK, F.-W. & ENGELHARDT, M. (1963). Dunnschichtchromatographische Untersuchungen uber die Auftrennung von Steroidgemischen und die Ausscheidung Intravenos Applizierter Glucocorticoidester in Galle und Duodenalsaft. Acta endocr., Copenh., 43, 227-245.

SCHELINE, R.R. (1973). Metabolism of Foreign Compounds by Gastrointestinal Microorganisms. Pharmacological Reviews, 25, 451-523.

SCHLOERB, P.R. & LUKERT, B.L. (1963). Further Observations on the Isolated Human Jejunum. Archs Surg., 86, 356-362.

SCHNELLER, G.H. (1970). Status Report on Drug Bioavailability. Am. J. hosp. Pharm., 27, 485-488.

SEARLE, S.R. (1971). Linear Models. New York: John Wiley and Sons, Inc.

SEEBALD, H. & FORTH, W. (1977). Determination of ^{14}C-Bumadizone and ^{14}C-Phenylbutazone in the Systemic Blood, the Blood of the Portal Vein, the Intestinal Lymph and the Bile of Cats after Intrajejunal Administration. Arzneim.-Forsch., 27, 804-809.

SHERLOCK, S. & WALSHE, V. (1946). The Use of a Portal Anastomotic Vein for Absorption Studies in Man. Clin. Sci., 6, 113-123.

SHIELDS, R. (1972). Comment. In Transport Across the Intestines, A Glaxo Symposium, ed. Burland, W.L. & Samuel, P.D. p. 75. Edinburgh and London: Churchill Livingstone.

SHIMADA, A. & ZIMMERMAN, D.R. (1973). Hepatic Portal Catheterization and Comparison of Portal and Inferior Vena Cava Free Plasma Amino Acid Patterns in the Baby Pig. J. Anim. Sci., 36, 245-251.

SHOEMAKER, W.C., WALKER, W.F., VAN ITALLIE, T.B. & MOORE, F.D. (1959). A Method for Simultaneous Catheterization of Major Hepatic Vessels in a Chronic Canine Preparation. Am. J. Physiol., 196, 311-314.

SHOEMAKER, W.C., YANOF, H.M., TURK, L.N. & WILSON, T.H. (1963). Glucose and Fructose Absorption in the Unanesthetized Dog. Gastroenterology, 44, 654-663.

SIEBER, S.M., COHN, V.H. & WYNN, W.T. (1974). The Entry of Foreign Compounds into the Thoracic Duct Lymph of the Rat. Xenobiotica, 4, 265-284.

SILBER, R.H. (1966). Fluorometric Analysis of Steroids. Methods of Biochemical Analysis, 14, 63.

SILBER, R.H. & MORGAN, E.R. (1956). Absorption and Excretion of 17, 21-Dihydroxy-20-ketosteroids in Dogs. Clin. Chem., 2, 170-174.

SILBER, R.H. & PORTER, C.C. (1954). The Determination of 17,21-Dihydroxy-20-ketosteroids in Urine and Plasma. J. biol. Chem., 210, 923-932.

SKELLERN, G.G., STENLAKE, J.B. & WILLIAMS, W.D. (1973). The Absorption, Distribution, Excretion and Metabolism of [2-^{14}C] Methimazole in Rat. Xenobiotica, 3, 121-132.

SLAUNWHITE, W.R. & SANDBERG, A.A. (1961). Disposition of Radioactive 17∝-Hydroxy-Progesterone, 6∝-Methyl-17∝-Acetoxy-Progesterone and 6∝-Methylprednisolone in Human Subjects. J. clin. Endocr. Metab., 21, 753-764.

SMITH, J.K. & CASE, M.T. (1978). Effect of Long Term Heparin Administration in Dogs. Drug and Chemical Toxicology, 1, 251-258.

SMITH, M.J.H. (1951). Plasma-Salicylate Concentration After Small Doses of Acetylsalicylic Acid. J. Pharm. Pharmac., 3, 409-414.

SMITH, R.L. (1971). Excretion of Drugs in Bile. In Handbook of Experimental Pharmacology, Volume XXVIII/1. Concepts in Biochemical Pharmacology. Part 1. ed. Brodie, B.B. & Gillette, J.R. pp.354-389. Berlin, Heidelberg and New York: Springer-Verlag.

SMITH, R.L. (1974). Biliary Excretion and Hepatotoxicity of Contraceptive Steroids. Acta endocr., Copenh., Suppl., 185, 149-168.

SMOLEN, V.F. (1971). Quantitative Determination of Drug Bioavailability and Biokinetic Behaviour from Pharmacological Data for Ophthalmic and Oral Administrations of a Mydriatic Drug. J. pharm. Sci., 60, 354-365.

SMOLEN, V.F. (1978). Bioavailability and Pharmacokinetic Analysis of Drug Responding Systems. Annual Reviews in Pharmacology and Toxicology, 18, 495-522.

SMOLEN, V.F. & SCHOENWALD, R.D. (1971). Drug-Absorption Analysis from Pharmacological Data I: Method and Confirmation Exemplified for the Mydriatic Drug Tropicamide. J. pharm. Sci., 60, 96-103.

SMOLEN, V.F. & WEIGAND, W.A. (1973). Drug Bioavailability and Pharmacokinetic Analysis from Pharmacological Data. J. Pharmacokinet. Biopharm., 1, 329-336.

SMYTH, D.H. (1974). Methods of Studying Intestinal Absorption. In Biomembranes, Volume 4A, Intestinal Absorption, ed. Smyth, D.H. pp.241-283. London and New York: Plenum Press.

SOLLMANN, T., HANZLIK, P.J. & PILCHER, J.D. (1910). Quantitative Studies on the Gastro-Intestinal Absorption of Drugs I. The Inhibitory Action of Phenol on Absorption. J. Pharmac. exp. Ther., 1, 409-444.

SRIVOSTAVA, L.S., WERK, E.E., THRASHER, K., SHOLITON, L.J., KOZERA, R., NOLTEN, W. & KNOWLES, H.C. (1973). Plasma Cortisone Concentration as Measured by Radioimmunoassay. J. clin. Endocr. Metab., 36, 937-943.

