A STUDY OF

THE INTERACTIONS BETWEEN PHENYTOIN

AND PHARMACEUTICAL ANTACIDS,

EXCIPIENTS AND ADSORBENTS

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ABBREVIATIONS

AUC	–	Area under blood level curve
B.P.	–	British Pharmacopoeia
B.P.C.	–	British Pharmaceutical Codex
I.P.A.	–	International Pharmaceutical Abstracts
S.D.	–	Standard deviation
U.S.P.	–	United States Pharmacopoeia

A STUDY OF THE INTERACTIONS BETWEEN PHENYTOIN AND PHARMACEUTICAL ANTACIDS, EXCIPIENTS AND ADSORBENTS

INTRODUCTION:

Phenytoin is a drug which is widely used in the treatment of epilepsy. Therapeutically, it is important that the correct concentration of phenytoin is maintained in the plasma because low plasma levels may result in the inadequate control of seizures, while elevated plasma levels may result in the appearance of symptoms of toxicity. Martindale's Extra Pharmacopoeia (1977) suggests that the dose of the drug be adjusted to the needs of the individual patient to achieve the adequate control of seizures.

Phenytoin sodium is a strongly alkaline compound which may cause gastric irritation unless each dose is taken with water after meals, a practice which may reduce the therapeutic effect. The alkalinity may lead patients to erroneously take antacids while being treated with phenytoin in an attempt to reduce the gastro-intestinal irritation caused by the drug.

A review of the literature has revealed that the administration of antacid preparations and medicinals which contain adsorbent powders such as kaolin and activated charcoal may alter the gastro-intestinal absorption of orally administered drugs. In addition, the excipients used in the preparation of pharmaceutical dosage forms can influence the absorption of drugs. Phenytoin interactions

have been observed by Decker *et al.* (1968), who reported on the adsorption of phenytoin from solution onto activated charcoal; by Tyrer *et al.* (1970), who attributed cases of phenytoin toxicity to phenytoin/excipient interactions, and by Pippenger (1975), who observed an interaction between orally administered phenytoin and antacids. It appears that further investigations into the phenomena studied by both Tyrer and Pippenger might prove useful because of the sparcity of information concerning these interactions available in current literature.

Two separate areas of study were therefore proposed:

(1) An assessment of the *in vitro* removal of phenytoin from solution by insoluble particulate matter;

(2) *In vitro* studies to determine the effect of selected antacids and adsorbents on the passage of phenytoin through a biological membrane at various pH values.

CHAPTER 1 LITERATURE SURVEY

1.1 PHENYTOIN

According to Delgado and Isaacson (1970), phenytoin was first prepared by Biltz in 1908. The drug was evaluated by Merritt and Putnam in 1938 and found to be effective in protecting cats against convulsive seizures induced by electrical stimulation. Since its introduction as a medicinal agent, phenytoin has become a prime drug for use in the treatment of epilepsy.

Martindale's Extra Pharmacopoeia (1977) states that phenytoin has little hypnotic action and is believed to stabilise rather than to elevate the seizure threshold and to limit the spread of seizure activity. It is of value in the control of grand mal and psychomotor epilepsy but is of little value in petit mal attacks. It can also be used in the treatment of status epilepticus, in the control of seizures in neurosurgery and in the treatment of cardiac arrhythmias.

Orally, phenytoin may be administered as the free acid or as the sodium salt. The suggested initial dose for adults is 100 mg of phenytoin sodium three times daily, progressively increasing the dose at intervals of a few days to a maximum of 600 mg daily.

1.2 PHYSICOCHEMICAL PROPERTIES OF PHENYTOIN

The physicochemical properties of phenytoin have been reviewed by Feldman et al. (1975) and Schwartz et al. (1977). Phenytoin is a weak organic acid with a pK_a of 8,3. The solubility of the drug in water is dependent on pH; it is poorly soluble in water but readily dissolves in alkali. Phenytoin is a sufficiently

weak acid to exist mainly in the unionised form at pH values less than eight.

Dill et al. (1956) and Schwartz et al. (1977) both give ranges for the solubility of phenytoin. For example, Schwartz quotes solubilities of 56,8 $\mu g/cm^3$ at pH 8,3 falling to 18,4 $\mu g/cm^3$ at pH 4,9 at 25 ^{o}C.

Schwartz warns that it is necessary for investigators to have a clear understanding of the physicochemical characteristics of phenytoin. "Because of its limited solubility, hydro-alcoholic solutions have been used in the past to broaden the experimental range of concentrations. Alcohol would affect in vitro binding experiments; studies intended to be of clinical relevance should be conducted at physiologic temperature, pH, ionic strength in aqueous buffer."

1.3 ABSORPTION OF PHENYTOIN

Glazko (1972 a) states that the absorption of phenytoin after oral administration is slow, somewhat variable and occassionally incomplete and that significant differences in the bio-availability of phenytoin from oral pharmaceutical preparations have been reported. The absorption of phenytoin has been reviewed by Woodbury and Swinyard (1972), who state that phenytoin is mainly absorbed through the intestinal mucosa by passive diffusion, a phenomenon which is governed by the pK_a and lipid solubility of the compound and the pH of the medium in which the substance is dissolved.

In the stomach, phenytoin (pK_a 8,3) will be predominantly in the non-ionised form but absorption will be limited by the low solubility of the drug in the gastric juice (pH about 2,0). In the duodenum (pH 7,0 to 7,5) the proportion of phenytoin which is ionised increases and the drug becomes more soluble in the intestinal fluids. This

increased solubility (which is enhanced by the presence of bile salts) and the large surface area of the small intestine both contribute to the rapid absorption of the drug from this region of the gastro-intestinal tract. However, even at the higher pH of the intestinal fluid, phenytoin is relatively insoluble, (100 $\mu g/cm^3$ at pH 7,8). The rate of absorption depends mainly on the rate at which phenytoin can enter the blood stream. The solubility of phenytoin in plasma is only 75 $\mu g/cm^3$ at 37 ^{o}C. Thus absorption can occur only at the rate at which the phenytoin is removed from the blood by storage in fat, binding to tissue and plasma constituents, metabolism by the liver, and excretion into bile and urine.

1.4 INTERACTIONS OF PHENYTOIN

Phenytoin is known to interact with a number of other drugs. These interactions are summarised in the Extra Pharmacopoeia (1977) and have been well documented by Martin et al. (1971), Hartshorn (1972), Kutt (1972), Buchanan and Sholiton (1972) and Kutt (1975). Kutt (1975) states that a variety of interactions may take place between anti-epileptic drugs or between anti-epileptic drugs and other therapeutic or chemical agents. These interactions will result in alterations in the solubility, absorption, metabolism, elimination and tissue distribution of the drug in question. No attempt will be made to discuss the numerous interactions of phenytoin with other drugs, but the specific interactions of phenytoin with antacids, excipients, adsorbents and metal ions will be dealt with individually.

1.4.1 Interaction of phenytoin with antacids

The field of drug-antacid interactions has been reviewed by Hurwitz (1977), who states that antacids are commonly used drugs which are often considered by many patients and

physicians to be inert and free from pharmacological effect. The Extra Pharmacopoeia (1977) cautions against the administration of other medication to patients on antacid therapy because, "Gastro-intestinal absorption can be reduced by adsorption on insoluble antacids or changes in gastric emptying time and the effects of a drug may be diminished or enhanced by alterations in the intestinal pH or by the formation of complexes".

The interaction between antacids and phenytoin was observed by Pippenger (personal communication 1978). "In 1973 we did observe clinically that patients receiving antacid therapy showed a marked reduction in the serum concentrations of phenytoin which were achieved on standard dosage regimens. ... Further investigation into this matter clearly demonstrated that the low serum concentration was directly due to a blockage of phenytoin absorption by the antacid, Mylanta." (A dried aluminium hydroxide gel - magnesium hydroxide, dimethicone combination.)

In further studies Pippenger (personal communication 1978) reports that three different antacid preparations "when given simultaneously with the total daily dosage of phenytoin, caused a marked reduction in serum phenytoin concentrations". In conclusion, Pippenger states, "There is no question on the basis of these studies, that there is indeed a clear-cut interaction between antacids and the absorption of phenytoin. Further clinical studies on patients receiving these two drugs in combination indicated that the effect of antacid on the absorption of phenytoin could be blocked simply by administering the antacid four hours away from the time the phenytoin was administered."

Pippenger attributes the observed phenytoin-antacid

interactions to a combination of factors, including :

(1) incomplete breakdown of the phenytoin capsule;

(2) the possible chelation of the phenytoin by the magnesium and calcium salts present in the antacids; and

(3) a blocking of the absorptive sites of the phenytoin within the intestinal mucosa.

Three further studies pertaining to the interaction of phenytoin and antacids were published by O'Brien et al. (1978), Kulshrestha et al. (1978) and Garnett et al. (1979).

O'Brien et al. studied the effects of antacids on the pharmacokinetics of phehytoin and observed that neither 10 cm^3 of Aluminium Hydroxide Gel B.P. nor a magnesium hydroxide mixture altered significantly either the rate or the extent of the absorption of a single oral dose of phenytoin. The authors attribute their failure to show an effect of antacids on the kinetics of phenytoin to the design of the study and to the fact that changes in the steady state concentrations of phenytoin in the plasma may be a more sensitive index than changes in the single dose kinetics studied.

In a more meaningful study, Kulshrestha et al. (1978) administered two antacid preparations (one containing calcium carbonate and the other a mixture of aluminium hydroxide and magnesium trisilicate - Gelusil ^{R}) to groups of epileptic patients who were stabilised on phenytoin therapy. The concentration of phenytoin in the serum was monitored. Analysis of the results revealed that there was no change in the mean concentration of phenytoin in the serum within the group treated with

calcium carbonate. The group treated with Gelusil [R] experienced a slight but statistically significant fall in the concentration of phenytoin in the serum (from 40,4 to 35,4 $\mu m/dm^3$.

The following factors are mentioned as the possible causes for the fall in concentration of phenytoin in the serum :

(1) the antacids may release metal ions which chelate with phenytoin and result in reduced absorption;

(2) aluminium hydroxide and magnesium trisilicate may form gels in the gastro-intestinal tract. These gels could possibly adsorb the phenytoin, thus reducing the absorption of the latter;

(3) the antacids may increase the pH of the upper region of the gastro-intestinal tract, thus increasing the ionisation of the phenytoin and hence reducing its absorption. However, the brief duration of the pH change would perhaps be insufficient to reduce the absorption of the relatively slowly absorbed drug;

(4) the presence of aluminium salts may reduce the gastro-intestinal motility, a condition which could delay absorption if not reduce the amount of drug absorbed.

Garnett et al. (1979) investigated the bio-availability of phenytoin given concomitantly with antacids. The antacids used were a combination of aluminium hydroxide and magnesium hydroxide gel, calcium carbonate and a combination of aluminium hydroxide and magnesium trisilicate. Eight subjects were used and served as their own controls. On analysis all the antacids decreased the mean AUC for phenytoin. These authors state that their data support the reports of an interaction between

phenytoin and antacids.

From these sources, two areas of study emerged :

(1) an investigation of the adsorption of phenytoin onto the particulate matter, this phenomenon could delay the absorption of the drug;

(2) an assessment of the possible interactions between phenytoin and the antacids with respect to their influence on the permeation of the phenytoin through a membrane.

1.4.2 Interaction of phenytoin with excipients

Phenytoin is frequently administered as the sodium salt in capsule form. The Pharmaceutical Codex (1979) states that it is customary to issue capsules almost completely filled, an inert diluent being necessary when the dose of the medicament is less than 100 mg and if the capsules are machine filled. The Codex lists the following diluents as being suitable: lactose, magnesium carbonate, magnesium oxide, calcium carbonate, starch, mannitol and kaolin. This list includes three antacids and kaolin, which is acknowledged by the Codex to have adsorbent properties. In addition, pharmaceutical grade starches may interact with medicinals.

The interaction of antacids and starches, used as excipients, with dicoumarol was demonstrated by Akers et al. (1973). In studies conducted in dogs, significantly higher plasma levels were observed when dicoumarol was administered with magnesium oxide or hydroxide. The use of talc, aluminium hydroxide and starch resulted in significantly lower plasma levels of the drug. The interaction of starch with pharmaceuticals has been reported by Zografi and Mattocks (1963), who report on the adsorption of anionic dyes by

starch. Goudah and Guth (1965) found that potato starch and arrowroot starch form complexes with benzoic acid and p-hydroxybenzoic acid esters. Salicylic acid was found to form an insoluble complex with both starches. Caffeine, however, did not interact with the starches tested. Mansour and Guth (1968) further found that preservatives such as benzoic acid, p-hydroxybenzoic acid and sorbic acid form complexes with potato starch, arrowroot starch, corn starch, rice starch and a number of commercial starches rich in amylose and that the starches show different affinities for the same compound according to their content of amylose.

Since starches act as adsorbents and form complexes with medicinals, it appeared that an investigation of the interactions of phenytoin with starch could prove of value.

A specific interaction between phenytoin and an excipient has been widely reported as a result of an outbreak of phenytoin intoxication in Australia in 1968. Tyrer et al. (1970) studied the outbreak in Brisbane and report that, in all, 51 persons were affected.

Investigations led to the conclusion that a change in the excipient in the phenytoin sodium capsules was the cause of the observed intoxication. The excipient had been changed from calcium sulphate dihydrate to lactose prior to the reported outbreak.

Further direct evidence was obtained from a series of determinations of the concentration of phenytoin in the blood of a single patient who was changed from phenytoin with lactose to phenytoin with calcium sulphate as excipient and then back to phenytoin with lactose as excipient. The concentration of phenytoin in the patient's

blood fell rapidly to a quarter of its former value when the pheyntoin with calcium sulphate was used instead of phenytoin with lactose. The concentration of phenytoin in the blood rose again when phenytoin with lactose as excipient was re-introduced. Similar tests carried out on a further three patients produced the same type of result.

Tyrer et al. (1970) draw the following conclusions from their study :

(1) the evidence presented showed that a change in the excipient in the phenytoin sodium capsules was associated with the alterations in concentration of phenytoin in the blood and with the clinical anticonvulsant intoxication observed;

(2) the mechanism through which the changed excipient exerted its effects seemed to be the same among epileptics;

(3) the excipients used in pharmaceutical preparations may not always be biologically inert. Excipients present in one preparation may theoretically alter the metabolism of another drug administered simultaneously.

A survey of the literature, however, has failed to reveal further information as to the nature of the calcium sulphate/phenytoin interaction, but it seems possible that the following explanations can be considered :

(1) an alteration in the dissolution rate of the capsules containing calcium sulphate dihydrate;

(2) since calcium sulphate dihydrate is moderately soluble in both water and dilute mineral acids, the

calcium ions released in the gastro-intestinal tract may possibly chelate with the phenytoin in solution within the gastro-intestinal tract and so reduce the absorption of the anticonvulsant.

1.4.3 Interaction of phenytoin with metal ions

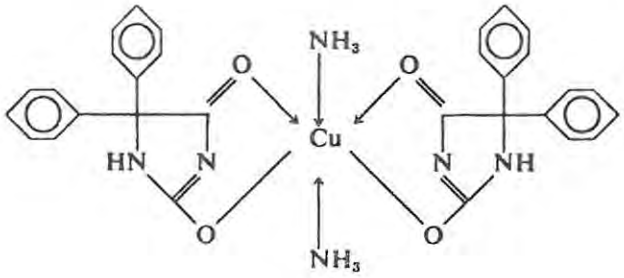
Antacids and other compounds containing polyvalent cations such as aluminium, calcium and magnesium may release these ions in the gastro-intestinal tract. Certain materials may form chelates or complexes with these cations. This phenomenon may result in the reduction in the amount of drug absorbed from the gastro-intestinal tract.

Neuvonen (1976) discusses the interactions influencing the absorption of tetracylines and states that the absorption of all tetracycline derivatives may be seriously affected by the simultaneous ingestion of antacids containing polyvalent cations such as Al(III), Mg(II) and Ca(II). The effect of this interaction was demonstrated *in vitro* by Juul Christensen *et al*. (1967). Their studies show that the diffusion of tetracycline hydrochloride through a membrane is diminished by magnesium oxide, aluminium hydroxide and calcium carbonate.

The elevated plasma levels of dicoumarol in dogs resulting from the co-administration of magnesium oxide or hydroxide reported by Akers *et al*. (1973) are attributed to chelate formation. The magnesium chelate of dicoumarol produces higher plasma levels than the drug administered alone.

Like many of the barbiturates, phenytoin forms a characteristic copper complex when treated with copper sulphate in pyridine solution. This reaction forms the basis for the B.P.C. identification test for phenytoin.

Glazko and Chang (1972) report on the formation of the metal chelates of phenytoin. A copper chelate is formed by adding $CuCl_2$ to a solution of phenytoin dissolved in 0,1 mol/dm^3 ammonium hydroxide and shaking continuously for 1 hour. The red precipitate formed contains 11 % copper. The molecular formula of $C_{30}H_{22}O_4Cu{-}2NH_3$ indicated chelation of two moles of phenytoin with one mole of copper. The following structural formula for the complex was proposed :



Phenytoin also appeared to form a complex with cobalt salts, but there was no indication of chelate formation with calcium, magnesium or ferrous salts. The authors, however, state that no conclusive evidence was obtained for the formation or the excretion of metal chelates of phenytoin in animals or in man.

It is important to note that this formation took place in ammonia solution. The question thus arises as to whether chelation of phenytoin with calcium, or magnesium ions, could not occur in the physiological pH ranges in the gastro-intestinal tract.

1.4.4 Interaction between phenytoin and adsorbents

A single reference was found which reported a clear interaction between phenytoin and an adsorbent powder.

This study is reported by Decker et al. (1968) and was carried out to assess the effectiveness of activated charcoal as an adsorbent in cases of poisoning by drugs and other toxic compounds.

One hundred cm^3 of artificial gastric juice was placed in a flask and incubated at 37 ^{o}C in a metabolic shaker. For the medicinals, capsules or tablets were used and these were emptied or crushed before dispersion in a small volume of water and addition to the flask. A slurry of 5 g of charcoal in 50 cm^3 of water was transferred to the flask and incubation (with shaking) was continued. After 20 min, the charcoal was separated from the contents of the flask by rapid filtration or centrifugation and the amount of drug remaining in solution was determined by the appropriate analytical method. The results of Decker et al. show that more than 80 % of the phenytoin is removed from solution in tests conducted with 1, 7 and 20 phenytoin tablets of an unspecified strength.

1.5 ANALYTICAL METHODS FOR PHENYTOIN

The various analytical methods available for the determination of phenytoin in both pharmaceutical dosage forms and in bio-availability studies are reviewed by Glazko (1972 b). In addition to the methods listed by Glazko, a number of more recent procedures such as radio-immunoassay, enzyme-immunoassay, spectrofluorimetry and high pressure liquid chromatography are available. In view of the many methods available, the following criteria were set for the choice of the most suitable procedure :

(1) The solubility of phenytoin is limited at physiological pH values, therefore the method had to be sufficiently accurate to measure concentrations up to 100 $\mu g/cm^3$ of the drug;

(2) The method should be rapid and simple enough for a large number of determinations to be performed daily by a single worker;

(3) The availability and cost of the apparatus and reagents.

On assessment, the spectrophotometric methods and in particular the benzophenone-extraction procedure seemed most suitable and therefore a detailed investigation of these were carried out.

1.5.1 Spectrophotometric determination of phenytoin

Glazko (1972 b) states that the first major breakthrough in these methods was reported in 1956 with the development of a spectrophotometric method by Plaa and Hine and the colorimetric procedure by Dill et al. The method of Dill et al. (1956) has been widely used but according to Glazko, it is time consuming. The practicalities were therefore not investigated but the method was borne in mind as a useful alternative.

According to Clarke (1969), phenytoin in methanol exhibits an absorption maximum at 258 nm. This property has been used by a number of workers for the measurement of phenytoin and the barbiturates in both pharmaceutical dosage forms and the blood. Examples of such work include the determination of phenytoin and phenobarbitone in serum by Westerink and Glerum (1964), and Huisman and Dek (1964). The spectrophotometric determination of phenytoin and barbiturates in pharmaceutical dosage forms has been reported by Diliberto (1969) and Amer et al. (1976) among others. These workers used various solvents at different pH's in order that the mixture of compounds could be determined.

The benzophenone-extraction procedure was reviewed by

Glazko (1972 b). This method was originally introduced by Wallace et al. (1965) and is based upon the formation of benzophenone from phenytoin by heating with alkali and further treatment with bromine. The reaction product is separated by steam distillation, followed by extraction of the benzophenone from the distillate with an organic solvent and measurement of the ultraviolet absorbance. Glazko (1972 b) states that the advantage of this method is that phenobarbitone, and most other drugs, do not interfere with the assay, and normal plasma blanks are low. However, the original procedure required large volumes of blood and a great many modifications were made in order to provide a technique suitable for clinical applications.

Wallace (1966, 1968 and 1969) published further modifications which incorporate the use of potassium permanganate for the oxidation step, and the elimination of the distillation procedure by refluxing with n-heptane during the oxidation of the sample. The procedure was scaled down by Lee and Bass (1970), who achieved a sensitivity of 1 $\mu g/cm^3$ with 2 cm^3 samples of plasma. Morselli (1970) used a scaled-down procedure and refluxing to achieve an assay rate of 40 samples per 4 hours.

Further investigations and modifications of the method were published by Bock and Sherwin (1971), Meulenhoff and Kutsch Lojenga (1972), Glazko (1972 b), Saitoh et al. (1973), Wallace et al. (1974), and Wallace and Hamilton (1974).

Saitoh et al. (1973) describe a modification to the method proposed by Wallace (1968). Improvements were made possible by the introduction of changes in the reaction conditions and by the determination of the benzophenone concentration by the use of the expanded scale of a

recording spectrophotometer. The conversion of phenytoin to benzophenone was found to be quantitative and the blank value negligible. The method allows the use of plasma or whole blood samples as small as 0,1 cm^3. In addition, the method has proved useful for *in vitro* investigations and was successfully applied to a study of the dissolution behaviour of phenytoin from a ground mixture with microcrystalline cellulose by Yamamoto *et al.* (1976). It was therefore decided to carry out an in depth study of this method.

Saitoh *et al.* describe the method of Wallace (1968) as follows :

" 20 ml of 1 % $KMnO_4$ in 7N NaOH and 5 ml of n-heptane are added to diphenylhydantoin extracted from biological specimens, and the mixture is then refluxed for 30 min with constant magnetic stirring. After cooling, the n-heptane layer is separated from the aqueous permanganate layer and its absorbance is determined at 247 nm against pure n-heptane." Blank values from 10 cm^3 of oxalated whole blood containing no phenytoin were determined.

Saitoh *et al.* experienced problems with the blank readings and investigated this aspect. Higher blank readings were obtained when the n-heptane was added to the oxidation mixture prior to refluxing. A procedure was devised in which the phenytoin was converted to benzophenone and extracted in the n-heptane after cooling. This method yielded blank readings of an acceptably low absorbance.

The effect of reaction conditions on the yield of benzophenone was investigated by Saitoh *et al.* because the Wallace method of refluxing is time consuming if a number of samples are to be analysed. The results of

the study show that the oxidation of phenytoin with alkaline permanganate should be performed at temperatures lower than 80 °C. Furthermore, refluxing for 30 min with constant stirring as proposed by Wallace (1968) is unnecessary and that the benzophenone which forms quantitatively by the permanganate oxidation at 80 °C for 5 min is completely extracted by the n-heptane after shaking for 10 min.

The method devised by Saitoh et al. is as follows :

The phenytoin is extracted from the blood or plasma as described and to 0,5 cm^3 of the phenytoin extract is added 10 cm^3 of 1 % $KMnO_4$ in 7 mol/dm^3 NaOH. The tube is immersed in a water bath at 80 °C for 5 min. After cooling, 2 cm^3 of n-heptane is added to the solution and the mixture is shaken for 10 min and centrifuged for 5 min. The n-heptane layer is then transferred into a microcell and the absorbance recorded on a ten-fold scale of a recording spectrophotometer over the range of 220 to 270 nm against pure n-heptane. The peak reading at 247 nm is read on the recording paper.

Using this method, Saitoh et al. conclude that there is a linear relationship between the absorbance readings and the phenytoin concentrations up to 30 $\mu g/cm^3$. The average percentage phenytoin recovered from all samples with this method was found to be 100,1 with a standard deviation of 1,0.

The method devised by Saitoh et al. (1973) was reviewed by Wallace and Hamilton (1974), who state that, according to their investigations, the nonreflux technique does not provide the sensitivity obtained through reflux. However, the method does offer the advantages of not

requiring the more expensive reflux apparatus and of achieving its optimum sensitivity in less time. " Thus, for laboratories having a limited budget and not possessing reflux apparatus, the nonreflux technique may be the most appropriate method."

In addition, the publication of Wallace and Hamilton gives the following practicalities :

(1) The amount of potassium permanganate (0,1 to 0,4 g per 10 cm^3) and the concentration of the sodium hydroxide (5 - 11 mol/dm^3) are not critical for the quantitative oxidation of phenytoin;

(2) Centrifugation of the n-heptane/permanganate oxidising mixture is unnecessary for the recovery of the n-heptane. Separation is achieved by the addition of water to float the n-heptane to the neck of the oxidation flask or tube, from whence it is transferred to the spectrophotometer cell.

Bearing the above discussion in mind, it was decided to proceed with an investigation of the oxidation method as devised by Saitoh et al. This involved an investigation into the sensitivity of the method, the production of absorbance curves to assess the wavelength at which the peak absorbance occurred, and the preparation of standard curves for benzophenone in n-heptane and for the oxidation procedure for phenytoin.

1.6 METHODS FOR ASSESSING DRUG INTERATIONS

It has previously been shown that possible phenytoin-antacid and phenytoin-excipient interactions have been observed clinically but information is lacking as to the nature of these interactions. A brief survey of the feasibility of in vivo studies on human subjects

or in animals was made but practical difficulties relating to cost, availability of apparatus and the inconclusive clinical results obtained by O'Brien *et al.* (1978) mitigated against such studies. The various *in vitro* methods available were therefore investigated. These methods were classified into two separate categories, namely, the adsorption of drugs from solution onto particulate matter and the assessment of drug diffusion through various membranes of both natural and synthetic materials.

1.6.1 "In Vitro" adsorption studies

A number of successful *in vitro* studies have been performed to demonstrate the adsorption of drugs in solution onto dispersed insoluble powders. Furthermore, it has been possible to correlate these results to clinical studies on human subjects in some instances.

In a series of studies conducted between 1961 and 1968, Sorby and co-workers attempted to elucidate the effects of various adsorbents on the absorption of a number of phenothiazine derivatives. Preliminary studies by Sorby and Plein (1961) indicate that considerable adsorption of the phenothiazines onto kaolin, talc and activated charcoal takes place. The *in vitro* studies were extended by Sorby (1965) and by Sorby and Liu (1966) in an attempt to establish a more complete understanding of the relationship which exists between the adsorption of drugs onto powders and their bioavailability. The possibility of using *in vitro* data to predict *in vivo* effects was investigated.

Promazine and admixtures of either activated attapulgite, or activated charcoal were used in 1965. The rate and the extent of the absorption of the drug in humans was studied using urinary excretion data. It was found that

the initial rate of the appearance of the drug in the urine is slowed but there is little decrease in the total availability of promazine when it is administered in mixtures containing activated attapulgite. Activated charcoal is reported to decrease both the rate and the extent of the absorption of promazine. In the test, 50 mg of promazine hydrochloride was equilibrated with the adsorbent prior to administration.

In the 1966 study, the adsorbent and the drug were not equilibrated prior to administration. This change in procedure served the purpose of ensuring that the results would have maximum applicability to the clinical situation. In vivo tests were conducted on a single male subject using 50 mg of promazine hydrochloride and an antidiarrhoeal preparation containing attapulgite and citrus pectin. It was found that the presence of the antidiarrhoeal mixture within the gastro-intestinal tract is sufficient to retard the absorption of the promazine. Adsorption isotherms were determined before in vivo tests were conducted. Sorby et al. (1966) further demonstrated the effects of pH and electrolyte concentration on the adsorption of phenothazine onto kaolin, talc and activated charcoal.

However, in a later study, Sorby (1968) found that promazine absorption from the human gastro-intestinal tract is unaffected by small amounts of activated attapulgite and activated charcoal if the drug and the adsorbent are not equilibrated before administration of a test dose.

Blaug and Gross (1965) reviewed the adsorption of a number of drugs onto antacids. Their discussion of reports dating from 1938 to 1965 deals with the loss of atropine, belladonna alkaloids and anticholinergics from solution

onto various insoluble antacids. Blaug and Gross measured the adsorption of nine anticholinergic drugs in aqueous solution onto six different insoluble antacids. The results obtained were plotted and interpreted according to the Langmuir isotherm. It was found that the adsorptive power of the antacids varies with the anticholinergic drugs investigated; atropine sulphate, methantheline bromide, propantheline bromide and oxyphenonium bromide are adsorbed to the greatest extent, and that magnesium trisilicate shows the highest adsorptive capacity of the antacids studied. Blaug and Gross caution against the administration of strong adsorbents and anticholinergic drugs in combination.

El-Nakeeb and Yousef (1968) investigated the effect of twenty-two compounds used as adsorbing, suspending and solubilising agents on the activity of eight antibiotics. Kaolin was found to reduce the antibacterial activity of neomycin, streptomycin and tetracycline hydrochloride. The reduction in activity is attributed to adsorption of the antibiotics onto kaolin. Khalil et al. (1976), however, report that out of a group of powders tested with seven antibiotics, kaolin has the least adsorptive power and that the tetracyclines are more highly adsorbed than other antibiotics.

The adsorption of digoxin onto an aluminium hydroxide-magnesium hydroxide combination and onto a kaolin-pectin combination was demonstrated by Binnion and McDermott (1972) who observed that both medications interfere with the absorption of digoxin in humans.

Khalil and Moustafa (1973) studied the in vitro adsorption of some tranquilisers, sedatives and anticholinergic compounds by antacids. The results indicate that in most cases magnesium trisilicate and magnesium oxide have

relatively higher adsorptive capacities for the compounds used. Calcium carbonate and aluminium hydroxide have an intermediate capacity, while bismuth oxycarbonate has the least adsorptive power. It was found that phenobarbitone and meprobamate are not adsorbed to any significant extent. The authors conclude by stating that careful *in vitro* and *in vivo* testing of drug availability is advisable prior to the administration of combinations of drugs and antacids. However, *in vitro* experiments are usually simpler, easier to interpret and should, with careful consideration of results, help to predict possible interactions that might take place in a dosage form or in the gastro-intestinal tract following the simultaneous administration of drug-antacid combinations.

The adsorption of atropine and hyoscine onto magnesium trisilicate was studied by El-Masry and Khalil (1974). They report that at relatively low initial concentrations of the drug, the adsorption data is shown to fit a Langmuir plot while at higher concentrations the extent of adsorption increases due to multilayer formation. Further *in vitro* adsorption studies of hyoscyamine onto magnesium trisilicate in Magnesium Trisilicate and Belladonna Mixture B.P.C. were performed. It was found that about 93 % of the hyoscyamine content of the belladonna tincture is adsorbed onto the magnesium trisilicate and that adsorption is complete within 30 min.

The uptake of digoxin and digitoxin by antacids was assessed by Khalil (1974). Among the antacids tested, magnesium trisilicate exhibits the highest adsorptive effect. The relatively weak adsorptive capacity of calcium carbonate, magnesium oxide, magnesium carbonate and aluminium hydroxide gel appear of little significance since these antacids dissolve in the gastric juice *in vivo*.

Magnesium trisilicate reacts in acid media and forms hydrated silica gel, which has adsorbing properties. The adsorption of the two glycosides was found to be dependent on the level of hydrochloric acid in the medium. Adsorption was noted even when 94 % of the antacid was converted to the gel.

Khalil et al. (1976) investigated the in vitro adsorption of various antibiotics onto antacids. They report that magnesium trisilicate and magnesium oxide show the highest adsorptive capacity; calcium carbonate and aluminium hydroxide have intermediate power, while bismuth oxycarbonate has the least adsorptive power. Tetracyclines were found to be more highly adsorbed than other antibiotics while triacetyloleandomycin and chloramphenicol have intermediate values. It was found that ampicillin is only adsorbed to a slight extent, while cloxacillin is not adsorbed.

Naggar et al. (1976) investigated the in vitro adsorption of some antirheumatics onto antacids and kaolin. These workers show that magnesium oxide, followed by aluminium hydroxide and bismuth oxycarbonate have a "fairly high" adsorptive capacity for compounds such as the salicylates, mefenamic acid and flufenamic acid. Magnesium trisilicate was found to have a tendency to adsorb phenazone, aminophenazone, indomethacin and methiazinic acid. Indomethacin, methiazinic acid, mefenamic acid and flufenamic acid are significantly adsorbed by kaolin.

Yu et al. (1976) observed that patients suffering from chronic diarrhoea and who were stabilised with codeine phosphate suffered relapses when the codeine phosphate was added to a kaolin mixture prior to administration. In vitro adsorption studies conducted with synthetic

gastric or intestinal fluids revealed that codeine phosphate is adsorbed onto kaolin and that the amount of drug adsorbed depends on the time of contact with the kaolin.

Numerous other studies of the adsorption phenomena have been reported. For example, Ridout (1968 (a) and (b)) investigated the adsorption isotherms for atropine sulphate onto kaolin. Further investigations to elucidate the nature of the kaolin surface and the adsorption phenomenon were carried out by Armstrong and Clarke (1971, 1973 and 1976).

It has previously been reported that Decker et al. (1968) studied the adsorption of phenytoin and other toxic substances onto activated charcoal. A number of other workers have performed tests to assess the effectiveness of activated charcoal as an adsorbent for drugs. These include Sintek et al. (1978), Sellers et al. (1977) and Sanvordeker and Dajani (1975) who studied theophylline; various analgesics, sedatives, tranquilizers, antidepressants and diphenoxylate hydrochloride with respect to their adsorption onto activated charcoal in vitro.

1.6.2 "In vitro" drug diffusion studies through membranes

Bates and Gibaldi (1970) state that a variety of experimental methods are available for studying the absorption of drugs. In vitro techniques are often relatively simple and may provide valuable information on the gastro-intestinal absorption of drugs.

The techniques available for the study of drug diffusion in vitro may be broadly classified into those methods which make use of synthetic lipid membranes and those methods which make use of biological membranes. Both classifications are based on the creation of suitable conditions of pH on either side of the membrane and the

measurement of the diffusion of a substance across the membrane under the conditions created.

Schanker (1961) states that gastro-intestinal membranes and many other biological membranes act as lipid barriers to most drugs. Levy and Mroszczak (1968) report that physicochemical systems consisting of two aqueous phases of different pH separated by a lipid phase are employed as models for *in vitro* studies of drug absorption by passive diffusion. These *in vitro* models may be classified into two groups :

(1) Models in which the aqueous phases are separated by a lipid membrane. Such systems were used by Inui *et al*. (1977), Yano *et al*. (1974), Falk and Fuerst (1974) and Nakano (1971) among others. The Sartorius Membranefilter Company has developed a commercially available apparatus devised by Stricker. Stricker (1971 (a) and (b) and 1973) published reviews on works using this particular apparatus, while Iinuma *et al*. (1976), used the Sartorius apparatus to demonstrate the correlation between the permeation rate constants through the artificial membrane and the *in situ* absorption rate constant for a series of sulphonamides and barbiturates in the rat;

(2) Models employing a water-immiscible organic liquid as a barrier. These are discussed in the publications of Doluisio and Swintosky (1964), Lamy (1964), Perrin (1967), and Khalil and Martin (1967).

Levy and Mroszczak (1968) state that the former type appears more suitable for determining the effect of complex formation on drug permeation because of the relatively high aqueous-lipid phase volume ratio inherent in a system where

the lipid phase is located in a membrane. Systems in which the lipid phase is represented by a water-immiscible organic liquid interposed between two aqueous phases have the disadvantage of a low and biologically unrealistic aqueous-lipid phase volume ratio, with the organic phase acting as a very effective trap for the more lipid soluble components of a drug complex and thereby making it difficult to maintain an adequate concentration of that component in one or both of the aqueous phases.

The use of the Sartorius Absorption Simulator was considered but the apparatus was not suitable for use in these studies because it is not designed for use with suspensions which tend to cause sediment formation within the diffusion chamber. For this reason, methods which make use of biological membranes and which were suitable for use with suspensions were investigated. Bates and Gibaldi (1970) classify the methods which employ biological membranes as follows :

(1) Everted small intestinal sac technique

This method was devised by Wilson and Wiseman (1954) and involves isolating a small segment of the intestine of a laboratory animal, everting the segment, and filling the sac with a small volume of drug-free physiological buffer. Both ends of the segment are tied off and the sac is immersed in a flask containing a relatively larger volume of buffer solution that contains the drug. The flask and its contents are then oxygenated and agitated continuously at 37 ^{o}C for a specified period of time. After incubation, the inner or serosal fluid is assayed for drug content. Bates and Gibaldi (1970) state that the integrity of the preparation is usually maintained for periods of up to 1 hour. Eversion provides a

means of prolonging the viability of the epithelial cells which have a very high oxygen requirement. However, Tidball et al. (1967) found that the oxygen utilisation of normally orientated segments of the small intestine of the rat does not differ significantly from that of the everted segments. In addition, Mayersohn and Gibaldi (1969) showed that the passive transfer of salicylate across the everted rat intestine is virtually identical when the mucosal solution is gassed with either oxygen or nitrogen.

The major disadvantage of the sac method is the difficulty in obtaining more than one serosal sample per intestinal segment. This problem was overcome by Crane and Wilson (1958) who tied the intestinal segment to a cannula so that a series of serosal samples could be obtained. The everted gut technique has been used successfully by a number of workers including Aguiar and Fifelski (1966), Feldman et al. (1970), Kaplan and Cotler (1972) and Chowhan and Amaro (1977).

The main problem associated with the use of the everted gut in the present study was the anticipated difficulty in maintaining the uniformity of a suspension during the test. From an examination of the apparatus normally used, it did not appear practical to attempt to add a stirring mechanism which would not interfere with the intestinal segment. In addition, the apparatus is normally constructed from a large test-tube which is partially immersed in a water-bath at 37 ^{o}C. The lack of a suitable stirring unit for such a system necessitated the investigation of other techniques employing biological membranes.

(2) Circulation techniques

Bates and Gibaldi (1970) state that circulation techniques have been used infrequently for drug absorption studies. These methods involve the isolation of the entire small intestine of a laboratory animal or a segment thereof and establishing a closed circulation of oxygenated buffer through the lumen. The outer solution on the serosal side of the intestinal membrane is also circulated and oxygenated. Absorption rates from the lumen to the outer solution are followed by sampling both the fluid circulating through the lumen and the solution on the serosal side of the intestine. Both serosal and mucosal volumes are relatively large and comparable. The duration of the experiment is usually 1 hour.

Bates and Gibaldi state that the method is applicable to kinetic studies of the factors affecting drug absorption. The major advantages claimed for the method are that both surfaces of the intestine are oxygenated and that eversion of the membrane is not necessary. The method is described by Fisher and Parsons (1949), Wiseman (1953) and Darlington and Quastel (1953).

The method of Fisher and Parsons (1949) may be outlined as follows :

A rat is anaesthetised with ether and the abdomen is opened by a midline incision. Both ends of a segment of the small intestine are cannulated and a closed circulation of an oxygen-saturated, CO_2 - bicarbonate buffered fluid is established through the lumen before the circulation of blood to the intestinal wall is interrupted. The segment is suspended in a

bath of oxygenated Ringer solution, and the absorption processes followed by sampling the fluid circulating through the lumen and the fluid bathing the exterior of the intestine. Fisher and Parsons attribute the success of the technique to the fact that there is no time at which the mucosal cells are deprived of an adequate supply of oxygen.

Fisher and Parsons place great emphasis on the oxygen requirements of the isolated intestine. However, the results of Tidball et al. (1967) and Mayersohn and Gibaldi (1969) quoted earlier indicate that the oxygen requirements of the isolated intestine may not be as critical as was first thought by investigators.

Wiseman (1953) used a similar method to that devised by Fisher and Parsons for his study. The apparatus used by Darlington and Quastel (1953) is similar to that used by Wiseman but these investigators did not anaesthetise the animal prior to removal of the intestine. A male guinea-pig is killed by decapitation and allowed to bleed. The abdomen is opened in the midline and all of the intestine is removed except that portion adjacent to the stomach which adheres firmly to the surrounding tissue. The intestine is placed in a small beaker of the solution to be used in the experiment, all the solutions having previously been brought to 38 ^{o}C and gassed with 93 % O_2 + 7 % CO_2. In order to obtain a standardised procedure, the intestine in the beaker is removed and that part of the intestine which is 30 to 40 cm from the stomach in the intact animal is cut out, and the inside washed with the solution to be used. Care is taken to maintain the orientation of the intestine so that perfusion is carried out through the segment in the normal direction of flow.

CHAPTER 2 MATERIALS

2.1 ANTACIDS, ADSORBENTS AND STARCHES

Antacids used in the study were selected on the basis of their frequent administration as medicinals and on their being representative of the common cations and anions used in therapeutics. In addition, the literature shows that they have the ability to adsorb various drugs *in vitro* and that the cations released from these compounds by the action of hydrochloric acid may form complexes with drugs (such as the tetracyclines).

The following antacids were selected :

Dried Aluminium Hydroxide Gel (B.P.)
Calcium Carbonate (B.P.)
Magnesium Carbonate, Heavy and Light (B.P.)
Magnesium Oxide, Light (B.P.)
Magnesium Trisilicate (B.P.)

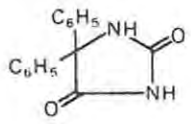
Light Kaolin (B.P.) and Activated Charcoal (Norit OL) were chosen as the adsorbents. Both substances are known to adsorb drugs from solution *in vitro* and are acknowledged by the Pharmaceutical Codex (1979) as possessing adsorbent properties. Activated charcoal is known to adsorb phenytoin and was used in the *in vitro* adsorption studies for comparative purposes.

Of the compounds listed above, the Codex states that all except magnesium trisilicate and activated charcoal are suitable diluents for capsules. In addition, starch is also included by the Codex and was therefore used in this study. The literature has shown that the affinity of starches for drugs may differ according to the amylose content of the starch. Because the source of Starch B.P. can be maize, rice, wheat or potato, it was decided to investigate these starches individually.

The preparation, composition and properties of the compounds selected are well known and will not be reproduced here. For this study use was made of pharmaceutical or laboratory grade materials which were readily available from local sources of supply. Identification and particle size analyses were not performed but all the powders were screened through a 125 μm sieve prior to use. This ensured that all the powders complied with the B.P. requirements for "Very Fine Powder". No further reprocessing or activation procedures were carried out on these agents prior to their use.

2.2 PHENYTOIN

Phenytoin B.P.C. is 5,5 - diphenylhydantoin (or 5,5 - diphenyl-2-4-imidazolidinedione). It has a molecular mass of 252,3 and possesses the following structural formula :



Phenytoin is a white or almost white, odourless or almost odourless, tasteless crystalline powder. It is very slightly soluble in water but is soluble in solutions of alkali hydroxides and in 70 parts of alcohol, 500 parts of chloroform and in 600 parts of ether. It is more soluble in hot alcohol.