STAUD, R., RIETBROCK, N. & FASSBENDER, H.P. (1975). Excretion of Methylproscillaridin in Patients with a Biliary Fistula. Eur. J. clin. Pharmac., 9, 99-103.

STEDMAN'S MEDICAL DICTIONARY. (1976). Twenty-third Edition. Baltimore: The Williams and Wilkins Company.

STEEL, J.D., TAYLOR, R.I., DAVIS, P.E., STEWART, G.A. & SALMON, P.W. (1976). Relationships Between Heart Score, Heart Weight and Body Weight in Greyhound Dogs. Aust. vet. J., 52, 561-564.

STEFFEY, E.P., GILLESPIE, J.R., BERRY, J.D., EGER, E.I. & SCHALM, O.W. (1976). Effect of Halothane and Halothane-Nitrous Oxide on Hematocrit and Plasma Protein Concentration in Dog and Monkey. Am. J. vet. Res., 37, 959-962.

STRANDELL, T., DELIN, A., ERWALD, R., KULLING, K.G., LUNDBERGH, P., MANONS, O. & WIECHEL, K.-L. (1973). Portal Blood Flow Measurement by a Catheter Tip Electromagnetic Velocity Probe in Awake Man. Acta chir. scand., 139, 7-10.

SULLIVAN, T.J., HALLMARK, M.R., SAKMAR, E., WEIDLER, D.J., EARHART, R.H. & WAGNER, J.G. (1976a). Comparative Bioavailability: Eight Commercial Prednisone Tablets. J. Pharmacokinet. Biopharm., 4, 157-172.

SULLIVAN, T.J., SAKMAR, E., ALBERT, K.S., BLAIR, D.C. & WAGNER, J.G. (1975). In vitro and in vivo Availability of Commercial Prednisone Tablets. J. pharm. Sci., 64, 1723-1725.

SULLIVAN, T.J., SAKMAR, E. & WAGNER, J.G. (1976b). Comparative Bioavailability: A New Type of in vitro - in vivo Correlation Exemplified by Prednisone. J. Pharmacokinet. Biopharm., 4, 173

SULLIVAN, T.J., STOLL, R.G., SAKMAR, E., BLAIR, D.C. & WAGNER, J.G. (1974). In vitro and in vivo Availability of Some Commercial Prednisolone Tablets. J. Pharmacokinet. Biopharm., 2, 29-41.

SUZUKI, T., ISOZAKI, S., ISHIDA, R., SAITOH, Y. & NAKAGAWA, F. (1974). Drug Absorption and Metabolism Studies by Use of Portal Vein Infusion in the Rat II. Influence of Dose and Infusion Rate on the Bioavailability of Propranolol. Chem. Pharm. Bull., 22, 1639-1645.

SWARTZ, S.L. & DLUHY, R.G. (1978). Corticosteroids: Clinical Pharmacology and Therapeutic Use. Drugs, 16, 238-255.

SWINTOSKY, J.V., FOLTZ, E.L., BONDI, A. JR. & ROBINSON, M.J. (1958). Sulfaethylthiadiazole III. Kinetics of Absorption, Distribution and Excretion. J. Am. pharm. Ass., Sci. Ed., 47, 136-141.

SWINTOSKY, J.V., ROBINSON, M.J., FOLTZ, E.L. & FREF, S.M. (1957). Sulfaethylthiadiazole I. Interpretations of Human Blood Level Concentrations Following Oral Doses. J. Am. pharm. Ass., Sci. Ed., 46, 399-403.

SYMCHOWICZ, S., ZEMAN, W.V., WILLIAMS, R.A. & TABACHNICK, I.I.A. (1965). Absorption of Betamethasone (Celestone) from the G.I. Tract in Dogs. Archs int. Pharmacodyn., 158, 360-370.

TAHER, EL-S. & IBRAHIM, T.M. (1969). Obliteration of the Intra-Abdominal Umbilical Vessels in Puppies. Zentbl. Vet Med., [A], 16, 185-192.

TAIT, J.F. (1963). The Use of Isotopic Steroids for the Measurement of Production Rates in vivo. J. clin. Endocr., 23, 1285-1297.

TAIT, J.F. & BURSTEIN, S. (1964). In vivo Studies of Steroid Dynamics in Man. In The Hormones. Physiology, Chemistry and Applications, Volume 5, ed. Pincus, G., Thimann, K.V. & Astwood, E.B. pp.441-557. New York and London: Academic Press.

TANNER, A.R., BOCHNER, F., CAFFIN, J.A., HALLIDAY, J.A. & POWELL, L.W. (1979a). Dose-dependent Prednisolone Kinetics. Clin. Pharmac. Ther., 25, 571-578.

TANNER, A.R., CAFFIN, J.A., HALLIDAY, J.W. & POWELL, L.W. (1979b). Concurrent Administration of Antacids and Prednisone: Effect on Serum Levels of Prednisolone. Br. J. clin. Pharmac., 7, 397-400.

TAYLOR, W. (1971). The Excretion of Steroid Hormone Metabolites in Bile and Feces. Vitamins and Hormones, 29, 201-285.

TEMBO, A.V., AYRES, J.W., SAKMAR, E., HALLMARK, M.R. & WAGNER, J.G. (1977a). Plasma Prednisolone Concentrations: Comparison of Radioimmunoassay and Competitive Protein Binding Assay. Steroids, 29, 679-693.

TEMBO, A.V., HALLMARK, M.R., SAKMAR, E., BACHMANN, H.G., WEIDLER, D.J. & WAGNER, J.G. (1977b). Bioavailability of Prednisolone Tablets. J. Pharmacokinet. Biopharm., 5, 257-270.

TEMBO, A.V., SAKMAR, E., HALLMARK, M.R., WEIDLER, D.J. & WAGNER, J.G. (1976). Effect of Food on the Bioavailability of Prednisone. J. clin. Pharmac., 16, 620-624.

TEORELL, T. (1937). Kinetics of Distribution of Substances Administered to the Body. I. The Extravascular Modes of Administration. Archs int. Pharmacodyn., 57, 205-225.

THIESSEN, J.J. (1976). Prednisolone. Bioavailability Monograph. J. Am. pharm. Ass., NS 16, 143-146.

THIRY, L. (1864). Uber eine neue Methode, den Dunndarm zu isolieren. Sitzber. Akad. Wiss. Wein. Math. - Naturw. Kl, I, 50, 77-96. Cited by Parsons, D.S. (1968).

TIDWELL, H.C. (1950). Mechanism of Fat Absorption as Evidence by Chylomicrographic Studies. J. biol. Chem., 182, 405-414.

TOMKINS, G.M. (1956). Enzymatic Mechanisms of Hormone Metabolism. I. Oxidation-Reduction of the Steroid Nucleus. Recent Progr. Horm. Res., 12, 125-133.

TREDGER, J.M., CHAKRABORTY, J. & PARKE, D.V. (1973). A Comparative Study of the Excretion of Corticosterone and Betamethasone in the Rat. Biochem. Soc. Trans., 1, 998-999.

TRUITT, E.B. JR., McKUSICK, V.A. & KRANTZ, J.C. JR. (1950). Theophylline Blood Levels After Oral, Rectal and Intravenous Administration, and Correlation with Diuretic Action. J. Pharmac. exp. Ther., 100, 309-315.

TSE, F.L.S. & WELLING, P.G. (1977). Prednisolone Bioavailability in the Dog. J. pharm. Sci., 66, 1751-1754.

TSE, F.L.S. & WELLING, P.G. (1979). Relative Bioavailability of Prednisone and Prednisolone in Man. J. Pharm. Pharmac., 31, 492-493.

TURNER, R.H., MEHTA, C.S. & BENET, L.Z. (1970). Apparent Directional Permeability Coefficients for Drug Ions: In vitro Intestinal Perfusion Studies. J. pharm. Sci., 59, 590-595.

UETE, T. & SHIMANO, N. (1971). A Simple Method for Separate Determination of Delta^{4}-and Delta^{1,4}-Corticosteroids in Urine. Clin. Chem., 17, 161-165.

UNITED STATES PHARMACOPEIA XVIII (1970). United States Pharmacopeial Convention, Inc., Bethesda, Maryland, p.934.

URIBE, M., GO, V.L.W. & SUMMERSKILL, W.H.J. (1976a). Kinetics and Interconversion of Prednisone and Prednisolone Compared in Chronic Active Liver Disease (CALD) After Oral Doses. Gastroenterology, 71, 932.

URIBE, M., SCHALM, S.W., SUMMERSKILL, W.H.J. & GO, V.L.W. (1976b). Effect of Liquid Diet on Serum Protein Binding and Prednisolone Concentrations After Oral Prednisone. Gastroenterology, 71, 362-364.

URIBE, M., SCHALM, S.W., SUMMERSKILL, W.H.J. & GO, V.L.W. (1978). Oral Prednisone for Chronic Active Liver Disease: Dose Responses and Bioavailability Studies. Gut, 19, 1131-1135.

USSING, H.H. (1949). The Active Ion Transport Through the Isolated Frog Skin in the Light of Tracer Studies. Acta physiol. scand., 17, 1-37.

VAN BERGE HENEGOUWEN, G.P. & HOFMANN, A.F. (1978). Nocturnal Gallbladder Storage and Emptying in Gallstone Patients and Healthy Subjects. Gastroenterology, 75, 879-885.

VAUGHAN WILLIAMS, E.M. (1951). The Measurement of Secretion and Absorption by Intestinal Loops in Conscious Dogs. J. Physiol., 112, 32p-33p.

VELLA, L. (1880). Nuovo methodo per avere il succo enterico puro e stabilirne le proprieta fisiologiche. Mem. Acad. Sci. Inst. Bologna, Ser. 4, 2, Fasc. 3e, 515-535. Cited by Parsons, D.S. (1968).

VELLA, L. (1888). Neues Verfahren zur Gewinnung reinen Darmsaftes und Feststellung seiner physiologischen Eigenschaften. Moleshott's Untersuch. Naturl. Mensch. Thiere, 13, 40-74.

VERMEULEN, A. (1959). The Metabolism of 4-^{14}C Prednisolone. J. Endocr., 18, 278-291.

VERMEULEN, A. & CASPI, E. (1959). Metabolism of 1-Dehydrosteroids in Man. II. Isolation of 20α- and 20β-Hydroxy Metabolites. J. biol. Chem., 234, 2295-2297.

WAGNER, J.G. (1971). Biopharmaceutics and Relevant Pharmacokinetics. 1st ed. Hamilton, Illinois: Drug Intelligence Publications.

WAGNER, J.G. (1975). The Fundamentals of Clinical Pharmacokinetics. 1st ed. Hamilton, Illinois: Drug Intelligence Publications, Inc.

WAGNER, J.G., CARPENTER, O.S. & COLLINS, E.J. (1960). Sustained Action Oral Medication I. A Quantitative Study of Prednisolone in Man, in the Dog and in vitro. J. Pharmac. exp. Ther., 129, 101-107.

WAGNER, J.G. & NELSON, E. (1963). Per Cent Absorbed Time Plots Derived from Blood Level and/or Urinary Excretion Data. J. pharm. Sci., 52, 610-611.

WAGNER, J.G. & NORTHAM, J.I. (1968). Lag Time Before Essentially Constant Urinary Excretion Rate is Attained. J. pharm. Sci., 57, 994-999.

WALDERN, D.E., FROST, O.L., HARSCH, J.A. & BLOSSER, T.H. (1963). Improved Equipment for Catheterization of Blood Vessels of the Portal System of Cattle. Am. J. vet. Res., 24, 212-214.

WALSH, C.T. & LEVINE, R.R. (1974). Drug Absorption in Rats Pretreated With Antibiotics. J. Pharmac. exp. Ther., 188, 277-286.

WANGSNESS, P.J. & McGILLIARD, A.D. (1972). Evaluation of a Direct Method for Measuring Absorption from the Gut. J. Dairy Sci., 55, 1432-1438.

WARE, A.J. & COMBES, B. (1973). Influence of Sodium Taurocholate, Cholestyramine, and Mylanta on the Intestinal Absorption of Glucocorticoids in the Rat. Gastroenterology, 64, 1150-1155.