According to the Merck Index the melting point is between 295 ^{o}C and 298 ^{o}C. Martindale and the U.S.P. (XIX) give the melting point at about 295 ^{o}C with decomposition.

Atherden (1969) states that Phenytoin Sodium B.P. is the sodium derivative of the tautomeric (lactim) form of phenytoin and has a molecular mass of 274,3. It is a white, odourless, somewhat hygroscopic powder which on exposure to air absorbs carbon dioxide with the liberation of phenytoin. It is soluble in water and in alcohol but is insoluble in chloroform and ether. Solution in water is often incomplete unless the pH is raised to above 11,7.

The phenytoin used for this study was supplied by Parke Davis (Batch No. 76049). Lennon Limited supplied the sample of Phenytoin Sodium (Batch No. 62782), and a B.P. Commission Authentic Specimen of Phenytoin (Batch No. 556) which was used as a primary reference standard.

The primary method of identification adopted for the phenytoin was by means of the infra-red absorption spectra. The spectrum of the Authentic Specimen was compared to that of the samples. Clarke (1969) gives the principle absorption peaks measured in potassium bromide. Potassium bromide discs were produced on a Jasco press according to the manufacturer's instructions and the spectra were recorded between 2000 and 600 cm^{-1} with potassium bromide as reference on a Bèckman IR10 Spectrophotometer.

The phenytoin sodium provided by Lennon Limited was received with a certificate of analysis showing the sample to be 99,3 % pure. Since this sample complied with the identification test, no further analytical procedures were performed on it.

The sample of phenytoin obtained from Parke Davis was subjected to a melting point determination and assay by means of non-aqueous titration to determine its purity. The method for the melting point was that of Class 1a compounds of the U.S.P. (XVIII) using a commercial Gallenkamp melting

point apparatus. Non-aqueous titration was performed according to the method of the U.S.P. (XIX). It was found that the Parke Davis sample complied with the identification test, the melting point criterion and the purity specified in the Pharmacopoeia.

2.3 SOLUTIONS FOR ADSORPTION STUDIES

A number of solutions were considered for use in this study. These included simulated gastric or intestinal juice U.S.P. and various citrate and phosphate buffer formulations. The buffer system selected was similar to that used by Sorby et al. (1966). These investigators used buffers of pH 2,5 and 6,5 containing 0,03 mol/dm^3 of total phosphate. Since initial investigations indicated that the Sartorius Solubility Simulator Model 16751 could be of value in estimating the effects of powders on the absorption of phenytoin (Stricker, personal verbal communication 1978), it was decided to use the solutions recommended in the operating instructions to this apparatus. Table 1 reflects the composition of the solutions used and the total phosphate concentrations, which compare favourably with those used by Sorby.

TABLE 1

COMPOSITION OF ARTIFICIAL GASTRIC AND INTESTINAL JUICE FOR "IN VITRO" ADSORPTION STUDIES

	Artificial Gastric Juice	Artificial Intestinal Juice
pH (± 0,2)	2,8	6,5
Mass Na_3PO_4-$12H_2O$ added (g)	9,50	12,50
0,06 mol/dm^3 HCl	to 1000 cm^3	
Phosphate concentration mol/dm^3	0,025	0,033

2.4 APPARATUS FOR "IN VITRO" DIFFUSION STUDIES

An examination of the Sartorius Absorption Simulator led to the belief that it would be possible to modify the apparatus in such a manner that the diffusion chamber could be replaced with a length of ileum of a small laboratory animal according to the method of Fisher and Parsons (1949). The following features of the Absorption Simulator can be used advantageously for the construction of the apparatus for the circulation technique. Namely,

(a) thermostatically controlled chambers surrounded by heated jackets and equipped with separate thermometers;

(b) magnetic stirrers for each chamber to ensure homogeniety of the solutions in the chambers;

(c) a dual peristaltic pump which enables two solutions to be circulated simultaneously.

2.4.1 Construction of apparatus

In order to perform the circulation studies it was necessary to construct a modified distribution head to replace the standard units supplied with the Absorption Simulator. The modified distribution head required the following materials :

(a) a tapered rubber bung approximately 28 mm thick and having an upper diameter of 48 mm and a lower diameter of 41 mm;

(b) two glass eye droppers 70 mm long and having an external diameter of 7 mm. The lower end of the dropper is shaped to form a nipple and the upper end is grooved;

(c) approximately 140 mm of 0,325 mm diameter stainless

steel wire;

(d) a Luer Lock blunt-ended syringe needle 115 x 2,0 mm.

Two holes 7 mm in diameter and two holes 12 mm in diameter were bored through the bung and positioned in such a manner that holes of the same diameter were radially opposite each other. The glass droppers were fitted into the smaller holes so that the tips of the droppers protruded through the bung to a distance of approximately 30 mm. One end of the stainless steel wire was formed into a ring 8 mm in diameter. The free end was positioned in one of the larger holes in the bung and held in position by a plastic tube 13 mm in diameter and approximately 20 mm long forced into the hole so trapping the wire between the wall of the bung and the plastic tube itself. The wire was positioned in such a manner that the loop protruded approximately 95 mm below the lower surface of the bung into the chamber. The plastic tube retaining the wire support had an internal diameter of 11 mm and served as an outlet for the gas and as an access so that samples could be withdrawn from the chamber and fresh buffer added. The other larger hole was used as a socket for the thermometer supplied with the Absorption Simulator.

A fifth hole of 2 mm in diameter was bored through the bung adjacent to the thermometer socket and the 115 mm needle was inserted so that the Luer fitting rested on the upper surface of the bung.

The detailed layout of the circulation chamber and the distribution head is shown in Figure 1.

A diagramatic representation of the apparatus is shown in Figure 2 and the detailed tube connections to the peristaltic pump of the Absorption Simulator in Figure 3.

FIGURE 1 CIRCULATION CHAMBER AND MODIFIED DISTRIBUTION HEAD FOR THE SARTORIUS ABSORPTION SIMULATOR (x 0,7)

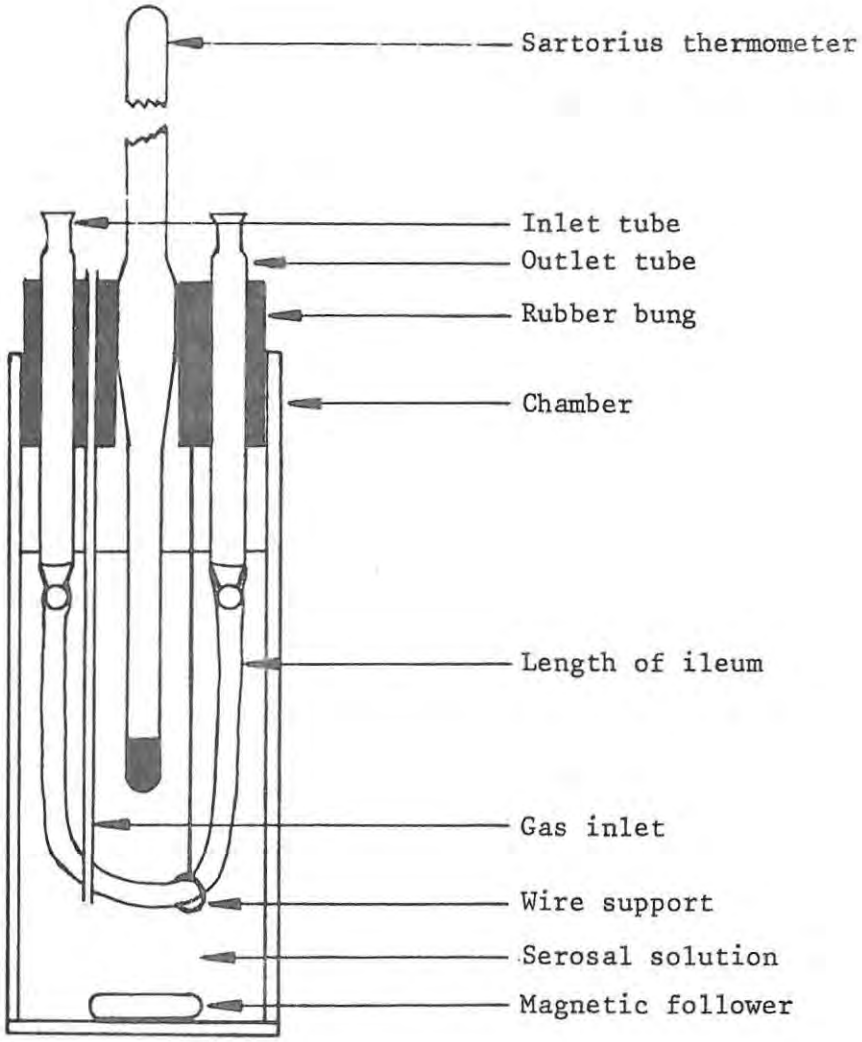


FIGURE 2 DIAGRAM OF MODIFICATIONS TO SARTORIUS ABSORPTION SIMULATOR (x 0,4)

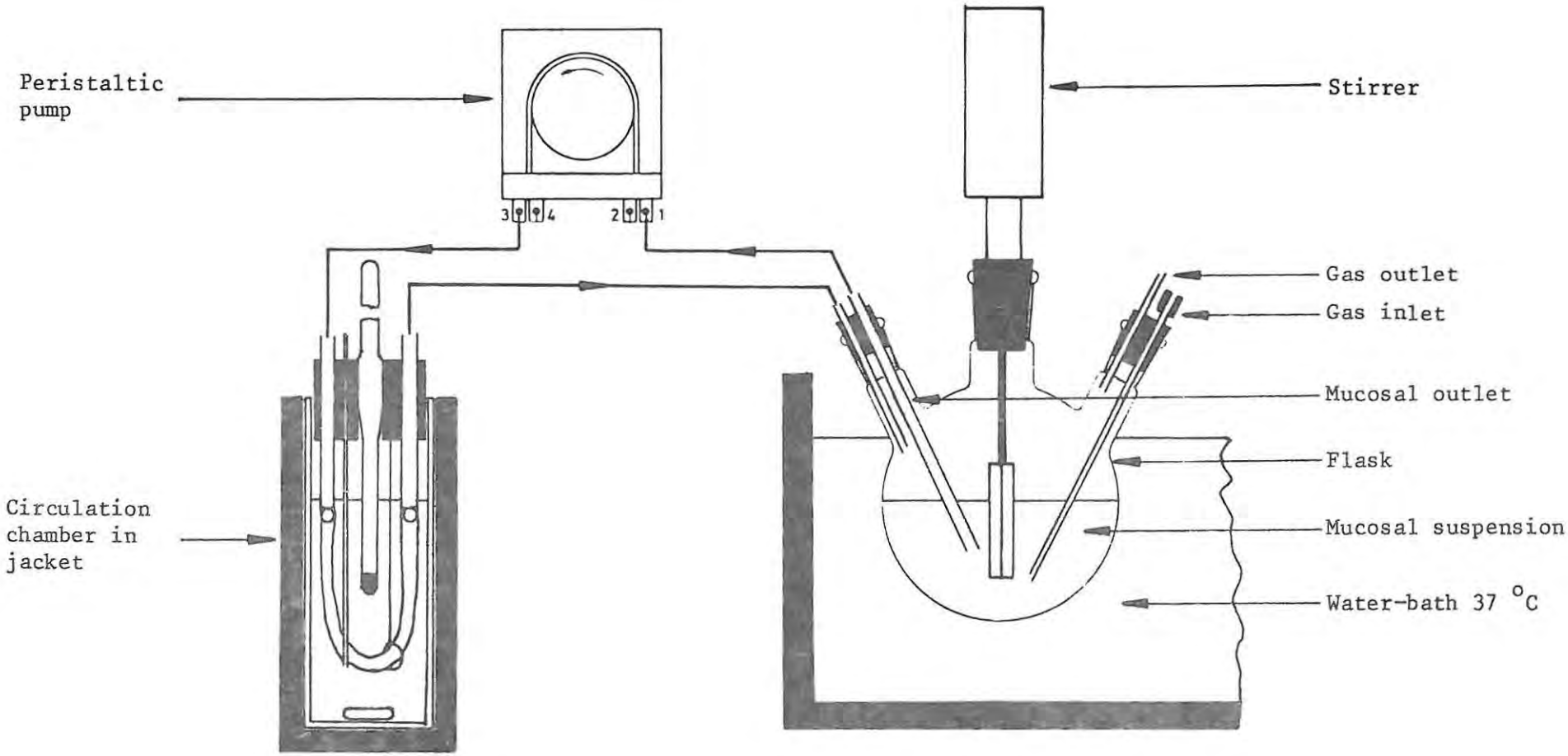
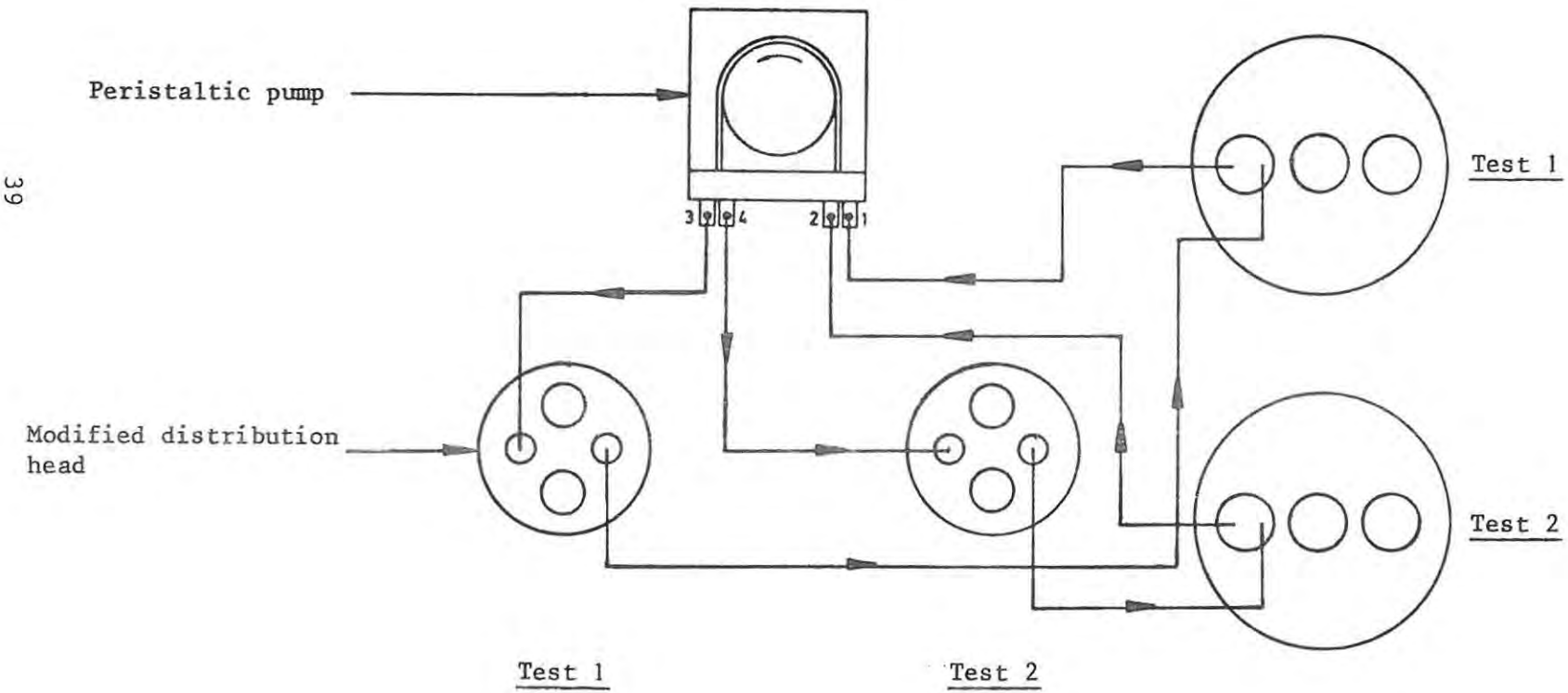


FIGURE 3 TUBE CONNECTIONS FOR MODIFIED SARTORIUS ABSORPTION SIMULATOR



By making use of this configuration it was possible to conduct two tests simultaneously employing adjacent segments of ileum from the same animal.

Figure 3 shows that both chambers of the Absorption Simulator were fitted with the modified distribution heads. The drug suspensions were held in 500 cm^3 three-necked flasks positioned in a water-bath maintained at 37 ^{o}C immediately alongside the Absorption Simulator. The central neck of each flask was fitted with an Electrothermal Mini-Stirrer. One neck of each flask was fitted with a rubber bung into which a 115 x 2,0 mm syringe needle had been fitted to serve as a gas inlet. An additional hole was bored in this bung to serve as a gas outlet. The third neck of the flask was fitted with a bung through which two plastic tubes were passed to serve as the inlet and outlet for the suspension.

2.4.2 Selection of animal

For work of this type, it is common practice to use animals of one strain and preferably of the same sex and approximately the same age and mass throughout. At the time of conducting the experimental work, suitable laboratory rats were not available in sufficient number or at reasonable cost. Darlington and Quastel (1953) used guinea-pigs for their study. These animals were readily obtainable from the local branch of the South African Institute for Medical Research and were used for this study. The gut of the guinea-pig proved to be of a suitable size for the apparatus. Although not of standard mass and of both sexes, the results of the work indicate that they were suitable for use in this type of study.

2.4.3 Physiological solutions

A number of formulae for various physiological solutions

are reported suitable for use with isolated mammalian organs when performing the everted intestinal sac technique or the circulation technique. An examination of the formulae for these solutions reveals that most of them contain glucose. The benzophenone-extraction procedure for determination of phenytoin cannot be performed in the presence of reducing sugars. It was therefore necessary to use a solution which was glucose free. A search of the literature showed that Aguiar and Fifelski (1966) had used a suitable buffer in their study of the effect of pH on the in vitro absorption of flufenamic acid. The advantages of this solution are that it contains no glucose and that the pH can be adjusted over a wide range. Aguiar and Fifelski used a mixture of 95 % oxygen and 5 % carbon dioxide to gas the solution.

The composition of the solution used in this work and prepared in accordance with the formula of Aguiar and Fifelski is shown in Table 2. This formula yields a solution of pH 7,2. Solutions of a lower pH were prepared by the addition of 20 mmoles of aspartic acid to the original solution, the pH was then adjusted to the desired value by the addition of sodium hydroxide or hydrochloric acid solution.

For this work the physiological solution was freshly prepared daily from sterile concentrated stock solutions which were suitably diluted in a volumetric flask. The volume of the calcium chloride solution used was determined by the Mohr titration according to Vogel (1966). In addition, all stock solutions were inspected regularly to ensure that they were free from microbial growth.

TABLE 2 COMPOSITION OF PHYSIOLOGICAL SOLUTION ACCORDING TO AGUIAR AND FIFELSKI (1966).

Components	Molecular Formula	Relative Molecular Mass	Conc. Required mmoles/dm^3	g/dm^3
Sodium Chloride	NaCl	58,44	145,0	8,473
Potassium Chloride	KCl	74,56	4,56	0,340
Calcium Chloride	$CaCl_2,2H_2O$	147,00	1.25	0,184
Sodium Phosphate (Dibasic)	$Na_2HPO_4,12H_2O$	358,14	1,33	0,476
Sodium Phosphate (Monobasic)	$NaH_2PO_4,2H_2O$	156,01	0,33	0,051
Distilled Water				to 1 dm^3
Aspartic Acid	$NH_2.CH(COOH).CH_2.COOH$	133,11	20,0	2.662

CHAPTER 3 METHODS

3.1 ANALYTICAL PROCEDURE FOR PHENYTOIN

The analytical method was based on the benzophenone-extraction procedure developed by Saitoh et al. (1973). The following parameters listed by Saitoh were used as a basis for the methods which were developed :

(a) There is a linear relationship between the absorbance readings and phenytoin concentrations up to 30 $\mu g/cm^3$;

(b) The molar absorptivity of benzophenone is $1,895 \times 10^4$;

(c) If quantitatively converted, 5 µg of phenytoin corresponds to 5 cm^3 of $3,97 \times 10^{-6}$ mol/dm^3 benzophenone.

In order that a workable assay method be established, it was necessary to assess the shape of the absorbance curve of benzophenone in n-heptane, the wavelength at which the maximum absorbance occurred and whether benzophenone was the only product resulting from the oxidation process.

1. Determination of the shape of the absorbance curve of benzophenone in n-heptane

By means of the molar absorptivity value for benzophenone it was calculated that an optimum absorbance of 0,4 would be given by a solution containing 3,846 $\mu g/cm^3$ of benzophenone in n-heptane. To establish the shape of the absorbance curve, a 5 $\mu g/cm^3$ dilution of benzophenone in

n-heptane was prepared and the absorbance spectrum was recorded for a 1 cm path length against n-heptane between 220 and 300 nm on a Beckman DB Spectrophotometer. The absorption spectrum shown in Figure 4 has a well defined maximum at 247 nm. The absorbance spectrum for 1 cm of pure n-heptane against water is also shown. The percentage transmission of the pure n-heptane recorded at 245 nm was 97 % (Manufacturer's specification 98 % at 245 nm).