WATSON, A.R.J. & EGERTON, J.R. (1977). Effect of Feeding on Plasma Antibiotic Concentrations in Greyhounds Given Ampicillin and Amoxycillin by Mouth. J. small Anim. Pract., 18, 779-786.

WEBSTER, A.J.F. (1974). Clearance by the Hepatic Portal Circulation of the Products of Digestion and Metabolism in the Ruminant Gut. Proc. Nutr. Soc., 33, 155-161.

WEBSTER, A.J.F., OSUJI, P.O., WHITE, F. & INGRAM, J.F. (1975). The Influence of Food Intake on Portal Blood Flow and Heat Production in the Digestive Tract of Sheep. Br. J. Nutr., 34, 125-139.

WEST, H.J. (1959a). Prevention of Peptic Ulcer During Corticosteroid Therapy. Br. med. J., 1, 680.

WEST, H.J. (1959b). Steroid Therapy and Peptic Ulceration. Lancet, 1, 1149-1150.

WIECHEL, K.-L. (1979). Direct Selective Catheterization of the Portal Venous System. Scand. J. Gastroent., 14, 123-130.

WILLIAMS, R.T. (1972). Toxicologic Implications of Biotransformation by Intestinal Microflora. Toxicol. appl. Pharmac., 23, 769-781.

WILSON, C.G., MAY, C.S. & PATERSON, J.W. (1977). Plasma Prednisolone Levels in Man Following Administration in Plain and Enteric-Coated Forms. Br. J. clin. Pharmac., 4, 351-355.

WILSON, C.G., SSENDAGIRE, R., MAY, C.S. & PATERSON, J.W. (1975). Measurement of Plasma Prednisolone in Man. Br. J. clin. Pharmac., 2, 321-325.

WILSON, T.H. (1956). A Modified Method for Study of Intestinal Absorption in vitro. J. appl. Physiol., 9, 137-140.

WILSON, T.H. & WISEMAN, G. (1954). The Use of Sacs of Inverted Small Intestine for the Study of the Transference of Substances from the Mucosal to the Serosal Surface. J. Physiol., 123, 116-125.

WINNE, D. & REMISCHOVSKY, J. (1970). Intestinal Blood Flow and Absorption of Non-Dissociable Substances. J. Pharm. Pharmac., 22, 640-641.

WINNE, D. & REMISCHOVSKY, J. (1971). Der EinfluB der Durchblutung auf die Resorption von Harnstoff, Methanol und Athanol aus dem Jejunum der Ratte. Arch. Pharmak., 268, 392-416.

WISEMAN, G. (1953). Absorption of Amino-Acids Using an in vitro Technique. J. Physiol., 120, 63-72.

WOLFE, D.L., FORLAND, S.C. & BENET, L.Z. (1973). Drug Transfer Across Intact Rat Intestinal Mucosa Following Surgical Removal of Serosa and Muscularis Externa. J. pharm. Sci., 62, 200-205.

WRIGHT, A.S. & CROWNE, R.S. (1972). The Alimentary Absorption of 2,4,6-Tri(3,5-di-tert-butyl-4-hydroxybenzyl) phenol (Ionox 312) in the Rat. Xenobiotica, 2, 1-5.

ZEMAN, W.V. & SYMCHOWICZ, S. (1970). A Biomedical Model for Absorption Studies from Various Gastrointestinal Sites in Dogs. Am. J. vet. Res., 31, 1293-1296.

ZIERLER, K.L. (1961). Theory of the Use of Arteriovenous Concentration Differences for Measuring Metabolism in Steady and Non-Steady States. J. clin. Invest., 40, 2111-2125.

ZIPSER, R.D., SPECKART, P.F., ZIA, P.K., EDMISTON, W.A., LAU, F.Y.K. & HORTON, R. (1976). The Effect of ACTH and Cortisol on Aldosterone and Cortisol Clearance and Distribution in Plasma and Whole Blood. J. clin. Endocr. Metab., 43, 1101-1109.

APPENDIX A1

Equipment for surgery

The following equipment and materials were used for surgery and preparation of chronic dogs.

Surgical instruments. Figures in brackets are the number of instruments packed in the instrument package for use in the surgical operation. (See Figure 2.5, page 115.)

Scalpel handles No. 4 Swann-Morton (2)
Mosquito haemostats (8) } For controlling bleeding points
Straight haemostats (4) }
Aneurism needles (2)
Iris scissors (2)
Mayo scissors (2)
Iris forceps (2)
Curved forceps (2)
Artery clamp (1) (Can be helpful in catheterising carotid artery)
Mayo needle holders (1)
Large pointed haemostat 20 cm (1)
Suture needles - Round bodied curved size 17 (3)
Morrison's round bodied curved size 10 (3)
Stainless steel, Alcester Surgical Needle Co. Ltd.
Tissue forceps (2)
Towel clamps (9) For holding drapes in place
Kidney dish (1)
Large Gauze Swabs 30 cm x 20 cm (6)
"Mersilene" - Black-braided suture 2/0 B.P.C. RR 923, Ethicon Ltd. Ready-cut lengths 40 cm long plus reel
Catheters - 4 carotid artery or jugular vein type
2 portal vein type

Obturators	-	5 carotid artery or jugular vein type (14-gauge) 3 portal vein type (17-gauge)
Joiners	-	1 of each size catheter. Length of stainless steel tube (lengths of cut off needle as for obturators, but not filled) in case catheter severed accidentally
Needle Adaptors	-	4 14-gauge 2 17-gauge (made from stainless steel Luer-Lok needles cut approximately 3 cm from the hub and the ends smoothed.)
Swabs		All gauze swabs B.P.C. 10 cm x 10 cm x 8 ply: Johnson & Johnson Pty.Ltd. and Smith & Nephew (Malaysia) Sdn. Berhad.

The above equipment constituted the instrument pack. It was wrapped in a drape in the manner that enabled sterile opening and overwrapped in paper. The pack was sterilised by autoclaving at 121^{o} for 30 minutes.