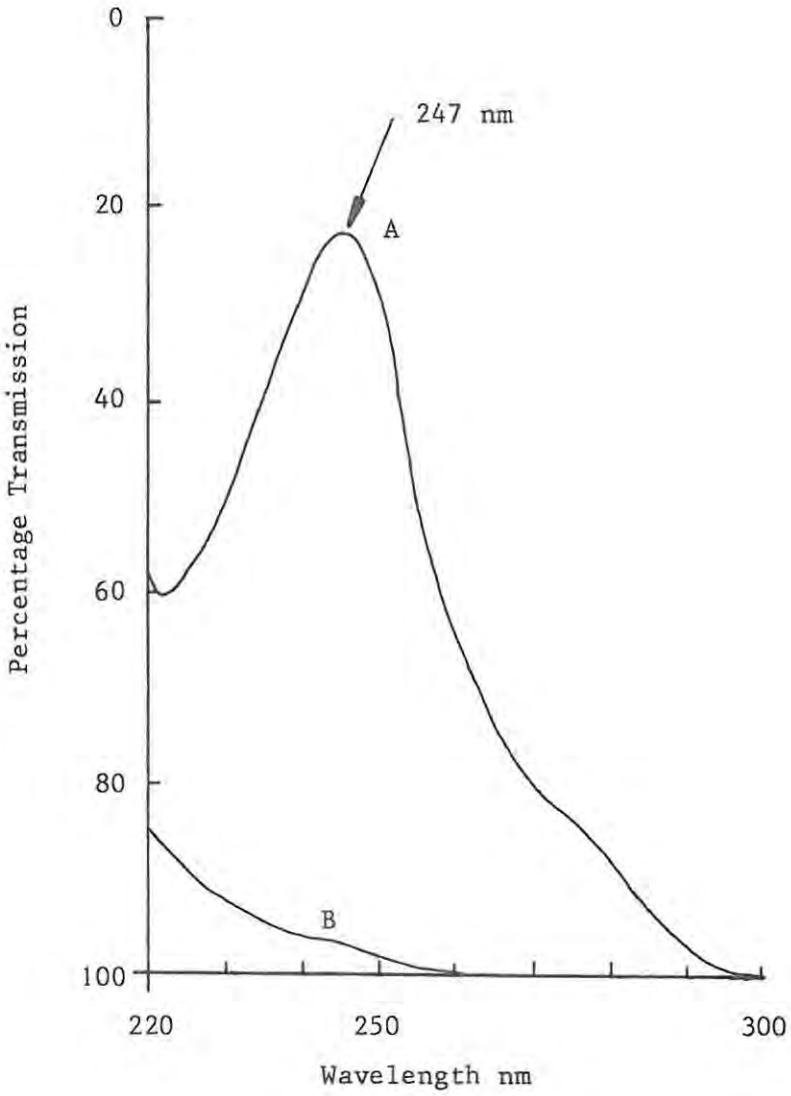
2. Preparation of calibration line for the determination of benzophenone in n-heptane

Solutions containing between 1 and 6 $\mu g/cm^3$ of benzophenone in n-heptane were prepared by dilution. The absorbance of each solution was measured at 247 nm against n-heptane as reference and the procedure was repeated in triplicate. Figure 5 shows the calibration curve obtained from the procedure. From these results it was concluded that there is a linear relationship between the absorbance readings and the benzophenone concentrations up to 6 $\mu g/cm^3$.

3. Verification of absorbance spectrum of phenytoin oxidation product

A solution containing 40 $\mu g/cm^3$ of phenytoin in 0,1 mol/dm^3 NaOH was prepared. A 1 cm^3 sample of this solution was oxidised by heating with 10 cm^3 of 1 % $KMnO_4$ in 7 mol/dm^3 NaOH for 5 min at 80 °C. The solution was cooled to room temperature and the oxidation product was extracted in 5 cm^3 of n-heptane. Figure 6 shows a well defined spectrum for the oxidation product with a maximum at 247 nm and a shape which is identical to that recorded for benzophenone in n-heptane. The blank showed 99 % transmission at 247 nm. It was

FIGURE 4 ABSORPTION SPECTRUM FOR BENZOPHENONE IN n-HEPTANE



A - Absorption spectrum for benzophenone in n-heptane.

B - Absorption spectrum for n-heptane against reference water.

FIGURE 5 CALIBRATION CURVE FOR THE DETERMINATION OF BENZOPHENONE IN n-HEPTANE

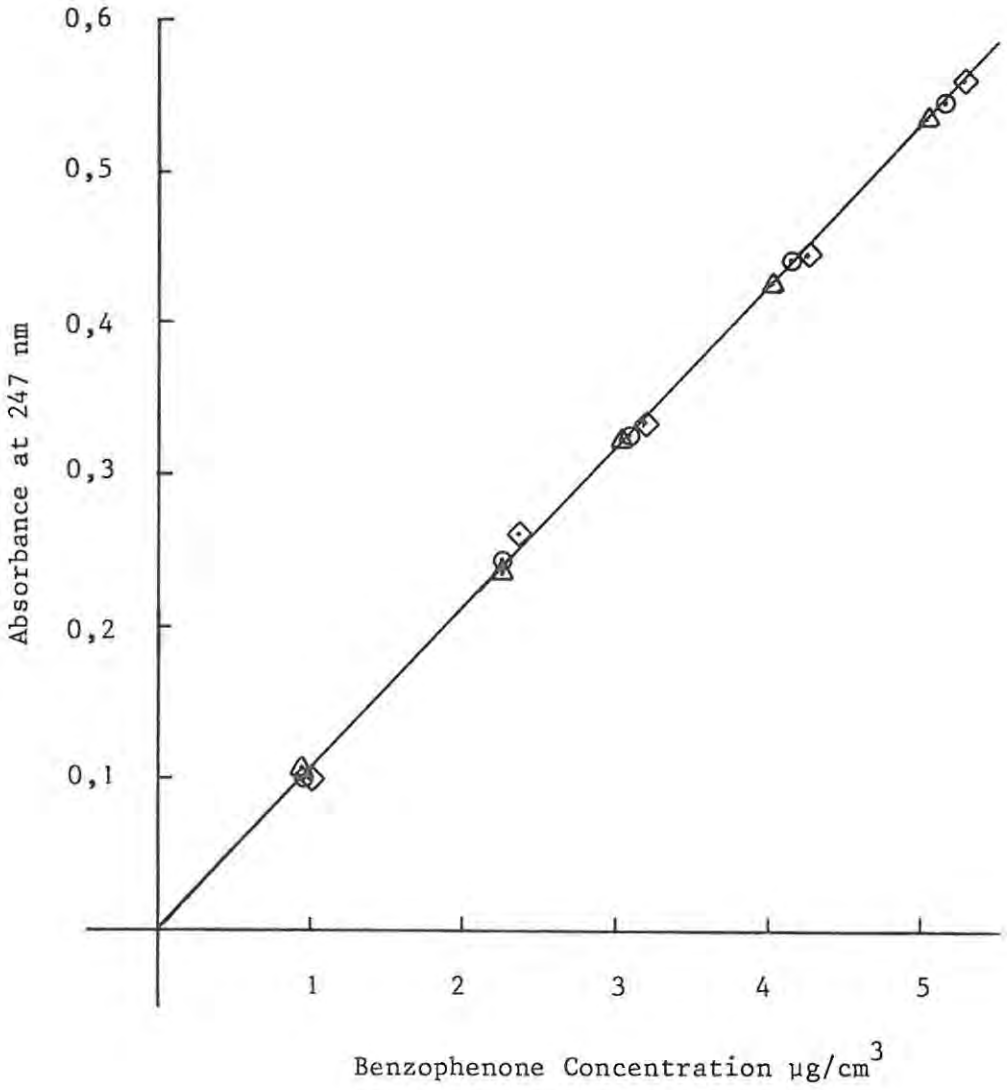
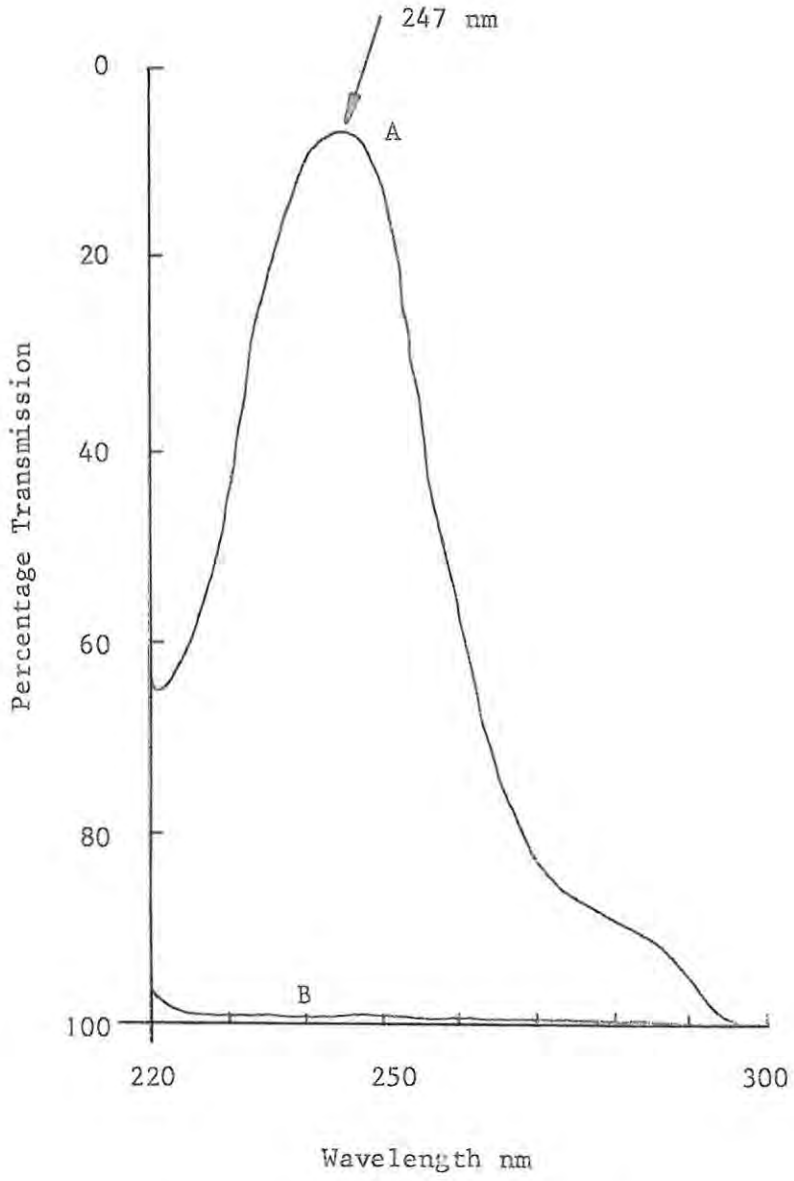


FIGURE 6 ABSORPTION SPECTRUM FOR PHENYTOIN OXIDATION PRODUCT IN n-HEPTANE



A – Absorption spectrum for phenytoin oxidation product in n-heptane.

B – Absorption spectrum for blank.

3.1.1 Benzophenone-extraction procedure : Method 1

This assay method was developed for the measurement of phenytoin concentrations up to 40 µg/cm^3. Concentrations exceeding this value could be determined by accurately diluting the phenytoin solution prior to the assay procedure being carried out. This method was suitable for use in the *in vitro* adsorption studies and in situations where higher phenytoin concentrations were encountered.

(a) The 1 cm^3 sample to be assayed was added to 10 cm^3 of 1 % $KMnO_4$ in 7 mol/dm^3 NaOH in a 50 cm^3 pear-shaped flask fitted with a ground-glass stopper;

(b) The flask was inverted ten times to ensure mixing and then heated at 80 °C for 5 min in a water-bath;

(c) The flask was cooled to room temperature in running water and 5 cm^3 of n-heptane was added and the flask shaken on a mechanical shaker for 10 min;

(d) The n-heptane was floated free of the oxidising solution with distilled water and raised to the neck of the flask;

(e) The n-heptane was removed with a Pasteur pipette and transferred to a spectrophotometer cell. The absorbance was measured at 247 nm against pure n-heptane;

(f) The blank value was determined by repeating the procedure omitting the phenytoin.

A calibration curve was prepared to assess the effectiveness of the procedure and for use in the determination of the phenytoin concentrations of unknown samples.

Approximately 50 mg of phenytoin was weighed out accurately and dissolved in and made up to 50 cm^3 with 0,1 mol/dm^3 NaOH.

This solution was used to prepare phenytoin dilutions of the following strengths in 0,1 mol/dm^3 NaOH :

10 µg/cm^3 (1 to 100)	20 µg/cm^3 (1 to 50)
30 µg/cm^3 (3 to 100)	40 µg/cm^3 (2 to 50)

1 cm^3 aliquots of the dilutions were treated as previously outlined. Sixfold repeats were performed for each dilution and the procedure was repeated in triplicate. A blank determination was carried out in sixfold with the phenytoin omitted from the procedure.

Table 3 shows the results from the oxidation procedure. The mean blank absorbance obtained was - 0,001 (S.D. 1,7 x 10^{-3}). This value was so low that its influence on the calibration curve appeared insignificant and, therefore, no correction was made to the results obtained. The results shown in columns 1 to 5 reflect the mean values obtained from the six repeats in each case. Results were calculated individually and then averaged, the following method being used to obtain the figures reflected in the Table :

Column 1: Benzophenone concentrations were obtained by means of a polynomial regression programme and the use of the co-ordinates for the benzophenone calibration curve.

Column 2: The equivalent phenytoin concentration was calculated from the following formula :

$$\frac{\text{Molecular Mass Phenytoin}}{\text{Molecular Mass Benzophenone}} \times \text{Benzophenone Concentration} \times 5$$

i.e. $$\frac{252,26}{182,21} \times \text{Benzophenone Concentration} \times 5$$

= 6,922 x Benzophenone Concentration µg/cm^3

The factor of 5 is introduced because the benzophenone derived from the phenytoin solution was extracted into 5 cm^3 of n-heptane.

This solution was used to prepare phenytoin dilutions of the following strengths in 0,1 mol/dm^3 NaOH :

10 µg/cm^3 (1 to 100)	20 µg/cm^3 (1 to 50)
30 µg/cm^3 (3 to 100)	40 µg/cm^3 (2 to 50)

1 cm^3 aliquots of the dilutions were treated as previously outlined. Sixfold repeats were performed for each dilution and the procedure was repeated in triplicate. A blank determination was carried out in sixfold with the phenytoin omitted from the procedure.

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$$\text{i.e.} \quad \frac{252,26}{182,21} \times \text{Benzophenone Concentration} \times 5$$

$$= \quad 6,922 \times \text{Benzophenone Concentration} \quad \mu g/cm^3$$

The factor of 5 is introduced because the benzophenone derived from the phenytoin solution was extracted into 5 cm^3 of n-heptane.

TABLE 3 ANALYSIS OF RESULTS FOR BENZOPHENONE-EXTRACTION PROCEDURE :

METHOD 1

				Calculation of Results				
				Benzophenone Calibration Curve			Molar Absorptivity	
Test Number	Mass Phenytoin mg	Phenytoin Conc $\mu g/cm^3$	Mean Absorbance at 247 nm	Benzophenone Conc $\mu g/cm^3$	Phenytoin Conc $\mu g/cm^3$	% Phenytoin Recovered	Phenytoin Conc $\mu g/cm^3$	% Phenytoin Recovered
1	50,1	10,02	0,140 (0,003)	1,287	8,907	88,9 (1,9)	9,285	92,7 (1,9)
		20,04	0,278 (0,004)	2,610	18,063	90,1 (1,3)	18,525	92,4 (1,3)
		30,06	0,417 (0,006)	3,933	27,230	90,6 (1,3)	27,777	92,4 (1,3)
		40,08	0,558 (0,003)	5,272	36,493	91,1 (0,5)	37,128	92,7 (0,5)
2	51,4	10,28	0,143 (0,001)	1,322	9,147	89,0 (0,8)	9,525	92,7 (0,8)
		20,56	0,290 (0,005)	2,724	18,855	91,7 (1,5)	19,325	94,0 (1,5)
		30,84	0,432 (0,005)	4,073	28,195	91,4 (1,1)	28,753	93,2 (1,1)
		41,12	0,576 (0,003)	5,447	37,707	91,7 (0,5)	38,347	93,3 (0,5)
3	51,3	10,26	0,139 (0,002)	1,282	8,875	86,5 (1,4)	9,247	90,2 (1,5)
		20,52	0,285 (0,005)	2,670	18,478	90,1 (1,7)	18,948	92,3 (1,7)
		30,78	0,421 (0,002)	3,964	27,437	89,1 (0,5)	27,988	90,9 (0,5)
		41,04	0,570 (0,007)	5,387	37,285	90,9 (1,2)	37,930	92,4 (1,2)
				1	2	3	4	5

Column 3: Percentage phenytoin recovered

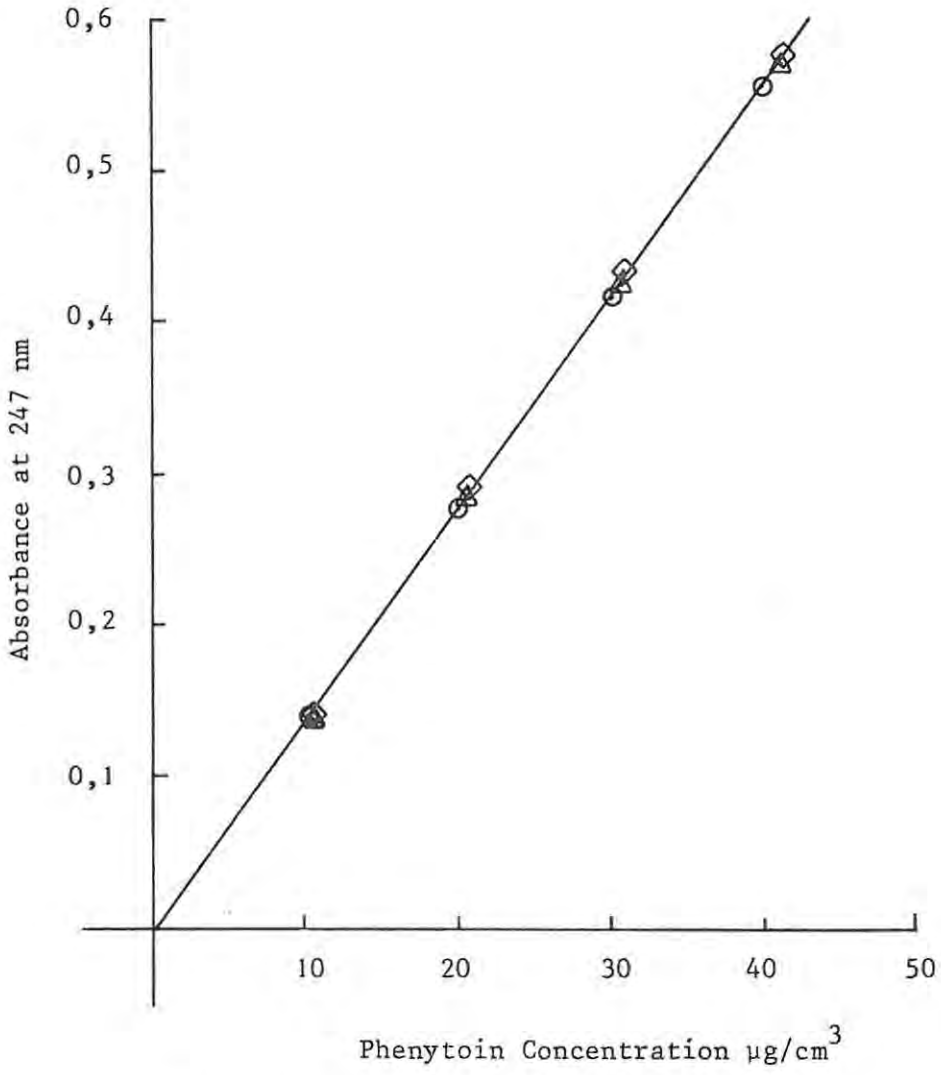
$$= \frac{\text{Calculated Concentration}}{\text{Actual Concentration}} \times 100$$

Columns 4 and 5: By use of the molar absorptivity for benzophenone it was calculated that the absorptivity value for benzophenone is 104,00 and that the equivalent benzophenone concentration may be determined from the measured absorbance. The equivalent phenytoin concentration may be calculated by applying the conversion factor of 6,922 as above and the percentage recovery of phenytoin is calculated as per Column 3.

The calibration curve prepared from the triplicate results is shown in Figure 7. Analysis of the co-ordinates of these points revealed a correlation coefficient of 0,999. The gradient obtained was 0,014 and the y intercept -0,0012. The plot obtained was a straight line and was so close to the origin that it was concluded that the product of the oxidation procedure obeyed Beer's Law for phenytoin concentrations up to 40 $\mu g/cm^3$. The percentage recovery differed slightly, depending on the method of assessment. By use of the benzophenone calibration plot, a mean percentage recovery for the 72 determinations of 90,09 (S.D. 1,89) was achieved and by use of the molar absorptivity value for benzophenone, the percentage recovery was 92,44 (S.D. 1,57).

A comparison of the above results with those of Saitoh et al. (1973) and Wallace and Hamilton (1974) proved useful in assessing the method. It must be borne in mind that both groups extracted the phenytoin from biological fluids prior to assay, whereas no prior extraction was necessary for the method devised for this study. Saitoh achieved a percentage recovery of between 99,6 to 100,9 % in the range

FIGURE 7 CALIBRATION CURVE FOR THE DETERMINATION OF PHENYTOIN BY BENZOPHENONE-EXTRACTION PROCEDURE : METHOD 1



5 to 30 µg/cm^3 of phenytoin, whereas Wallace and Hamilton's semi-micro procedure yielded a percentage phenytoin recovery of between 103,5 and 91,4 with a mean of 94,1 % over the range 5 to 80 µg/cm^3. Wallace and Hamilton achieved a sensitivity of 70 % when assessing the work of Saitoh et al. It was concluded that, although the phenytoin yield obtained was less than that obtained by Saitoh et al., it compared favourably with the recovery by the method of Wallace and Hamilton. It also appears that the Saitoh method is more accurate and sensitive than the assessment of Wallace and Hamilton indicates.