Drapes and gowns. Standard hospital operating gowns (4), together with 4 surgeons towels, folded and placed for sterile use in a pack similar to above. Assorted size standard hospital drapes (14 approximately) constituted the drapes pack. Both packs were sterilised by autoclaving at 121^{o} for 30 minutes.

Pre-sterilised equipment

Surgical gloves	"Gammex" Sterile Latex Surgical Gloves (Ansell Rubber Co.,Pty.Ltd.) Sizes 7 and 7½.
Syringes	Sterile Disposable Syringes. Pharma-Plast (Australia) Pty.Ltd. 10 ml, 5 ml and 1 ml (Tuberculin type)
Needles	Yale Sterile Disposable Needle (Becton, Dickinson and Company Ltd.) 19 G x 1¼, 21 G x 1½ and 26 G x ½

Scalpel blades — Swann-Morton Sterile Surgical Blade No.21.

Chromic catgut — (absorbable) "Ethicon" Surgical Catgut BP, (Ethnor Pty.Ltd.) Chromic 3/0, S-112 and Chromic 2, S-116

Straight atraumatic closure needle with silk, "Atraloc-Eyeless" needle sutures 623 B.B. silk 2/0. Ethicon-Black Braided Silk B.P.C. (Ethnor Pty.Ltd.)

Three-way stopcocks, Sterile K-75. Pharmaseal, Inc.

Solution administration set with filter AHC0026, Travenol Laboratories Pty.Ltd.

The above equipment was obtained pre-sterilised. The packages were opened by the unsterile helper and the sterile contents emptied onto the sterile instruments trolley as needed. The surgical gloves were emptied onto the opened pack of gowns.

Other equipment

Oster animal hair clippers with 0.1 mm blade

McGill endotracheal tube 1 cm i.d.

Masks, "Deseret E-Z Breathe Filtermask" No.510 (Deseret Pharmaceutical Co. Inc.)

Caps, standard hospital

Swabs (for rubbing on "Povidine K")

"Elastoplast", Elastic adhesive bandage B.P.C. 10 cm x 3 metres Smith & Nephew (Australia) Pty.Ltd.

Crepe bandage, 8 cm wide (Johnson & Johnson Ltd.)

Small safety pins

Combine dressing roll (Johnson & Johnson Pty.Ltd.)

Dog coat (made from drape), safety pins and dog collar.

"Zephiran" concentrate 1:10. 10 per cent aqueous solution of alkyldimethylbenzyl ammonium chloride. (Winthrop Laboratories Division of Sterling Pharmaceuticals Pty.Ltd., Sydney) For the sterile storage of needle adaptors and spare obturators between use for catheter flushing and sampling.

APPENDIX A2

Plasma concentrations of prednisolone (ng/ml) in the portal vein, carotid artery and jugular vein followign oral administration of formulations (K and N) to dogs with either short-life or nonpatent portal vein catheters.

Explanatory note:

nd represents values below the limit of detection of the assay method (50 ng/ml); these values were treated as 0 where necessary for calculations.

10 mg hand-filled capsule (K)

Dog	Run	Time (minutes)	Plasma prednisolone concentrations (ng/ml) Portal vein	Carotid artery	Jugular vein
P 5	1	20	123	nd	nd
		35	90	nd	nd
		55	258	135	137
		90	295	265	260
		120	530	450	451
		150	330	340	347
		210	61	60	60
P 9	1	5	nd	nd	nd
		15	nd	nd	nd
		30	nd	nd	nd
		40	54	nd	nd
		50	203	201	201
		60	508	201	201
		75	465	435	436
		90	201	207	205
P 9	2	5	nd	nd	nd
		15	nd	nd	nd
		30	nd	nd	nd
		40	nd	nd	nd
		50	159	102	101
		60	345	223	227
		70	-	183	182
		80	-	164	161
		90	-	196	196

2 x 10 mg hand-filled capsules (N)

Dog	Time (hours)	Plasma prednisolone concentrations (ng/ml) Carotid artery	Jugular vein
P 6	0.5	384	50
	1.25	179	211
	2.25	110	102
	3.25	101	119
	4.5	90	89
	5.5	289	259
	6.5	55	60
	7.5	nd	nd

10 mg hand-filled capsule (K)

Dog	Time (minutes)	Plasma prednisolone concentrations (ng/ml) Carotid artery	Jugular vein
P 7	20	nd	nd
	30	nd	nd
	45	61	60
	60	124	130
	70	259	258
	80	303	307
	90	332	332
	105	286	280
	150	149	145
	180	91	95
	210	nd	nd
	240	nd	nd

APPENDIX A3

Plasma concentrations of prednisolone (ng/ml) in the portal vein (P) and carotid artery (A) after administration of formulations (A to N) to greyhounds I to III.

Explanatory notes:

1. nd represents values below the limit of detection of the assay method (50 ng/ml); these values were treated as 0 for statistical calculations.

2. ^{b} indicates value estimated from plasma concentration curves.

5 mg commercial tablet (A)

Dog	Run		Time (minutes) 15	30	45	60	75	90	105	120	135	150	180	240
I	1	P	-	-	-[b]	-	-	-	-	-	-	-	-	-
		A	nd	126	115[b]	110	-	98	-	87	-	nd	-	-
	2	P	nd	167	448	306	-	311	-	273	-	275	-	-
		A	nd	nd	nd	56	-	207	-	120	-	126	-	-
	3	P	nd	nd	nd	136	-	448	-	447	-	196	-	-
		A	nd	nd	nd	129	-	299	-	218	-	196	-	-
	4	P	100[b]	293	135	124	-	120	-	59	-	118	-	-
		A	50[b]	147	53	39	-	235	-	119	-	111	-	-
	5	P	111	278	439	326	57	nd	-	nd	-	nd	-	-
		A	91	266	445	188	103	nd	-	nd	-	nd	-	-
II	1	P	nd	283	222	nd	nd	nd	nd	nd	208	201	nd	nd
		A	nd	228	63	nd	nd	nd	nd	nd	nd	227	nd	nd
	2	P	nd	172	522	339	189	114	68	nd	96	289	178	111
		A	nd	91	475	264	205	96	79	96	139	327	178	111
	3	P	nd	nd	nd	nd	nd	133	177	76	nd	446	302	53
		A	nd	nd	nd	nd	nd	63	65	55	nd	nd	464	313
III	1	P	426	833	255	232	156	147	118	nd	nd	nd	302	241
		A	371	621	400	195	143	136	118	nd	nd	nd	220	149
	2	P	nd	51	170	242	nd	nd	606	159	525	99	51	77
		A	nd	56	nd	nd	61	nd	427	240	nd	58	53	98
Mean		P	71	231	243	189	67	141	194	113	166	180	167	96
± S.E.M.			32	56	58	61	43	46	77	47	67	38	44	25
		A	51	154	155	98	85	113	138	94	28	105	183	134
± S.E.M.			25	39	48	43	38	35	55	31	37	27	53	36