It was further concluded that the use of the calibration curve for the oxidation method (Figure 7) would give results of adequate accuracy and sensitivity when applied to the in vitro studies contemplated.

3.1.2 Benzophenone-extraction procedure : Method 2

Because of the low rate of diffusion of phenytoin through biological membranes it was necessary to develop the assay procedure further in order that lower concentrations of phenytoin could be measured more accurately during the diffusion studies. It appeared possible to measure the concentrations of these dilute solutions by increasing the sample size of the phenytoin solution from 1 cm^3 to 5 cm^3 and by reducing the volume of n-heptane from 5 cm^3 to 2 cm^3. Because of the small volume of n-heptane it was necessary to obtain and use semi-micro quartz spectrophotometer cells having a capacity of approximately 1,5 cm^3 and a path length of 1 cm.

The assay method originally developed made use of 1 cm^3 phenytoin solutions which were oxidised by use of 10 cm^3 volumes of 1 % $KMnO_4$ in 7 mol/dm^3 NaOH. The use of a 5 cm^3 phenytoin solution resulted in the dilution of the

oxidising solution to 15 cm^3. In order to preserve the oxidising capacity of the solution it was necessary to increase the concentration of the $KMnO_4$ to 1,5 % and the strength of the alkali to 10 mol/dm^3. This dilution of the concentrated oxidising solution resulted in concentrations of $KMnO_4$ and NaOH which fell within the limits set by Wallace and Hamilton (1974).

A further factor to be borne in mind was that the diffusion studies were carried out in physiological solution, the formula of which is given in Table 2. It was therefore necessary to assess the effectiveness of the assay procedure in the presence of the constituents of this solution.

Because of the altered conditions for the oxidation procedure a second calibration curve for the determination of phenytoin was prepared as follows :

1. Approximately 25 mg of phenytoin was accurately weighed and dissolved in and made up to 25 cm^3 with 0,1 mol/dm^3 NaOH;
2. 1 cm^3 of this solution was diluted to 100 cm^3 with physiological solution of pH 7,2 and the pH of the solution was readjusted by the dropwise addition of HCl;
3. 1, 2, 3, 4 and 5 cm^3 volumes of solution (2) were diluted to 25 cm^3 with physiological buffer to provide solutions containing 2, 4, 6, 8 and 10 µg/ 5 cm^3 of phenytoin respectively;
4. 5 cm^3 aliquots of the diluted solutions were assayed for phenytoin content by use of 10 cm^3 of 1,5 % $KMnO_4$ in 10 mol/dm^3 NaOH. The oxidation procedure was performed as previously described and the benzophenone extracted in 2 cm^3 n-heptane;

5. The absorbance of the benzophenone solutions was read at 247 nm against pure n-heptane;

6. Blank values were determined by repeating the procedure with the phenytoin omitted;

7. Each dilution was assayed in triplicate and the procedure was repeated in triplicate.

Table 4 shows the analysis of results of the second method. The mean blank absorbance obtained with the physiological buffers was 0,006 (S.D. 4,5 x 10^{-3}). The mean blank was used to correct the measured absorbances. The results shown in Columns 1 to 5 reflect the mean values obtained from the triplicate repeats, the results being calculated individually and then averaged. The figures reflected in Table 4 were obtained as follows :

Column 1: Benzophenone concentrations were obtained by means of a polynomial regression programme and the use of the co-ordinates for the benzophenone calibration curve (Figure 5);

Column 2: The equivalent phenytoin concentration was calculated by making use of the relative molecular masses as shown under Section 3.1.1. A factor of 2 was, however, used because the benzophenone derived from the phenytoin solution was extracted into 2 cm^3 of n-heptane;

Column 3: The method used to calculate the percentage phenytoin recovered has been shown under Section 3.1.1;

Columns 4 and 5: The concentration of phenytoin was calculated from the measured absorbance at 247 nm and the molar absorptivity of benzophenone. The concentration was converted to µg/5 cm^3. The percentage recovery was calculated as previously outlined.

An examination of Table 4 revealed that there was a marked

TABLE 4 ANALYSIS OF RESULTS FOR BENZOPHENONE-EXTRACTION PROCEDURE :

METHOD 2

				Calculation of Results				
				Benzophenone Calibration Curve			Molar Absorptivity	
Test Number	Mass Phenytoin mg	Phenytoin Conc $\mu g/5\ cm^3$	Mean Absorbance at 247 nm	Benzophenone Conc $\mu g/cm^3$	Phenytoin Conc $\mu g/5\ cm^3$	% Phenytoin Recovered	Phenytoin Conc $\mu g/5\ cm^3$	% Phenytoin Recovered
1	25,4	2,032	0,059	0,520	1,440	70,86	1,571	77,30
		4,064	0,116	1,063	2,943	72,42	3,088	75,99
		6,096	0,184	1,711	4,736	77,69	4,899	80,36
		8,128	0,231	2,158	5,977	73,53	6,150	75,66
		10,160	0,254	2,378	6,583	64,79	6,762	66,56
2	26,1	2,088	0,074	0,663	1,835	87,88	1,970	94,35
		4,176	0,136	1,253	3,469	83,08	3,621	86,70
		6,264	0,198	1,844	5,106	81,52	5,271	84,15
		8,352	0,243	2,273	6,293	75,35	6,469	77,46
		10,440	0,276	2,587	7,164	68,62	7,348	70,38
3	26,8	2,144	0,067	0,596	1,651	76,98	1,783	83,20
		4,288	0,133	1,225	3,391	79,09	3,541	82,58
		6,432	0,194	1,806	5,001	77,75	5,165	80,30
		8,576	0,245	2,292	6,346	74,00	6,523	76,06
		10,720	0,271	2,540	7,032	65,60	7,215	67,30
				1	2	3	4	5

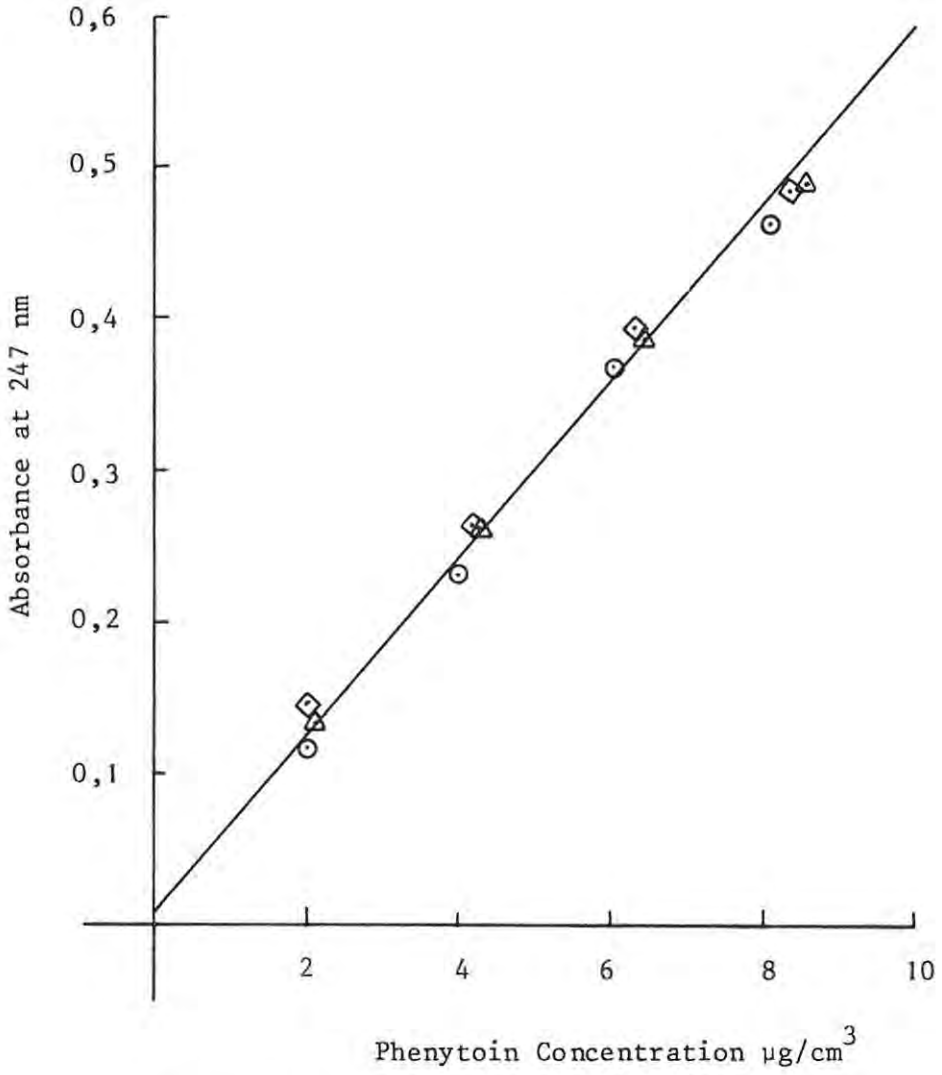
reduction in the efficiency of the assay method when concentrations of 10 µg/5 cm^3 were tested. This reduction in sensitivity was attributed to less efficient extraction of the benzophenone in the reduced volume of n-heptane. It has previously been shown that Beer's Law is obeyed for benzophenone in n-heptane up to 6 µg/cm^3 and for phenytoin concentrations up to 40 µg/cm^3 for Method 1. Preliminary investigations with the diffusion studies showed that concentrations exceeding 8 µg/5 cm^3 were unlikely to be encountered and therefore the absorbance readings for the phenytoin solutions of 10 µg/5 cm^3 were not taken into account in constructing the calibration curve. The calibration curve was prepared from 32 readings and is shown in Figure 8. Analysis of the co-ordinates of these points revealed a correlation coefficient of 0,994 with a gradient of 0,028 and the y intercept 0,011.

The mean percentage recovery of phenytoin was 77,5 (S.D. 4,9) when calculated from the benzophenone calibration curve and 81,2 (S.D. 5,5) when calculated from the molar absorptivity value for benzophenone with phenytoin concentrations up to and including the values for 8 µg/5 cm^3. Although the sensitivity of Method 2 is less than that of Method 1, it was nevertheless an improvement on the 70 % sensitivity achieved by Wallace and Hamilton (1974) when reviewing the method of Saitoh et al. (1973). It was concluded that Method 2 would be satisfactory for solutions of phenytoin in physiological solution not exceeding 8 µg/5 cm^3 in concentration.

3.1.3 Calculation of results

The analyses of the points of the calibration curve of the benzophenone in n-heptane and of the benzophenone-extraction procedures Methods 1 and 2 were performed by use of a polynomial regression programme on a Hewlett-

FIGURE 8 CALIBRATION CURVE FOR THE DETERMINATION OF PHENYTOIN BY BENZOPHENONE-EXTRACTION PROCEDURE : METHOD 2



Packard 9830 A calculator. A triangular factorisation of the X'X metric is used to calculate the coefficients and the statistics according to Graybill (1961). With the use of this programme it was possible to obtain the correlation coefficient for the points, the best line to fit the points, the y intercept and the gradient. By means of the co-ordinates of the points of the calibration curves and the use of the polynomial regression programme it was possible to obtain the concentration of phenytoin in unknown solutions directly from the absorbance values. Visual reading of data from the calibration curves was, therefore, unnecessary.

3.2 "IN VITRO" ADSORPTION STUDIES

Most workers in this field have based their in vitro adsorption studies on the work of Batuyios and Brecht (1957). The following factors were considered in the selection of a standardised technique :

(1) Solute concentration: The concentration of phenytoin selected was determined by the solubility of the drug in the gastro-intestinal fluids and was based upon the values given by Schwartz et al. (1977). Concentrations of 25 $\mu g/cm^3$ were used for most studies as this was within the solubility of phenytoin at the pH's of the tests;

(2) Ratio of insoluble powder to drug solution: The literature shows that the ratio chosen by different workers varies. For example, Blaug and Gross (1965) used 1 g of antacid per 50 cm^3 of drug solution. El-Masry and Khalil (1974) used 100 cm^3 of aqueous alkaloidal solution to 1 g of adsorbent. For the present study 100 mg of powder per 10 cm^3 of drug solution was used. This was the same ratio as that

used by El-Masry and Khalil (1 to 100) and in addition made it possible for the study to be conveniently carried out in 10 cm^3 test tubes fitted with ground-glass stoppers.

(3) Nature of solvent and pH: The effect of pH is of significance in the design of *in vitro* adsorption studies for phenytoin because of the low solubility of the drug in simulated gastro-intestinal fluids. The problem is further compounded by the fact that the antacid powders react with the hydrochloric acid used in simulated gastric juice. Because of the solution of the antacids in artificial gastric juice, Blaug and Gross (1965) performed their studies using water as solvent. Naggar *et al.* (1976), Khalil (1977), El-Masry and Khalil (1974) and Khalil and Moustafa (1973) adopted a similar approach to this problem. However, Khalil (1974) demonstrated the adsorbing power of hydrated silica gel formed by the interaction of magnesium trisilicate and 0,2 mol/dm^3 HCl. The problem may thus not simply be circumvented by the use of water as solvent since the by-products of the acid neutralisation may themselves have adsorbing properties.

The presence of ions such as phosphate or citrate in the solution was shown to suppress the adsorption of atropine and hyoscine onto magnesium trisilicate by El-Masry and Khalil (1974). It was therefore necessary to design the tests in such a manner that any reduction in adsorption by the phosphate component of the simulated gastro-intestinal solutions could be detected.

(4) Temperature and duration of incubation: All adsorption studies were carried out at 37 $^{o}C \pm 0,5\ ^{o}C$. The time of incubation has been varied greatly by

investigators. Batuyios and Brecht (1957) state that 1 h of mixing was adequate but the experimental time used was 3 h. Blaug and Gross (1965) allowed equilibration to proceed for 24 h. Decker et al. (1968) used 20 min in an attempt to simulate the length of time taken between the administration of a dose of activated charcoal and gastric lavage. El-Masry and Khalil (1974) show that the adsorption of atropine and hyoscine onto magnesium trisilicate takes place within 10 min and adopted 30 min for their experiments. Preliminary studies of 30 min, 1 h, 2 h and 3 h suggested that if adsorption did occur, 30 min of incubation was adequate and this duration was adopted for most studies.

3.2.1 General method

1. 100 mg quantities of the powders were placed in the 10 cm^3 test tubes;
2. The phenytoin solution was prepared by dilution and 10 cm^3 aliquots were pipetted into the test tubes;
3. The test tubes were stoppered and shaken in a horizontal position on a mechanical shaker for 10 min and then transferred to a water-bath at 37 ^{o}C ($\pm$ 0,5 ^{o}C) and allowed to incubate for the specified time;
4. Because of the lack of a shaker water-bath it was necessary to remove the test tubes at 5 min intervals and to shake them briefly but vigorously to maintain the suspension;
5. After incubation, the test tubes were centrifuged at 3000 r p m for 30 min;
6. The supernatant was removed by use of a glass/metal syringe and filtered through a syringe filter-holder fitted with a prefilter and a 0,8 µm membrane

filter into a clean dry test tube;

7. 1 cm^3 aliquots of the filtrate were assayed in duplicate by means of the benzophenone-extraction procedure Method 1;

8. The remaining filtrate and suspension was remixed and the pH was measured with a Metrohm E520 pH meter;

9. A blank was performed by repeating the procedure with the solvent and the powders but omitting the phenytoin;

10. A control was performed by use of the phenytoin solution which was treated in the same manner as the tests but lacked the powder.

3.2.2 Methods for specific tests

1. Studies on the adsorption of phenytoin onto the particulate matter from simulated gastric and intestinal solutions

The phenytoin solution was prepared by dissolving approximately 25 mg of the accurately weighed drug in and making up to 10 cm^3 with 0,1 mol/dm^3 NaOH. 1 cm^3 aliqots of this solution were diluted to 100 cm^3 with either simulated gastric or intestinal solution and 10 cm^3 samples of these solutions were used for the studies with 100 mg of the appropriate powder.

The test method adopted was performed in accordance with the general method as outlined under Section 3.2.1.

2. Studies on the adsorption of phenytoin onto particulate matter from aqueous solution

Phenytoin concentrations of 25, 40 and 60 $\mu g/cm^3$ were used. The solutions were prepared by dissolving the appropriate mass of phenytoin in 50 cm^3 of 0,1 mol/dm^3

NaoH. A 5 cm^3 sample of the phenytoin solution was then diluted to 500 cm^3 with distilled water. Ten cm^3 aliquots of the diluted phenytoin solution were used for the tests, which were conducted in accordance with the method previously outlined. For the assay of those solutions containing 40 and 60 $\mu g/cm^3$ of phenytoin, 3 cm^3 of the filtrate from the tests was diluted with an equal volume of 0,1 mol/dm^3 NaOH. This ensured that the concentration of phenytoin assayed fell well within the limits of accuracy of the benzophenone-extraction procedure Method 1.

During this set of tests, it was decided to measure the possible loss of phenytoin onto the filter system. Samples of the controls which comprised the phenytoin solution without the powder were assayed before and after filtration and the results compared. It was found that no loss of phenytoin took place onto the filter system.

3.2.3 Calculation of results

The absorbance at 247 nm of the tests and the controls was corrected by taking into consideration the blank readings :

i.e. Corrected absorbance = Measured absorbance - blank.

By use of the benzophenone-extraction procedure Method 1, the phenytoin concentrations were obtained from the calibration curve by means of the polynomial regression programme.

In each instance, the concentration of phenytoin remaining in the supernatant was compared to the phenytoin concentration of the controls. The percentage phenytoin remaining in solution was calculated as follows :

% phenytoin remaining in supernatant =

$$\frac{\text{Phenytoin conc. in supernatant } \mu g/cm^3}{\text{Phenytoin conc. of control } \mu g/cm^3} \times 100$$

The results were then tabulated and compared.

3.3 "IN VITRO" DIFFUSION STUDIES

3.3.1 General method

1. Preparation of physiological solution and drug suspension

   1. The physiological solution was prepared in accordance with Table 2 and was adjusted to pH 7,2;
   2. 125 cm^3 of physiological solution of pH 7,2 was pipetted into compartments of the simulator to serve as the serosal solutions;
   3. 250 cm^3 of physiological solution was pipetted into the 500 cm^3 flasks which served as reservoirs for the mucosal solution. 30 mg of phenytoin sodium was then added to each flask followed by the substance under test. The pH of the mucosal flasks was then adjusted to the desired value;
   4. The temperature of both the serosal and mucosal compartments was maintained at 37 $^{o}C \pm 0,5\ ^{o}C$;
   5. Sufficient oxygenated physiological solution was maintained at 37 ^{o}C for the preparation of the intestinal segments.

2. Preparation of the intestinal segments

   1. An adult guinea-pig was isolated for 24 h prior to use. Food was withheld but the animal was

allowed water ad libitum;

2. The animal was stunned by a sharp blow to the base of the head and sacrificed by decapitation;

3. The intestine was removed via a midline incision in the abdomen and was transferred to a beaker which contained oxygenated physiological solution at 37 ^{o}C;

4. Both the inside and the outside of the intestine were gently rinsed with the solution using a syringe to remove any intestinal contents and blood;

5. The ileum was identified and two adjacent segments 17 cm $\pm$ 0,5 cm long were measured and cut to length. A pair of blunt forceps were used to open the cut end of the ileum sufficiently to allow the inlet tube of the distribution head to be inserted into the lumen. The ileum was securely fixed over the formed end of the glass tube with waxed dental floss. The free end of the ileum was then threaded through the wire loop and attached to the outlet tube in a similar manner. Care was taken to ensure that the proximal and distal ends of the ileum were in accordance with the direction of flow. The procedure was repeated with the second distribution head;

6. The intestinal loops attached to the distribution heads were suspended in the physiological solution in the serosal compartments and a mixture of 95 % oxygen and 5 % carbon dioxide was passed through the contents of both the serosal and mucosal compartments.

3. Experimental procedure

1. The tubing was connected according to Figure 3, the stirrers and peristaltic pump were started and the drug suspensions were circulated through the intestinal segments;
2. Two 5 cm^3 samples were withdrawn by pipette from each serosal compartment at 15 min intervals for 90 min. The volumes of solution in the compartments were maintained by replacing the solution withdrawn with 10 cm^3 of fresh physiological solution at 37 ^{o}C. The samples withdrawn were run into the pear-shaped flasks ready for assay;
3. The pH of the contents of the mucosal flasks was monitored and adjusted when necessary to maintain a constant pH in the mucosal compartment throughout the test;
4. At the end of the experiment 10 cm^3 of the suspension was withdrawn from the mucosal flasks and clarified by filtration through a prefilter/ 0,8 µm membrane filter system fitted to a glass/ metal syringe;
5. The 5 cm^3 samples from the serosal compartments were assayed for the content of phenytoin by means of the benzophenone-extraction procedure Method 2;
6. The concentration of phenytoin present in the filtrate of the mucosal suspensions was determined by the assay of 1 cm^3 aliquots by means of the benzophenone-extraction procedure Method 1.

3.3.2 Precautions

The following precautions were necessary when the

circulation technique was used :

1. The physiological solution was prepared and used on the day of the experiment;
2. Care was taken to ensure that the guinea-pig was not excited prior to being killed;
3. Care was taken in the correct identification of the ileum and that the correct orientation of the intestine was maintained;
4. Care was taken to ensure that the contents remaining in the intestine were gently flushed from the lumen. This procedure prevented blockage of the apparatus during the experiment;
5. All fat and mesentary adhering to the intestine were gently removed but care was taken to avoid tearing of the membrane;
6. Care was taken that the segments of ileum did not become twisted during attachment to the distribution head;
7. All air was removed from the segments and the tubing of the apparatus;
8. The time taken for the preparation of the intestinal segments did not exceed 10 min.

3.3.3 Specific test methods

The methods adopted for the various tests and the tests conducted using the modified Sartorius Absorption Simulator are given in Table 5.

3.3.4 Calculation and presentation of results

Sticker (1973) reminds us that the passive diffusion of a drug through a membrane with time can be divided into

three periods :

1. In the first period, diffusion of the drug occurs mainly from the mucosal solution to the serosal solution;

2. During the second period there is a simultaneous back-diffusion to a greater or lesser extent from the serosal solution to the mucosal solution;

3. During the third period of diffusion, the rates are the same in both directions, so that there is no further change in the conditions.

Stricker states that the rate of diffusion of a substance from the mucosal to the serosal side can, therefore, best be determined during the first period for which there is a linear relationship between drug diffusion and time.

From the data obtained with the circulation technique it was, therefore, possible to determine the diffusion rates from the slope of the lines obtained when the amount of phenytoin which diffused through the membrane was plotted against time. From these plots, an in vitro permeability profile for phenytoin under the various experimental conditions could be determined in terms of -

(a) diffusion rate;

(b) lag time;

(c) the amount of phenytoin transferred in 90 min.

In order to calculate the amount of phenytoin which diffused through the membrane it was necessary to take into consideration the amount of drug removed in each sample of the serosal fluid. This was based upon the fact that at the end of each 15 min interval, two 5 cm^3 samples were removed from the 125 cm^3 volume of the serosal compartment and that 10 cm^3 of fresh solution was added to replace the volume

removed. The corrected amounts of phenytoin which diffused in each time interval were calculated in accordance with Table 6.

To aid in this calculation, the programme shown in Table 7 was written in BASIC for the Hewlett-Packard 9830 A calculator. For values of C_1 to C_6 supplied, values for E_1 to E_6 were computed. The values for t_1 to t_6 were then plotted against the values for E_1 to E_6. Because each test was repeated a number of times it was possible to calculate the standard deviation (σ_n) for the phenytoin concentrations at each sampling time.

From the standard deviation the standard error of the mean ($\sigma_{\bar{x}}$) was calculated as follows :

$$\sigma_{\bar{x}} = \frac{\sigma_n}{\sqrt{n}}$$

where n = number of results.

Both the standard deviation and the standard error of the mean were calculated on a Casio *f*x-29 calculator according to the method of Harper (1977). The standard error of the mean is represented on the graphs by a vertical line through the mean of the E values. By use of the co-ordinates of the points for the values for t and E and the polynomial regression programme the following could be determined :

1. the best line to fit the available data;
2. the gradient which represents the rate of diffusion of the phenytoin through the ileum;
3. the point at which the line cuts the x axis which represents the lag time or the time taken for the phenytoin to appear in the serosal solution after the commencement of the experiment.

TABLE 5 SPECIFIC TEST METHODS FOR ASSESSMENT OF DIFFUSION OF PHENYTOIN ACROSS THE ISOLATED GUINEA-PIG ILEUM

Nature of test	Mucosal Solution	Remarks
1. Studies on the effect of the calcium component of physiological solution on diffusion of phenytoin.	Physiological solution pH 7,2	Three tests were performed with the standard physiological solution which contained 125 mmoles/dm^3 of calcium chloride and three tests with physiological solution which lacked the calcium chloride component.
2. Studies on the effect of pH on the diffusion of phenytoin.	Physiological solution adjusted to pH 7,5; pH 6,5; pH 5,2 and pH 3,0	The pH's of these tests were determined by the pH's at which the tests with the antacids were conducted. They served as a demonstration of the effect of pH on the diffusion of phenytoin and also as controls against which the tests conducted with the antacids and other compounds could be compared. For solutions of pH 7,5 and 6,5 adjustments were made with 0,1 mol/dm^3 NaOH and HCl. Solutions of pH 5,2 and 3,0 contained aspartic acid to aid in the adjustment of pH. No results were recorded at pH 3,0 because at this low pH the ileum degraded rapidly and became unusable.

TABLE 5 (Continued)

Nature of test	Mucosal Solution	Remarks
3. Studies on the effect of antacids on diffusion of phenytoin.	Physiological solution + 2 g of antacid. The suspension was adjusted to the following pH's: Aluminium hydroxide pH 7,5 Calcium carbonate pH 6,5 Magnesium carbonate light pH 7,5 Magnesium trisilicate pH 7,5	Adjustment of the pH was carried out by the dropwise addition of 1 mol/dm^3 HCl. This was necessary to bring the pH of the antacid containing suspensions to an acceptable physiological level. Initially all tests were to be conducted at pH 6,5. However, the magnesium and aluminium containing compounds presented a greater resistance to pH change and tests were therefore conducted at the more easily maintainable level of pH 7,5.
4. Studies on the effect of light kaolin on diffusion of phenytoin.	Physiological solution + 2 g light kaolin adjusted to pH 7,5	pH 7,5 was selected for convenience because most tests were conducted at this pH value and therefore comparison with the other compounds was possible.
5. Studies on the effect of calcium chloride on the diffusion of phenytoin.	Physiological solution + 250 mg of Ca(II) added in the form of $CaCl_2$ solution. Adjusted to pH 6,5.	pH 6,5 was used so that results could be compared with those obtained with calcium carbonate.

TABLE 5 (Continued)

Nature of test	Mucosal Solution	Remarks
6. Studies on the effect of magnesium chloride on the diffusion of phenytoin.	Physiological solution + 250 mg of Mg(II) added in the form of $MgCl_2$. Adjusted to pH 7,5	pH 7,5 was used so that results could be compared with those obtained with the magnesium containing compounds.

NOTE: 1. SEROSAL SOLUTION. All tests were conducted with 125 cm^3 physiological solution of pH 7,2 prepared according to Aguiar and Fifelski (1966).

2. MUCOSAL SOLUTION. All tests were conducted with 250 cm^3 physiological solution to which was added 30 mg phenytoin sodium prior to pH adjustment.

TABLE 6

CIRCULATION TECHNIQUE : METHOD FOR CALCULATING CORRECTED AMOUNTS OF PHENYTOIN IN SAMPLES

Time t min	Conc. $\mu g/5\ cm^3$	Conc. $\mu g/10\ cm^3$	Conc. $\mu g/125\ cm^3$	Corrected Phenytoin Concentration $\mu g/125\ cm^3$	Corrected Phenytoin Concentration $\mu g/cm^3$
t_1 15	C_1	$2C_1$	A_1	$D_1 = A_1$	E_1
t_2 30	C_2	$2C_2$	A_2	$D_2 = A_2 + 2C_1$	E_2
t_3 45	C_3	$2C_3$	A_3	$D_3 = A_3 + 2C_1 + 2C_2$	E_3
t_4 60	C_4	$2C_4$	A_4	$D_4 = A_4 + 2C_1 + 2C_2 + 2C_3$	E_4
t_5 75	C_5	$2C_5$	A_5	$D_5 = A_5 + 2C_1 + 2C_2 + 2C_3 + 2C_4$	E_5
t_6 90	C_6	$2C_6$	A_6	$D_6 = A_6 + 2C_1 + 2C_2 + 2C_3 + 2C_4 + 2C_5$	E_6

where : t_1 to t_6 = time interval after which samples were taken

C_1 to C_6 = mean quantity of phenytoin (µg) in a 5 cm^3 sample obtained from duplicate assay results

$2C_1$ to $2C_6$ = quantity of pheytoin (µg) per 10 cm^3

A_1 to A_6 = total quantity of phenytoin (µg) in 125 cm^3 of solution ($A_1 = 12,5 \times 2C_1$)

D_1 to D_6 = corrected quantity of phenytoin (µg) in 125 cm^3 of solution

E_1 to E_6 = corrected quantity of phenytoin (µg) in 1 cm^3 of solution ($E_1 = D_1 \times 0,008$)

TABLE 7 "BASIC" PROGRAMME FOR CALCULATION OF CORRECTED AMOUNTS OF PHENYTOIN IN SAMPLES

```
10  DIM  C[6],A[6]
20  DISP  "HOW MANY READINGS?";
30  INPUT  N
40  FOR  I=1 TO N
50  DISP  "INPUT READING!";
60  INPUT  C[I]
70  A[I]=2xC[I]x12.5
80  NEXT I
90  PRINT  A[1]x0.008
100  PRINT  0.008x(A[2]+2xC[1])
110  PRINT  0.008x(A[3]+2x(C[1]+C[2]))
120  PRINT  0.008x(A[4]+2x(C[1]+C[2]+C[3]))
130  PRINT  0.008x(A[5]+2x(C[1]+C[2]+C[3]+C[4]))
140  IF  N=6  THEN  160
150  GOTO  170
160  PRINT  0.008x(A[6]+2x(C[1]+C[2]+C[3]+C[4]+C[5]))
170  STOP
180  END
```

CHAPTER 4 RESULTS

4.1 ADSORPTION STUDIES

4.1.1 Adsorption of phenytoin onto particulate matter suspended in simulated gastric and intestinal solutions

The amounts of phenytoin, expressed as a percentage of the control, remaining in solution after incubation with the various agents are presented in Tables 8 and 9. For each time period, Column A represents the percentage phenytoin remaining in the supernatant and column B the pH of the suspension. The columns labelled "blank" reflect the pH of the suspensions of the particulate matter in the simulated gastric or intestinal solutions prepared without the phenytoin. The columns labelled "control" reflect the pH of the simulated gastric and intestinal solutions containing phenytoin used for the tests and lacking the powders. The concentration of phenytoin in the solutions used for the tests are also shown in $\mu g/cm^3$ and were calculated from :

(a) the mass of phenytoin used in preparing the dilutions (Column C);

(b) the assay results of the control solutions (Column D).

It is to be noted that for each agent tested, a dilution of phenytoin in 0,1 mol/dm^3 NaOH was prepared. 1 cm^3 aliquots were then diluted to 100 cm^3 with the simulated gastric and intestinal solutions. This accounts for the fact that both Tables 8 and 9 reflect the same phenytoin concentrations according to mass. The concentration of phenytoin according to the assay represents the mean of duplicate concordant results. Columns C and D show a good correlation between the phenytoin concentration as

determined by mass and by assay of the solution incubated at 37 ^{o}C for 180 min. It is to be noted that the solutions used for these assays were the same as those used for the tests and were centrifuged and filtered in an identical manner to the tests. According to the assay results 99,2 % (S.D. $\pm$ 2,8) of the phenytoin was recovered from the simulated gastric solutions of measured pH 3,0 (S.D. $\pm$ 2,7), and 97,8 % (S.D. $\pm$ 2,7) from the simulated intestinal solution of measured pH 6,6 (S.D. $\pm$ 0,1).

From these results it appears that no degradation of the phenytoin in solution takes place over the 180 min test period in either simulated gastric or intestinal solution. In addition precipitation of the phenytoin from solution at the pH of the gastric solutions did not take place. If precipitation had occurred, the concentrations shown in column D would have been detectably lower than those shown in column C. Therefore any loss of phenytoin from solution onto the particulate matter can be attributed to adsorption.

In the tests presented the antacid powders react with the acid content of the simulated gastric and intestinal solutions to establish their own pH environment. It was calculated that there is insufficient HCl present in 10 cm^3 of the simulated gastric or intestinal solution to inactivate 100 mg quantities of the antacid, therefore particulate matter remained in suspension in all the tubes at the completion of the experiment.

Preliminary studies were carried out with aluminium hydroxide, activated charcoal and magnesium trisilicate with incubation periods of 30, 60, 120 and 180 min. The remaining tests in this series were carried out with incubation periods of 30 and 180 min. Since these studies showed little difference between the results at the various time intervals, all subsequent tests were carried out with

an incubation period of 30 min.

4.1.2 Adsorption of phenytoin onto particulate matter suspended in distilled water

The conditions of the study and the results are shown in Table 10. Light magnesium oxide was included in this series of tests. Because the preliminary studies on the starches showed similar results, it was decided to confine this series of tests to maize starch.

4.2 STUDIES ON THE DIFFUSION OF PHENYTOIN ACROSS THE ISOLATED GUINEA-PIG ILEUM

The results of these studies are represented graphically in Figures 9 to 14 and are tabulated in Table 11. These results are presented in accordance with the discussion of Section 3.3.4.

TABLE 8 PERCENTAGE PHENYTOIN REMAINING IN SOLUTION AND MEASURED pH AFTER INCUBATION WITH PARTICULATE MATTER SUSPENDED IN SIMULATED GASTRIC SOLUTION

											Phenytoin Conc $\mu g/cm^3$	
	Test								Blank	Control	Mass	Assay
Length of Incubation Min	30		60		120		180		180	180	–	180
Particulate Matter	A	B	A	B	A	B	A	B	pH	pH	C	D
Aluminium Hydroxide	97	6,2	99	6,2	100	6,2	99	6,2	6,0	3,0	25,9	26,9
Calcium Carbonate	98	6,6	–	–	–	–	98	7,0	6,7	3,0	25,2	25,0
Charcoal, Activated	0	5,0	0	5,0	0	5,0	0	5,0	3,7	3,2	24,9	24,2
Magnesium Carbonate, Heavy	102	7,9	–	–	–	–	100	7,8	7,7	3,0	25,2	25,0
Magnesium Carbonate, Light	102	7,4	–	–	–	–	101	8,3	8,2	3,0	24,8	25,6
Magnesium Trisilicate	97	7,0	99	7,2	98	7,4	102	7,3	7,3	3,2	24,3	23,5
Kaolin Light	99	3,0	–	–	–	–	101	3,0	2,8	3,0	25,9	25,0
Starch, Maize	88	2,9	–	–	–	–	90	2,9	2,8	3,0	24,8	25,6
Starch, Potato	93	3,0	–	–	–	–	94	3,0	3,0	2,9	24,7	23,8
Starch, Rice	94	3,0	–	–	–	–	92	3,0	2,9	2,9	25,6	25,5
Starch, Wheat	93	3,1	–	–	–	–	95	3,0	2,8	2,9	24,7	23,8

N.B. See note on page 81

TABLE 9 PERCENTAGE PHENYTOIN REMAINING IN SOLUTION AND MEASURED pH AFTER INCUBATION WITH PARTICULATE MATTER SUSPENDED IN SIMULATED INTESTINAL SOLUTION

											Phenytoin Conc $\mu g/cm^3$	
	Test								Blank	Control	Mass	Assay
Length of Incubation Min	30		60		120		180		180	180	-	180
Particulate Matter	A	B	A	B	A	B	A	B	pH	pH	C	D
Aluminium Hydroxide	100	7,0	99	7,0	99	7,0	100	7,0	6,9	6,5	25,9	26,8
Calcium Carbonate	103	7,3	-	-	-	-	99	7,2	7,1	6,6	25,2	24,4
Charcoal, Activated	0	6,8	0	6,8	0	6,8	0	6,7	6,8	6,9	24,9	24,1
Magnesium Carbonate, Heavy	102	8,2	-	-	-	-	101	8,1	8,2	6,6	25,2	24,4
Magnesium Carbonate, Light	101	7,8	-	-	-	-	102	8,5	8,5	6,5	24,8	25,0
Magnesium Trisilicate	101	7,4	103	7,5	101	7,6	97	7,7	7,6	6,7	24,3	23,0
Kaolin, Light	99	6,6	-	-	-	-	101	6,6	6,5	6,6	25,9	24,3
Starch, Maize	91	6,4	-	-	-	-	95	6,5	6,4	6,5	24,8	25,0
Starch, Potato	94	6,5	-	-	-	-	95	6,5	6,5	6,6	24,7	23,9
Starch, Rice	97	6,6	-	-	-	-	96	6,6	6,5	6,6	25,6	25,2
Starch, Wheat	96	6,6	-	-	-	-	94	6,6	6,5	6,6	24,7	23,9

N.B. See note on page 81

NOTES RELEVANT TO TABLES 8 AND 9

Column A - represents the percentage phenytoin remaining in the supernatant.

Column B - represents the pH of the suspension with the phenytoin.

Blank - this column represents the pH of the suspensions after 180 min in the absence of phenytoin.

Control - this column represents the pH of the phenytoin solution in the absence of the particulate matter.

Column C - initial concentration of phenytoin in test solution calculated from the mass used.

Column D - concentration of phentyoin in test solution after 180 min according to assay.

TABLE 10

PERCENTAGE PHENYTOIN REMAINING IN SOLUTION AND MEASURED pH AFTER INCUBATION WITH PARTICULATE MATTER SUSPENDED IN WATER

Phenytoin Conc $\mu g/cm^3$	25		40		60	
Particulate Matter	A	B	A	B	A	B
Aluminium Hydroxide	100	8,2	99	8,2	98	8,1
Calcium Carbonate	99	10,2	99	10,2	98	10,2
Charcoal, Activated	0	7,7	0	7,6	0	7,8
Magnesium Carbonate, Light	100	10,2	98	10,2	99	10,3
Magnesium Oxide, Light	95	10,5	98	10.5	96	10,4
Magnesium Trisilicate	100	10,1	99	10,1	99	10,1
Kaolin, Light	100	9,6	99	9,6	100	9,5
Starch, Maize	101	10,0	99	10,3	98	10,0

NOTE:

1. Duplicate concordant results were obtained for each concentration of phenytoin used.
2. Length of incubation 30 min.
3. Column A represents the percentage phenytoin remaining in the supernatant after 30 min.
4. Column B represents the pH of the suspension after 30 min.

TABLE 11 RESULTS OF DIFFUSION OF PHENYTOIN ACROSS THE ISOLATED GUINEA-PIG ILEUM

Test	Number of tests	Correlation coefficient	Mean diffusion rate ($\mu g/cm^3/min$)	Mean lag time (min)	Corrected phenytoin conc. in serosal soln after 90 min ($\mu g/cm^3$)	Phenytoin conc. in mucosal solution after 90 min ($\mu g/cm^3$)
Calcium-free Buffer pH 7,2	3	0,893	0,016 (0,002)	18,8 (10,6)	1,14 (0,15)	32,8 (-) (single result obtained)
Standard Physiological Buffer pH 7,2	3	0,967	0,015 (0,003)	18,3 (4,7)	1,06 (0,14)	31,9 (1,9) (mean of duplicate tests)
pH 7,5	14	0,910	0,017 (0,003)	16,2 (4,9)	1,29 (0,29)	33,4 (3,9)
pH 6,5	6	0,912	0,014 (0,002)	20,6 (4,6)	0,93 (0,20)	23,8 (2,5)
pH 5,2	3	0,969	0,010 (0,001)	25,6 (4,6)	0,63 (0,08)	22,3 (1,2)
pH 3,0	2	No results obtained because of denaturation of proteins of the membrane during the course of the experiments				
Aluminium Hydroxide Gel pH 7,5	3	0,981	0,025 (0,002)	15,5 (1,3)	1,89 (0,14)	46,1 (3,0)
Calcium Carbonate pH 6,5	3	0,941	0,010 (0,001)	30,0 (5,8)	0,53 (0,10)	26,5 (1,0)
Light Magnesium Carbonate pH 7,5	3	0,931	0,012 (0,002)	30,1 (4,7)	0.73 (0.15)	27,6 (2,4)
Magnesium Trisilicate pH 7,5	2	0,993	0,016 ($3,5x10^{-4}$)	15,9 (1,1)	1,16 (0,04)	42,4 (1,1)

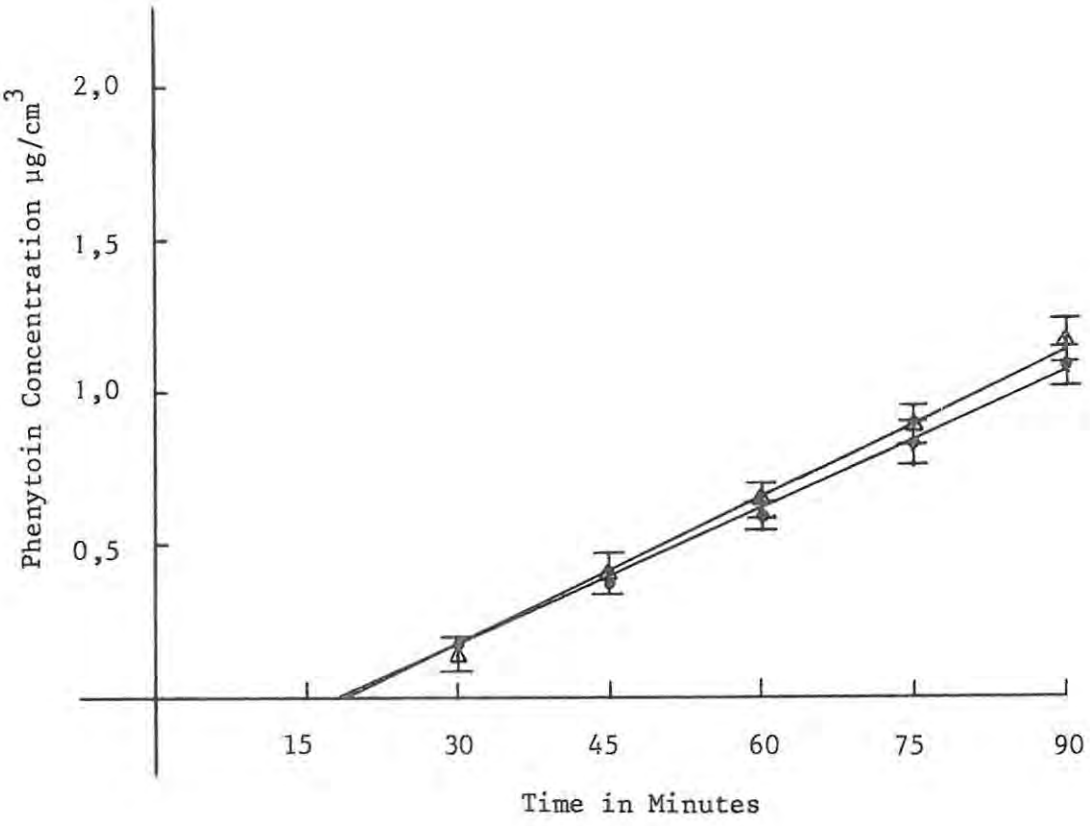
TABLE 11 (Continued)

Test	Number of tests	Correlation coefficient	Mean diffusion rate ($\mu g/cm^3/min$)	Mean lag time (min)	Corrected phenytoin conc. in serosal soln after 90 min ($\mu g/cm^3$)	Phenytoin conc. in mucosal solution after 90 min ($\mu g/cm^3$)
Light Kaolin pH 7,5	3	0,952	0,018 (0,003)	12,4 (6,6)	1,43 (0,33)	36,1 (4,5)
Calcium Chloride pH 6,5	3	0,931	0,013 (0,002)	25,7 (3,2)	0,80 (0,18)	32,3 (1,2)
Magnesium Chloride pH 7,5	3	0,941	0,013 (0,001)	23,7 (6,6)	0,88 (0,16)	34,5 (1,9)

NOTE : The mean results were obtained from the combined results by use of the polynomial regression programme.