5 mg commercial tablet (B)

Dog	Run		Time (minutes) 15	30	45	60	75	90	105	120	135	150	180	240
I	1	P	-	nd	225	99	-	109	-	86	-	nd	-	-
		A	-	nd	118	104	-	100	-	98	-	nd	-	-
	2	P	-	nd	566	652	-	159	-	90	-	nd	-	-
		A	-	nd	nd	652	-	94	-	74	-	nd	-	-
	3	P	nd	74	-	-	-	-	-	-	-	-	-	-
		A	nd	nd	nd	183	-	-	-	-	-	-	-	-
II	1	P	nd	61	nd	nd	nd	59	nd	377	139	75	nd	nd
		A	nd	88	nd	nd	nd	nd	nd	288	130	73	nd	nd
	2	P	nd	nd	58	341	104	159	313	73	nd	328	nd	80
		A	nd	nd	91	89	80	144	146	nd	nd	111	nd	75
	3	P	132	135	nd	nd	nd	nd	273	273	nd	nd	nd	nd
		A	96	nd	nd	nd	nd	60	246	143	56	nd	nd	nd
III	1	P	nd	nd	nd	357	193	356	372	333	157	109	nd	nd
		A	nd	nd	nd	208	250	312	325	259	158	104	nd	nd
	2	P	nd	nd	nd	nd	nd	nd	nd	nd	254	199	108	nd
		A	nd	nd	nd	nd	nd	nd	nd	nd	233	123	101	58
Mean		P	22	34	121	207	59	120	192	176	110	102	22	16
± S.E.M.			40	59	66	69	47	52	77	53	67	43	44	25
		A	16	11	26	155	66	101	143	123	115	59	20	27
± S.E.M.			32	44	54	48	42	42	55	37	37	32	53	36

5 mg commercial tablet (C) These had been claimed to be a therapeutic failure

Dog	Run		Time (minutes) 15	30	45	60	75	90	105	120	135	150	180	240
I	1	P	-	nd	119	225	-	263	-	200	-	nd	-	-
		A	-	nd	91	195	-	118	-	75	-	69	-	-
	2	P	-	nd	105	344	-	91	-	nd	-	nd	-	-
		A	-	nd	71	122	-	120	-	67	-	nd	-	-
II	1	P	nd	83	114	329	162	nd	67	217	104	82	57	nd
		A	nd	57	104	87	73	69	103	119	277	58	nd	nd
	2	P	nd	nd	nd	nd	nd	128	141	90	nd	nd	nd	nd
		A	nd	nd	nd	nd	nd	176	124	57	nd	55	nd	nd
	3	P	nd	nd	nd	107	132	258	182	188	213	280	nd	nd
		A	nd	nd	nd	nd	nd	204	97	185	132	179	nd	nd
III	1	P	nd	nd	218	nd	nd	61	nd	nd	nd	nd	nd	nd
		A	nd	nd	nd	nd	68	nd	nd	nd	nd	nd	nd	nd
	2	P	nd	52	588	80	208	nd	nd	nd	79	nd	nd	87
		A	nd	nd	nd	125	198	301	54	63	nd	nd	nd	72
Mean		P	0	19	163	155	100	114	78	99	79	52	11	17
± S.E.M.			44	63	66	69	47	52	77	53	67	43	44	25
		A	0	8	38	76	68	141	76	81	82	52	0	14
± S.E.M.			35	47	58	52	42	42	55	37	37	32	53	36

5 mg commercial tablet (D)

Dog	Run		Time (minutes) 15	30	45	60	75	90	105	120	135	150	180	240
III	1	P	nd	nd	444	250	251	100	nd	nd	nd	nd	nd	nd
		A	nd	nd	nd	166	200	100	nd	nd	nd	nd	nd	nd
	2	P	nd	nd	197	54	151	140	159	341	52	333	112	nd
		A	nd	nd	nd	50	52	83	144[b]	205	109	218	100	nd
	3	P	nd	63	70	134	nd	86	83	88	57	73	nd	116
		A	nd	62	56	68	nd	nd	nd	65	nd	nd	nd	nd
	4	P	nd	579	267	70	nd	268	400	nd	51	nd	nd	nd
		A	nd	nd	183	66	nd	nd	nd	nd	nd	nd	nd	nd
	5	P	nd	nd	nd	nd	nd	76	81	69	nd	316	266	197
		A	nd	nd	nd	nd	nd	58	113	nd	nd	53	150[b]	nd[b]
Mean		P	0	129	196	102	80	134	181	100	32	145	76	63
± S.E.M.				113	77	43	51	35	75	62	12	74	52	40
		A	0	12	48	70	51	48	52	55	22	55	50	0
± S.E.M.				12	35	27	38	20	32	39	21	42	32	

5 mg commercial prednisone tablet (E)

Dog	Run		Time (minutes) 15	30	45	60	75	90	105	120	135	150	180	240
III	1	P	nd	77	346	536	700	643	625	177	143	81	nd	nd
		A	nd	nd	204	253	290	327	312	170[b]	130[b]	64	nd	nd
	2	P	nd	141	434	249	83	76	nd	69	nd	nd	nd	nd
		A	nd	96	119	76	64	58	nd	64	nd	nd	nd	nd
	3	P	nd	nd	nd	nd	nd	63	58	55	61	91	305	nd
		A	nd	nd	nd	nd	nd	57	67	51	50	92	67	nd
Mean		P	0	73	260	262	261	260	228	100	68	57	102	0
± S.E.M.				40	132	155	221	191	199	39	41	28	101	
		A	0	33	108	110	118	147	126	95	60	52	23	0
± S.E.M.				31	59	74	88	88	95	38	38	27	22	

5 mg commercial capsule (F)

Dog	Run		Time (minutes) 15	30	45	60	75	90	105	120	135	150	180	240
II	1	P	nd	nd	-	-	-	-	-	-	-	-	-	-
		A	nd	nd	132	nd	nd	51	nd	67	254	66	nd	nd
III	1	P	785	532	273	280	280	526	381	195	188	111	124	117
		A	232	156	131	217	230	265	200	181	144	160	129	120
	2	P	nd	nd	nd	nd	nd	nd	nd	72	107	nd	nd	nd
		A	nd	nd	nd	nd	nd	85	83	87	249	53	nd	nd
	3	P	312	nd	nd	75	nd	140	276	115	64	nd	nd	nd
		A	104	nd	nd	nd	nd	nd	242	227	100	83	nd	nd
Mean		P	274	133	92	118	94	222	217	128	119	38	42	40
± S.E.M.			185	67	90	83	93	157	113	36	36	36	41	38
		A	84	39	66	54	58	100	131	141	187	91	32	30
± S.E.M.			55	39	38	54	58	58	55	38	38	24	32	30

5 mg hand-filled capsule (G)

Dog	Run		Time (minutes) 15	30	45	60	75	90	105	120	135	150	180	240
II	1	P	318	256	208	512	97	132	nd	nd	584	173	112	nd
		A	430	163	166	484	227	119	nd	nd	323	182	82	nd
	2	P	530	342	321	199	90	128	599	103	150	464	78	nd
		A	222	384	223	72	85	167	350	105	159	259	128	nd
	3	P	119	307	263	736	476	314[b]	151	nd	nd	nd	nd	nd
		A	nd	nd	237	456	500	324[b]	148	nd	nd	nd	nd	nd
III	1	P	nd	171	60	195	58	158	65	136	178	500	326	nd
		A	51	nd	nd	nd	nd	nd	71	168	58	nd	nd	nd
	2	P	577	nd	nd	134	99	nd	78	98	382	577	77	nd
		A	177	nd	nd	nd	nd	70	231	102	82	nd	nd	nd
Mean		P	309	215	170	355	164	146	179	67	259	343	119	0
± S.E.M.			112	61	61	116	78	50	108	28	102	110	55	
		A	176	109	125	202	162	136	160	75	124	88	42	0
± S.E.M.			75	76	52	110	94	55	61	33	56	55	27	

5 mg hand-filled capsule containing ^{3}H-prednisolone (H)

Total radioactivity concentration expressed as prednisolone

Dog	Run		Time (minutes) 15	30	45	60	75	90	105	120	135	150	180	240	24 hours
III	1	P	5	388	150	85	134	96	116	81	112	71	96	80	-
		A	2	94	114	111	109	105	113	119	104	97	92	80	0
	2	P	53	81	115	137	141	128	122	126	112	122	114	94	-
		A	19	46	66	95	111	109	114	114	117	105	102	93	0
Mean		P	29	235	133	111	137	112	119	103	112	97	105	87	-
± S.E.M.			24	153	17	26	3	16	3	22	0	25	9	7	
		A	11	70	90	103	110	107	114	117	111	101	97	87	0
± S.E.M.			8	24	24	8	1	2	0	2	6	4	5	6	

5 mg intravenous bolus injection via the jugular vein catheter (I)

Dog	Run		Time (minutes) 4	5.5	10	15	20	30	40	50	60	75	90	120	150	180
II	1	P	516	-	68	nd	nd	nd	nd	nd	588	139	nd	104	nd	nd
		A	-	468	70	nd	nd	nd	nd	nd	nd	63	nd	nd	nd	nd
	2	P	96	-	145	102	nd	nd	111	93	156	nd	100	201	208	378
		A	-	149	278	nd	nd	nd	nd	116	202	nd	nd	88	116	364
	3	P	nd	-	572	251	68	nd	nd	nd	nd	nd	394	484	253	298
		A	-	400	601	267	67	87	78	nd	nd	nd	nd	101	208	316
III	1	P	nd	-	161	nd	nd	130	nd	279	416	nd	270	1141	251	nd
		A	-	440	168	nd	nd	nd	nd	130	435	nd	130	437	230	nd
	2	P	nd	-	1145	501	121	63	nd	nd	nd	289	nd	58	nd	nd
		A	-	nd	1145	393	68	nd	nd	87	nd	74	60	nd	51	nd
	3	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		A	-	667	640	556	151	86	nd	214	182	nd	234	nd	218	111
Mean		P	122	-	418	171	38	39	22	74	232	86	153	398	142	135
± S.E.M.			100		202	94	25	26	22	54	117	57	78	200	59	84
		A	-	354	452	203	48	29	14	91	137	23	71	104	137	132
± S.E.M.				98	178	98	25	18	13	34	71	15	39	69	40	68

50 mg hand-filled capsule (J)

Dog	Run		Time (minutes) 15	30	45	60	75	90	105	120	135	150	180	240
I	1[d]	P	-	135	169	143	-	186	-	78	-	85	-	-
		A	-	289	167	118	-	249	-	78	-	70	-	-
II	1	P	nd	nd	770	84	856	1101	448	311	198	222	64	nd
		A	nd	nd	320	nd	103	776	481	261	250	256	133	125
III	1	P	2191	869	585	329	156	66	89	84	70	nd	86	82
		A	206	342	263	243	154	126	82	81	72	nd	nd	nd
	2	P	1784	1064	644	357	375	59	344	161	251	302	128	nd
		A	887	395	715	254	124	69	55	119	96	151	80	57
Mean		P	1325	517	542	228	462	353	294	159	173	85	93	27
± S.E.M.			673	264	130	68	207	251	107	54	54	49	19	27
		A	364	257	366	154	127	305	206	135	139	119	71	61
± S.E.M.			268	88	120	60	15	161	138	43	56	55	39	36

[d] Dose administered about one hour after a substantial meal.