The standard deviations are shown in parentheses in each case.

FIGURE 9 EFFECT OF CALCIUM COMPONENT OF THE PHYSIOLOGICAL SOLUTION ON THE DIFFUSION OF PHENYTOIN ACROSS THE ISOLATED GUINEA-PIG ILEUM AT pH 7,2

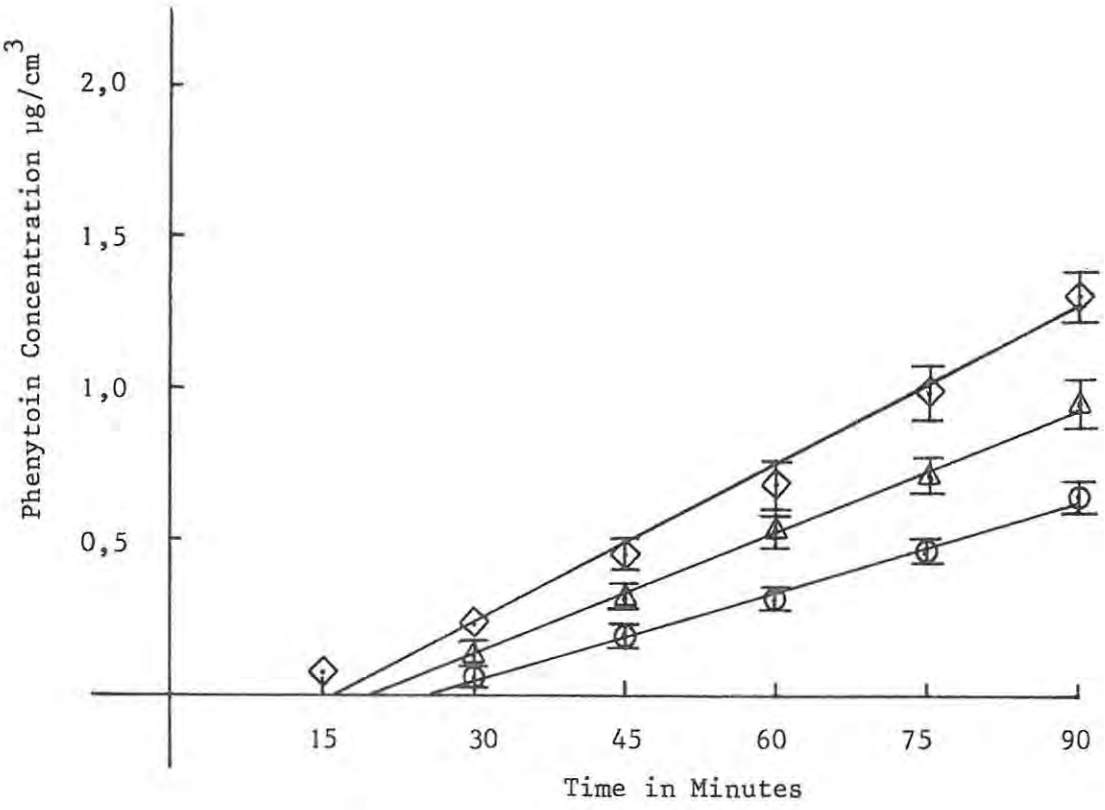


● Standard physiological solution pH 7,2 (3 tests)

△ Calcium-free physiological solution pH 7,2 (3 tests)

Vertical lines indicate standard error. The absence of vertical bars indicates that the standard error was too small to be shown.

FIGURE 10 EFFECT OF pH ON THE DIFFUSION OF PHENYTOIN ACROSS THE ISOLATED GUINEA-PIG ILEUM



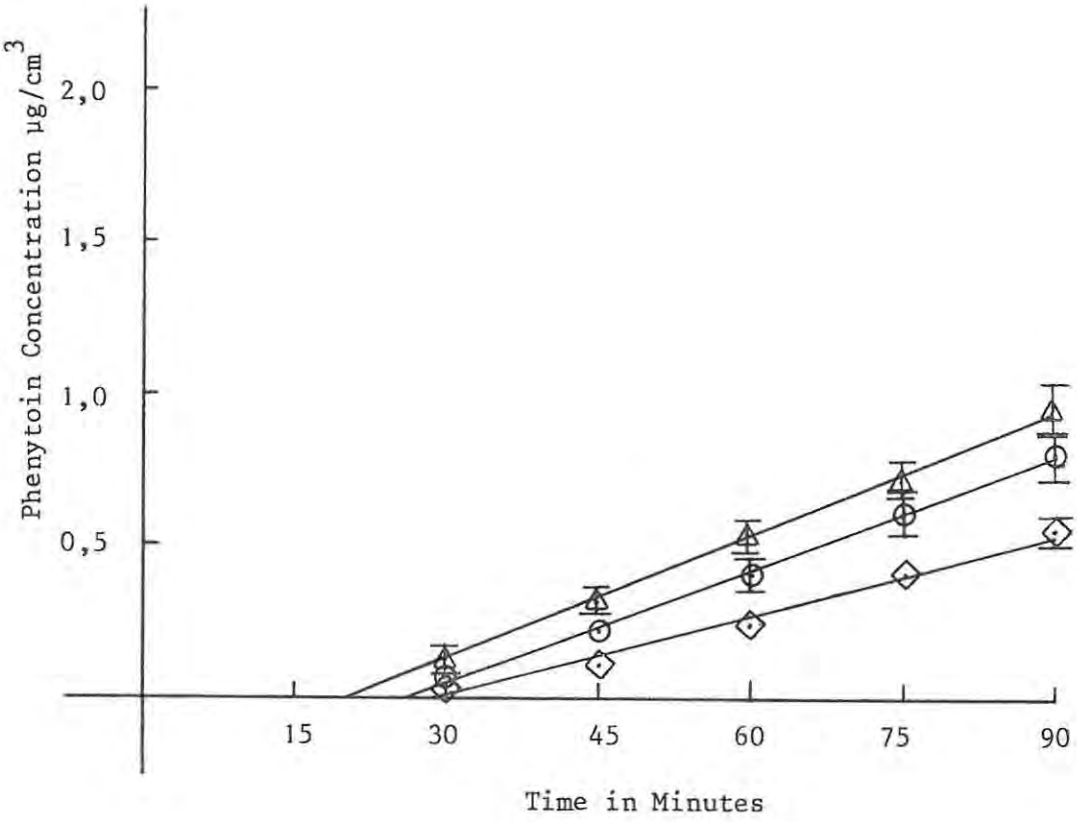
◇ pH 7,5 (14 tests)

△ pH 6,5 (6 tests)

⊙ pH 5,2 (3 tests)

Vertical lines indicate standard error. The absence of vertical bars indicates that the standard error was too small to be shown.

FIGURE 11 COMPARISON OF THE EFFECT OF CALCIUM CHLORIDE AND CALCIUM CARBONATE ON THE DIFFUSION OF PHENYTOIN ACROSS THE ISOLATED GUINEA-PIG ILEUM AT pH 6,5



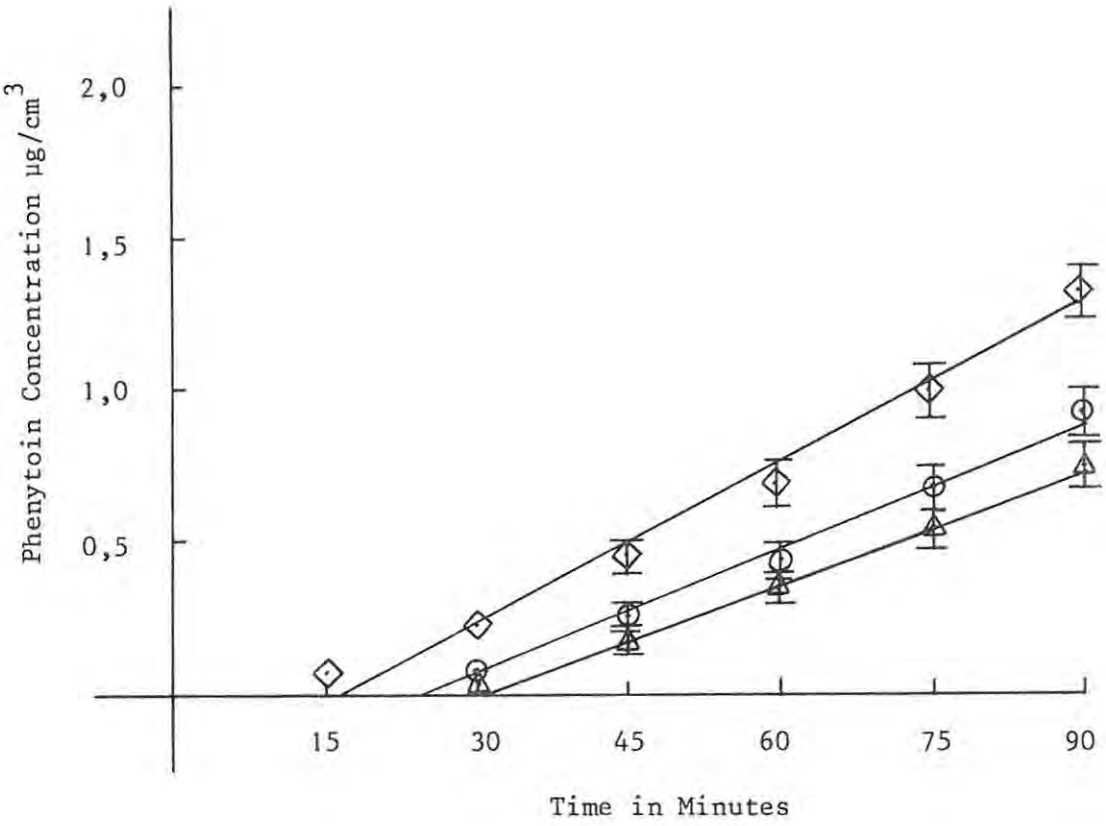
△ Diffusion at pH 6,5 (6 tests)

⊙ Calcium chloride pH 6,5 (3 tests)

◇ Calcium carbonate pH 6,5 (3 tests)

Vertical lines indicate standard error. The absence of vertical bars indicates that the standard error was too small to be shown.

FIGURE 12 COMPARISON OF THE EFFECT OF MAGNESIUM CHLORIDE AND LIGHT MAGNESIUM CARBONATE ON THE DIFFUSION OF PHENYTOIN ACROSS THE ISOLATED GUINEA-PIG ILEUM AT pH 7,5



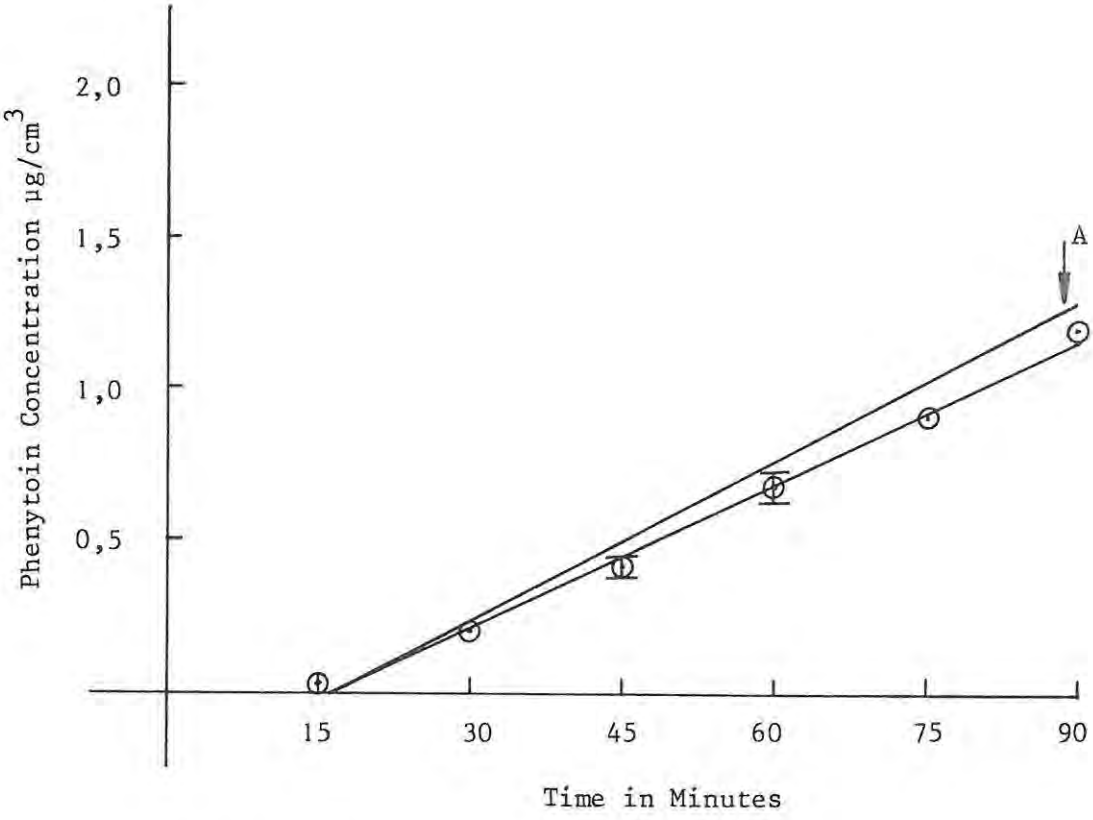
◇ Diffusion at pH 7,5 (14 tests)

⊙ Magnesium chloride (3 tests)

△ Light magnesium carbonate (3 tests)

Vertical lines indicate standard error. The absence of vertical bars indicates that the standard error was too small to be shown.

FIGURE 13 EFFECT OF MAGNESIUM TRISILICATE ON THE DIFFUSION OF PHENYTOIN ACROSS THE ISOLATED GUINEA-PIG ILEUM AT pH 7,5

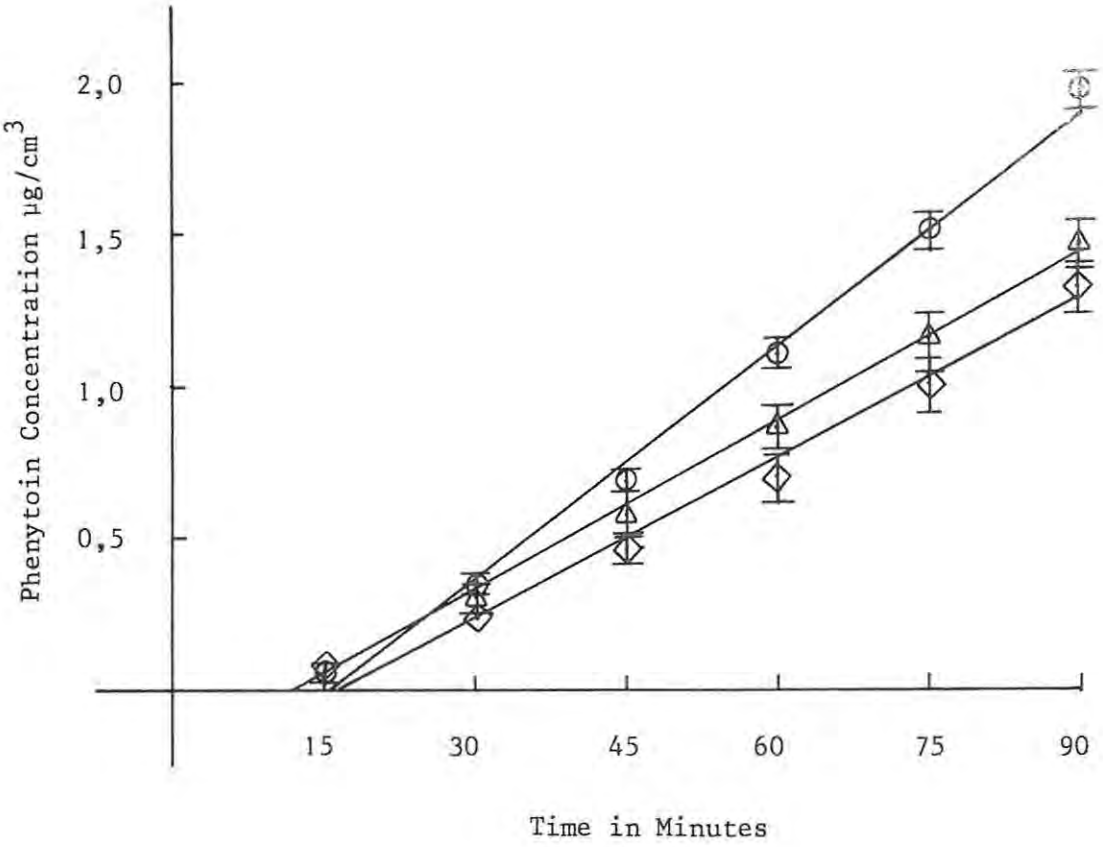


A Represents the diffusion at pH 7,5. Mean of 14 tests. For clarity the position of the points and the standard errors have been omitted.

⊙ Magnesium trisilicate (2 tests)

Vertical lines indicate standard error. The absence of vertical bars indicates that the standard error was too small to be shown.

FIGURE 14 EFFECT OF ALUMINIUM HYDROXIDE AND LIGHT KAOLIN ON THE DIFFUSION OF PHENYTOIN ACROSS ISOLATED GUINEA-PIG ILEUM AT pH 7,5



⊙ Aluminium hydroxide (3 tests)

△ Light kaolin (3 tests)

◇ Diffusion at pH 7,5 (14 tests)

Vertical lines indicate standard error. The absence of vertical bars indicates that the standard error was too small to be shown.

CHAPTER 5 DISCUSSION

5.1 ADSORPTION STUDIES

An examination of the results shown in Tables 8, 9 and 10 reveals that activated charcoal only is capable of adsorbing phenytoin to a significant extent *in vitro*. This finding confirms the results of Decker *et al.* (1968) who show that activated charcoal has a considerable adsorbing capacity for phenytoin.

Generally, there was no loss of phenytoin from solution onto the antacid substances (aluminium hydroxide gel, calcium carbonate, heavy and light magnesium carbonate and magnesium trisilicate). The results indicate that a small loss of phenytoin from solution occurs in the presence of the starches. This small decrease can be attributed to adsorption.

From Table 10 it may be seen that no significant adsorption takes place onto the particulate matter from aqueous solution at the pH's indicated in the table. Once again, some of the compounds tend to establish their own pH environment. This makes it impossible to compare the results at one and the same pH value. A small decrease in phenytoin concentration (5 %) was observed with the light magnesium oxide but this is unlikely to be of significance.

It is to be noted that at pH 2,9 the maize starch adsorbed phenytoin to the extent of 12 % but it failed to remove the phenytoin from aqueous solutions of pH 10 at the three concentrations tested. This may be due to the fact that, at pH 10, phenytoin is 98 % ionised, but at pH 6,5 it is only 1,6 % ionised and at pH 3,0 the drug is almost totally

unionised. Sanvordeker and Dajani (1975) demonstrated that the non-ionised species of diphenoxylate hydrochloride is preferentially adsorbed onto activated charcoal compared to its ionised species in solution and Tsuchiya and Levy (1972) demonstrated that the unionised forms of aspirin, salicylamide and phenylpropanonalime are more extensively adsorbed in vitro. The loss of phenytoin onto the starches may therefore be attributed to the prefential adsorption of the unionised form at pH 6,5 and pH 3,0.

An examination of a commercial sample of phenytoin sodium 100 mg capsules revealed that the product contains approximately 150 mg of diluent, which represents a significant proportion of the total capsule mass. Clinically the small loss of phenytoin from solution onto the pharmaceutical grade starches at pH 6,5 and pH 3,0 would not appear to be significant. The use of 150 mg of starch as diluent with 100 mg of phenytoin sodium is unlikely to result in any significant loss of phenytoin onto the starch at pH's below 6,5. In addition, the starches are digested during their passage through the gastro-intestinal tract and would therefore release any drug bound to their surface.

From the results of this study, it seems unlikely that the adsorption phenomenon is the cause of the reduced blood levels of phenytoin noted by Pippenger (personal communication 1978), Kulshrestha et al. (1978) and Garnett et al. (1979).