10 mg hand-filled capsule (K)

Dog	Run		Time (minutes) 15	30	45	60	75	90	105	120	135	150	180	240
I	1	P	-	86	-	78	-	93	-	141	-	nd	nd	-
		A	-	nd	-	nd	-	nd	-	69	-	nd	nd	-
II	1[d]	P	106	81	nd	nd	nd	408	156	493	667	317	228	nd
		A	96	117	303	240	323	nd	nd	291	204	163	267	239
III	1	P	nd	nd	80	nd	100	nd	81	nd	82	384	200	185
		A	nd	nd	nd	nd	nd	nd	89	nd	nd	100	94	134
Mean		P	36	56	54	27	62	167	118	212	273	234	143	62
± S.E.M.			35	28	27	26	31	123	22	146	197	118	71	61
		A	33	40	102	81	108	0	42	120	80	88	121	125
± S.E.M.			32	39	101	80	107		27	88	63	47	78	69

[d] Dose administered one hour after a substantial feed.

10 mg intravenous bolus injection via the jugular vein catheter (L)

Dog		Time (minutes) 4	6	10	15	20	30	40	50	60	75	90	120	150	180
III	P	2782	-	411	277	120	431	638	1345	728	721	701	439	234	341
	A	-	2711	416	270	61	129	721	357	526	488	377	291	238	356

10 mg intraportal bolus injection via the portal vein catheter (M)

Dog		Time (minutes) 4	6	10	15	20	30	40	50	60	90	120	150	180	240
II	P	nd	-	192	63	nd	nd	nd	72	452	232	88	409	189	1209
	A	-	nd	183	nd	nd	nd	nd	nd	320	200	57	313	223	538

2 x 10 mg hand-filled capsules (total dose 20 mg) (N)

Dog	Run		Time (minutes) 30	45	60	90	120	150	180
I	1	P	146	187[b]	228	510	217	194	170
		A	nd	69[b]	138	217	186	203	168
	2	P	164	148	115	97	74	nd	nd[b]
		A	63	113	122	83	76	56	nd[b]
Mean		P	155	168	172	304	146	98	85
± S.E.M.			9	19	56	206	71	96	84
		A	32	91	130	150	131	130	85
± S.E.M.			31	22	8	67	55	73	83

P - A concentration differences of prednisolone (ng/ml) after administration of formulations (A to C) to Dogs I to III.

5 mg commercial tablet (A)

Dog	Run	Time (minutes) 15	30	45	60	75	90	105	120	135	150	180	240
I	2	0	167	448	250	-	104	-	153	-	149	-	-
	3	0	0	0	7	-	149	-	229	-	0	-	-
	4	50	146	82	85	-	(-115)	-	(-60)	-	7	-	-
	5	20	12	(-6)	138	(-46)	0	-	0	-	0	-	-
II	1	0	55	159	0	0	0	0	0	208	(-26)	0	0
	2	0	81	47	75	(-16)	18	(-11)	(-96)	(-43)	(-38)	0	0
	3	0	0	0	0	0	70	112	21	0	446	(-162)	(-260)
III	1	55	212	(-145)	37	13	11	0	0	0	0	82	92
	2	0	(-5)	170	242	(-61)	0	179	(-81)	525	41	(-2)	(-21)
Mean		14	74	84	93	(-18)	26	56	18	138	64	(-16)	(-38)
± S.E.M.		6	23	55	36	18	34	34	27	69	42	23	34

5 mg commercial tablet (B)

Dog	Run	Time (minutes) 15	30	45	60	75	90	105	120	135	150	180	240
I	1	-	0	107	(-5)	-	9	-	(-12)	-	0	-	-
	2	-	0	566	0	-	65	-	16	-	0	-	-
II	1	0	(-27)	0	0	0	59	0	89	9	2	0	0
	2	0	0	(-33)	252	24	15	167	73	0	217	0	5
	3	36	135	0	0	0	(-60)	27	130	(-56)	0	0	0
III	1	0	0	0	149	(-57)	44	47	74	(-1)	5	0	0
	2	0	0	0	0	0	0	0	0	21	76	7	(-58)
Mean		7	15	91	57	(-7)	19	48	53	(-5)	43	1	(-11)
± S.E.M.		8	26	62	41	19	38	34	31	69	47	23	34

5 mg commercial tablet (C)

Dog	Run	Time (minutes) 15	30	45	60	75	90	105	120	135	150	180	240
I	1	-	0	28	30	-	145	-	125	-	(-69)	-	-
	2	-	0	34	222	-	(-29)	-	(-67)	-	0	-	-
II	1	0	26	10	256	89	(-69)	(-36)	98	(-173)	24	57	0
	2	0	0	0	0	0	(-48)	17	33	0	(-55)	0	0
	3	0	0	0	107	132	54	85	3	81	101	0	0
III	1	0	0	218	0	(-68)	61	0	0	0	0	0	0
	2	0	52	588	(-45)	10	(-301)	(-54)	(-63)	79	0	0	15
Mean		0	11	125	81	33	(-27)	2	18	(-3)	0	11	3
± S.E.M.		8	26	62	41	19	38	34	31	69	47	23	34

P - A concentration differences of prednisolone (ng/ml) of the mean data from administration of formulations D to N to Dogs I to III.

Formulation	Time (minutes) 10	15	20	30	40	45	50	60	75	90	105	120	135	150	180	240
D	-	0	-	117	-	148	-	32	29	86	129	45	10	90	26	63
E	-	0	-	40	-	152	-	152	143	113	102	5	8	5	79	0
F	-	190	-	94	-	26	-	64	36	122	86	(-13)	(-68)	(-53)	10	10
G	-	133	-	106	-	45	-	153	2	10	19	(-8)	135	255	77	0
H	-	18	-	165	-	43	-	8	27	5	5	(-14)	1	(-4)	8	0
I	(-34)	(-32)	(-10)	10	8	-	(-17)	95	63	82	-	294	-	5	3	-
J	-	961	-	260	-	176	-	74	335	48	88	24	34	(-34)	22	(-34)
K	-	3	-	16	-	(-48)	-	(-54)	(-46)	167	76	92	193	339	22	(-63)