5.2 DIFFUSION OF PHENYTOIN ACROSS THE ISOLATED GUINEA-PIG ILEUM

5.2.1 Use of the circulation technique and methods adopted for assessment of the trans-ileal diffusion of phenytoin

During the development of the circulation technique it was necessary to introduce a number of modifications to obtain

meaningful results. For initial investigations a solution containing 25 $\mu g/cm^3$ of phenytoin in physiological solution was used as the mucosal solution. However, this proved unsatisfactory as there was insufficient phenytoin present in the mucosal compartment to both saturate the membrane and diffuse into the serosal compartment in a measurable concentration. On consideration of this problem two solutions seemed possible :

1. The use of an increased volume of phenytoin solution in the mucosal compartment;
2. The use of phenytoin sodium in excess of its solubility in the mucosal compartment.

The first method is recommended by Stricker (1973) for use with the Sartorius Absorption Simulator in testing very lipid soluble compounds. With this system, an increased amount of drug is dissolved in a large volume of mucosal solution (e.g. 1 dm^3). Sufficient drug is therefore present in solution to both saturate and penetrate the lipid membrane in sufficient quantities for measurable concentrations to be reached in the serosal solution. Whilst this system is suitable for some diffusion studies, it was felt that it would not be possible for a meaningful comparison to be made between the in vitro results and published in vivo studies using this method.

By use of the second system it was possible to create a situation in vitro which closely resembled the in vivo conditions pertaining to the absorption of phenytoin as outlined by Woodbury and Swinyard (1972), i.e. phenytoin does not dissolve completely within the intestine. The undissolved phenytoin will go into solution only at the rate at which the drug can be absorbed.

The following points are of note concerning the method

adopted :

1. Phenytoin sodium is used largely in capsule form in medication in a dosage of 30 to 100 mg (B.P.C. 1973);

2. Schwartz et al. (1977) give the solubility of phenytoin at pH 7,4 and at 37 ^{o}C as 31 µg/cm^{3}. It was calculated that with the use of 250 cm^{3} of physiological solution of pH 7,5 this volume would be saturated by 7,75 mg of phenytoin;

3. A mass of 30 mg of phenytoin sodium was used in the mucosal solution. Phenytoin sodium dissolves more rapidly than phenytoin and this ensured that saturation of the mucosal solution occurred at a rapid rate. (Yamamoto et al. (1976));

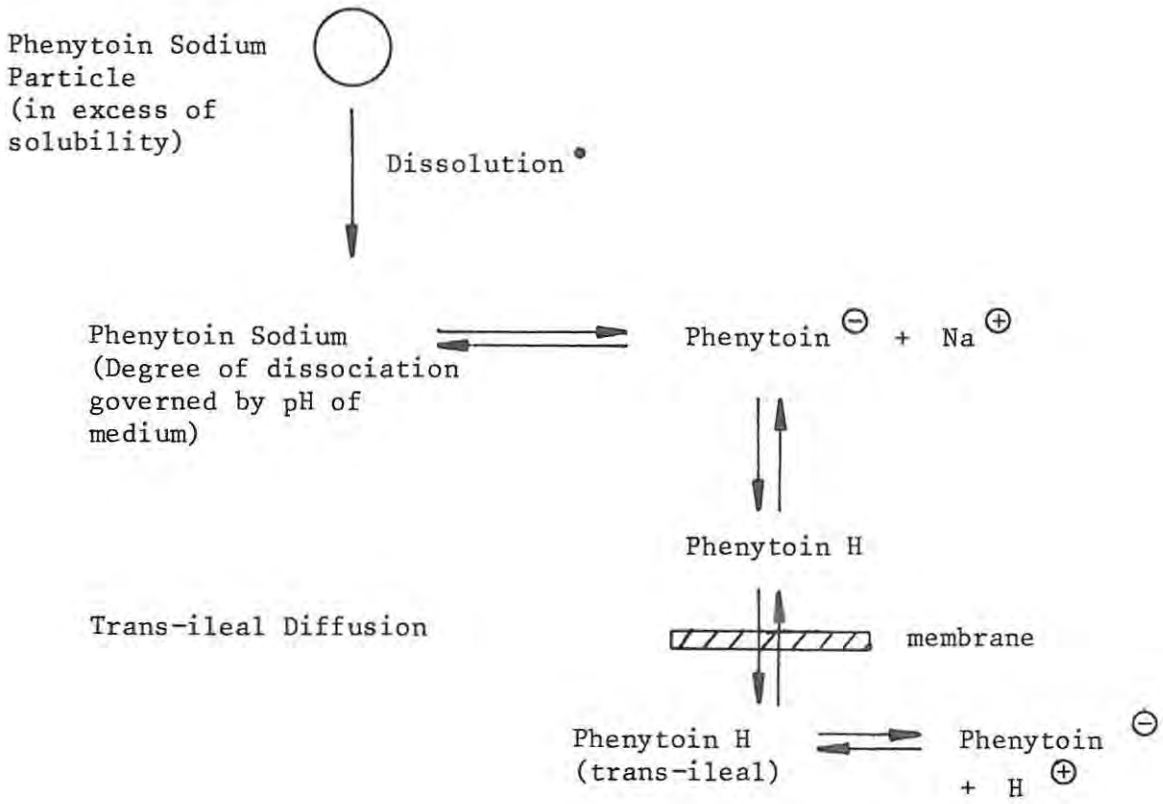
4. Phenytoin is reputed to have a high fat solubility and is protein bound. Sufficient phenytoin was thus present to saturate the isolated ileum before diffusion could occur;

5. In addition to saturating the membrane a "pool" of undissolved phenytoin would remain in the mucosal compartment. This "pool" resulted in a constant concentration of drug available for trans-ileal diffusion.

The process which takes place in the system adopted is represented diagramatically in Figure 15. An examination of the figure shows that two factors influence the diffusion of phenytoin, namely, the dissolution rate of the drug, and its degree of ionisation in the pH environment of the mucosal compartment.

With the use of the circulation technique it was possible to monitor the pH and by suitable adjustment to control, the degree of ionisation of the compound. Therefore it was possible to assess the influence of the antacids and other compounds on the dissolution and the eventual trans-ileal

FIGURE 15 SCHEMATIC REPRESENTATION OF THE TRANS-ILEAL DIFFUSION OF PHENYTOIN



• According to Yamamoto et al. (1976) dissolution is the rate determining step.

diffusion of phenytoin.

An examination of Table 11 shows that the trans-ileal diffusion of phenytoin takes place according to the first diffusion period as defined by Stricker (1973) in that correlation coefficients of between 0,893 and 0,993 were obtained for the points of the lines. These were regarded as being acceptable in terms of the system and demonstrate the validity of the circulation technique for the in vitro investigation of drug absorption.

5.2.2 Effect of pH on the trans-ileal diffusion of phenytoin

The studies on the influence of pH on the diffusion of phenytoin were conducted for two reasons :

1. To establish a pattern for the trans-ileal diffusion of phenytoin at specific pH values. These results serve as controls against which the tests performed in the presence of the substances can be compared;
2. To establish the validity of the circulation procedure for the assessment of drug absorption in vitro.

pH influences the diffusion of substances across a membrane in two ways :

1. by affecting the solubility of the compound;
2. by determining the degree of ionisation of the substance.

This statement is corroborated by Aguiar and Fifelski (1966) who state that the rate of absorption of a drug is dependent on two independent processes, namely, the rate of dissolution of the drug in the medium, and its rate of permeation through the gastric wall or intestinal lumen. "For a relatively insoluble drug (less than 0,01 mg/ml) the rate of solution becomes a fundamental factor affecting the

rate of absorption. The reason is that unless the drug dissolves at a sufficiently rapid rate, the necessary build-up of an effective concentration at the site of absorption will never occur."

From Table 11 it may be noted that for the tests conducted at pH 7,5; 6,5 and 5,2 the solubility of the phenytoin in the mucosal vessel at the completion of the experiment was 33,4 $\mu g/cm^3$, 23,8 $\mu g/cm^3$ and 22,3 $\mu g/cm^3$ for each pH value respectively. An examination of Figure 10 shows the influence of pH on the trans-ileal diffusion of phenytoin. As the pH is lowered, three observable differences result :

1. the mean rate of diffusion of phenytoin through the ileum falls from 0,017 $\mu g/cm^3$/min at pH 7,5 to 0,010 $\mu g/cm^3$/min at pH 5,2 which represents a reduction of 41 %;
2. the time which is needed for the phenytoin to penetrate the membrane and appear in the serosal solution (lag time) increases from 16,2 min at pH 7,5 to 25,6 min at pH 5,2 which represents an increase of 58 %;
3. the corrected concentration of phenytoin in the serosal solution after 90 min is lowered from 1,3 $\mu g/cm^3$ at pH 7,5 to 0,6 $\mu g/cm^3$ at pH 5,2 which represents a reduction of 54 %.

From the Henderson-Hasselbalch equation it may be calculated that phenytoin is 86,4 % unionised at pH 7,5; 98,4 % unionised at pH 6,5 and 99,9 % unionised at pH 5,2. If the pH-partition theory is used for evaluating the results it would appear that the present findings conflict with this theory in that the diffusion of phenytoin decreases as the percentage of the unionised species increases. The findings of this study will therefore need to be evaluated in terms of the absorption of phenytoin as outlined by Woodbury and Swinyard (1972). These authors state that little absorption

of phenytoin occurs in the stomach because of the low solubility of the drug in the gastric juice. Thus, despite the fact that phenytoin exists predominantly in the unionised form and should be absorbed readily by passive diffusion, it attains only a very low concentration in the gastric juice and consequently is poorly and slowly absorbed. In the duodenum where the pH is approximately 7 to 7,5, more drug exists in the ionised form, and hence is considerably more soluble in the intestinal fluid. It is at this site where the maximum absorption of phenytoin occurs.

From the results of this study it appears that the solubility of the phenytoin in the intestinal fluids is critical to the absorption of the drug. Although the solubility of the phenytoin exceeds the limit set by Aguiar and Fifelski (1966), the results demonstrate the validity of the observations of Woodbury and Swinyard (1972) regarding the factors influencing the absorption of orally administered phenytoin.

The absorption of phenytoin is acknowledged by Yamamoto et al. (1976) to be dissolution rate-limited. A further point to be borne in mind in the interpretation of these results is the dissolution rate of the phenytoin in the mucosal compartment. The dissolution rate of the phenytoin was not measured during these experiments. A lower dissolution rate at the lower pH values can be postulated as a contributing factor to the lowering of the trans-ileal diffusion of phenytoin with lowered pH. This factor would account for the differences in diffusion monitored at pH 6,5 and pH 5,2 which cannot wholly be explained by the solubility differences of the phenytoin measured at the conclusion of the experiment.

5.2.3 Effect of calcium component of the physiological solution on the trans-ileal diffusion of phenytoin

The results of this study are shown in Figure 9 and Table 11. This work was carried out in the form of a preliminary investigation using the physiological solution of Aguiar and Fifelski (1966) prepared with and without the $1,25 \times 10^{-3}$ moles/dm^3 of calcium chloride. From these tests it was apparent that the calcium component of the physiological solution did not influence the diffusion of phenytoin significantly. These tests were necessitated because of the observation of Tyrer et al.(1970) that the excipient calcium sulphate adversely affects the blood levels of phenytoin. It was felt that the calcium component of the physiological solution could adversely affect the diffusion of the drug.

During these preliminary studies, the decision was made to monitor the concentration of phenytoin remaining in solution on the mucosal side of the membrane at the conclusion of each experiment. A single result (from a duplicate assay procedure) was obtained for the calcium-free physiological solution and two results for the standard physiological solution. The final concentrations reached are shown in Table 11. In all other tests, the concentration of phenytoin in the mucosal solution was determined at the completion of the experiment.

From the results recorded it was apparent that the inclusion of calcium chloride in the physiological solution would not adversely influence future work and therefore the standard physiological solution was used for all subsequent tests.

5.2.4 Effect of calcium, magnesium and aluminium containing compounds on the trans-ileal diffusion of phenytoin

The result of these studies are shown in Figures 11 to 14.

The methods adopted for these tests are summarised in Table 5 and the results in Table 11. The reader is reminded of the difficulty which arose during the studies conducted with the antacid substances. In all these tests 2 g of the antacid was added to 250 cm^3 of physiological buffer which contained 30 mg of phenytoin sodium. The initial pH of the suspension was monitored and the pH was adjusted with HCl to an acceptable physiological value. Initially all these tests were to be conducted at pH 6,5. This method worked successfully for calcium carbonate. However, subsequent tests showed that the other antacids exhibited a greater resistance to this pH change and were more easily stabilised to pH 7,5. Both these pH values are acceptable in terms of the intestinal absorption of drugs. Bates and Gibaldi (1970) give the pH of the small intestine as approximately 6,5 and Woodbury and Swinyard (1972) as approximately 7 to 7,5. From the results presented it is therefore not possible to compare the effects of calcium and magnesium compounds as the tests were conducted at different pH values.

A further factor to be borne in mind is that the antacid compounds themselves act as buffers. Because of the presence of the biological membrane it was not practical to neutralise these compounds completely since the pH of the tests had to be maintained at physiologically acceptable levels. Therefore the system was adopted whereby surveylance was made of the mucosal pH and adjustments made when necessary during the duration of the tests.

The influence of these substances will be discussed on an individual basis. Comparisons will be made where possible using the parameters of lag time, diffusion rate, final concentration attained in the serosal solution and the final concentration of the phenytoin in solution in the mucosal compartment on completion of the experiment.

1. Calcium Carbonate

The presence of calcium carbonate produces a significant lowering in the trans-ileal diffusion of phenytoin as compared to the tests performed in the absence of the antacid at pH 6,5. From Table 11 three differences may by noted :

1. The mean diffusion rate of 0,010 $\mu g/cm^3$/min of phenytoin is 28,6 % lower than the tests at pH 6,5;
2. The lag time increases from 20,6 min to 30,0 min;
3. In the presence of calcium carbonate there is an increase in the solubility of the phenytoin from 23,8 to 26,5 $\mu g/cm^3$/min in the mucosal compartment at pH 6,5.

The initial pH of the mucosal suspension which contained 2 g of calcium carbonate with the phenytoin sodium was 8,0. This was adjusted to pH 6,5 with HCl. As a result of the pH adjustment soluble calcium was released into the mucosal compartment. Additional calcium would also be released from the calcium carbonate as a result of the formation of calcium bicarbonate by the action of the CO_2 content of the gassing mixture. It was felt that the release of soluble calcium would influence the trans-ileal diffusion of phenytoin; therefore a similar series of tests were performed with calcium chloride so that the influence of calcium ions could be assessed independently.

2. Magnesium Carbonate

An examination of the results reveals that the presence of light magnesium carbonate has an effect similar to that of calcium carbonate on the diffusion of the phenytoin, although the tests were conducted at different

pH values. The rate of diffusion was reduced from 0,017 to 0,012 $\mu g/cm^3$/min which represents a fall of 29 %. However, it is to be noted that there was a fall in the solubility of the phenytoin in the mucosal solution at pH 7,5 to 27,6 $\mu g/cm^3$. The initial impression was that the lowered diffusion rate of the phenytoin was caused by the reduced concentration of the drug in the mucosal solution but this fact does not explain the observation completely.

The rate of diffusion of the phenytoin measured in the presence of the magnesium carbonate was lower than in tests conducted without the antacids at pH 6,5. The diffusion rate for the tests in the absence of antacids at pH 6,5 was 0,014 $\mu g/cm^3$/min which is 14,3 % higher than the diffusion rate of phenytoin in the presence of magnesium carbonate at pH 7,5. Furthermore, the solubility of phenytoin in the mucosal solution at pH 6,5 was 23,8 $\mu g/cm^3$ as opposed to 27,6 $\mu g/cm^3$ in the presence of magnesium carbonate at pH 7,5. From these results it appears that the differences in concentration of phenytoin in the mucosal compartment could not alone explain the difference in diffusion rates observed in the experiments. It was felt, however, that the presence of magnesium ions released from the magnesium carbonate by the action of the acid could be the factor influencing the diffusion and the solubility of the phenytoin. Therefore a series of tests with magnesium chloride in the mucosal compartment were conducted in an attempt to clarify this observation.

3. Magnesium Trisilicate

The results of two tests are shown in Figure 13. A comparison of the two lines shows a slight reduction in the diffusion of phenytoin in the presence of magnesium

trisilicate at pH 7,5. Magnesium trisilicate is a slow acting antacid. During the tests with this compound, difficulty was experienced in maintaining the pH at a constant level. Difficulty was also experienced with the membranes which tended to contract sufficiently to interrupt the flow of mucosal fluid at times. Although there was very little difference between the diffusion rates in the presence and in the absence of magnesium trisilicate, the solubility of the phenytoin was found to increase to 42,4 $\mu g/cm^3$ at the controlled pH 7,5. While the results from the duplicate tests are insufficient to prove conclusively an effect of magnesium trisilicate on the diffusion of phenytoin, a greater diffusion than that recorded is expected in view of the increased solubility of the phenytoin in the presence of the antacid.

4. Aluminium Hydroxide Gel

Figure 14 illustrates how the presence of aluminium hydroxide gel increases markedly the diffusion of phenytoin at pH 7,5. The diffusion rate rose to 0,025 $\mu g/cm^3/min$ which represents an increase of 47 % over the tests conducted at pH 7,5 in the absence of the antacid. The increase in diffusion can be attributed to the increased solubility of the phenytoin in the presence of aluminium hydroxide gel. It therefore appears that even though Al(III) would be released into the mucosal compartment by the action of the acid, it does not adversely influence the diffusion of phenytoin under the test conditions.

5. Light Kaolin

Although kaolin contains aluminium in the form of hydrated aluminium silicate, unlike aluminium hydroxide gel, the substance does not dissolve in dilute mineral acids with

the formation of soluble aluminium (Al III). The trans-ileal diffusion of phenytoin in the presence of light kaolin is shown in Figure 14. The results obtained in the presence of both aluminium hydroxide gel and light kaolin are similar. The solubility of the phenytoin in the mucosal compartment increases in the presence of the light kaolin. Although this increase is lower than in the presence of aluminium hydroxide gel, it nevertheless was accompanied by an increase in the diffusion of the phenytoin at pH 7,5.

6. Calcium (Ca (II))

It has previously been shown that the presence of $1,25 \times 10^{-3}$ moles/dm^3 of calcium chloride in the mucosal solution did not produce a detectable change in the rate of phenytoin diffusion. However, when the calcium ion concentration was increased to 0,025 moles/dm^3, the solubility of the phenytoin was found to increase to 32,3 µg/cm^3 at pH 6,5. This higher concentration did not result in an increase in the diffusion rate of phenytoin. The diffusion rate fell by 7,1 % from 0,014 to 0,013 µg/cm^3/min at pH 6,5 and the lag time increased by 5,1 min.

From these results it appeared that the presence of free calcium ions in the mucosal compartment resulted in an alteration in the amount of phenytoin which appeared in solution at the conclusion of the experiments and adversely influenced the trans-ileal diffusion of phenytoin.

7. Magnesium (Mg (II))

The addition of 250 mg of Mg (II) in the form of magnesium chloride to the mucosal compartment (0,041 mol/dm^3 Mg (II)) resulted in a very slight increase in the solubility of

of the phenytoin in the mucosal compartment at the conclusion of the tests. (33,4 to 34,5 $\mu g/cm^3$). However, the diffusion rate fell from 0,017 to 0,013 $\mu g/cm^3/min$ which represents a reduction of 23,5 %. The lag time increased from 16,2 to 23,7 min, and the concentration in the serosal compartment at the completion of the tests was found to be 0,88 $\mu g/cm^3$ as opposed to 1,25 $\mu g/cm^3$ for the tests at pH 7,5 conducted in the presence of phenytoin alone.

From these results it appears that the presence of magnesium ions in the mucosal compartment results in an inhibition of the trans-ileal diffusion of phenytoin.

The findings of the effects of the antacids, kaolin, calcium chloride and magnesium chloride on the trans-ileal diffusion of phenytoin may be summarised as follows :

1. Magnesium and calcium ions cause a lowering of the trans-ileal diffusion of phenytoin as does the presence of calcium carbonate and magnesium carbonate. Calcium and magnesium ions released from the antacid may therefore account for the lowering of the diffusion rates by an unknown mechanism;
2. The effects of calcium and magnesium are not, however, directly comparable as the molar concentrations of these ions are different in the tests and the tests were conducted at different pH values;
3. The dissolution of the phenytoin sodium varied in the presence of the powders. This occurred in spite of the fact that the duration of the tests, the rate of agitation and the temperature were standardised;
4. When increased dissolution of the phenytoin sodium occurred in the absence of magnesium and calcium ions, it was accompanied by an increase in the trans-ileal diffusion;

5. In most instances, an increase in the dissolution of the phenytoin sodium in the presence of Ca(II) and Mg(II) resulted in a decrease in the trans-ileal diffusion.

These results may be compared with the findings of Juul Christensen et al. (1967) who demonstrated the influence of gastric antacids on the release in vitro of tetracycline hydrochloride. They showed that the diffusion of tetracycline hydrochloride through a membrane is diminished by magnesium oxide, aluminium hydroxide and calcium carbonate. This inhibition is attributed to the release of polyvalent metal ions into the system by the action of the acid in the simulated gastric juice. These ions are known to form non-diffusable complexes with tetracycline.

Although the formation of phenytoin-calcium and phenytoin-magnesium complexes have not been demonstrated, the formation of such complexes might account for the differences in phenytoin diffusion observed in the present study. In the light of the lack of evidence in this area it would seem unwise to speculate further concerning the formation of such complexes.

From the present study it has been possible to demonstrate qualitatively the differences in the trans-ileal diffusion of phenytoin in the presence and in the absence of some calcium and magnesium containing compounds. These observed differences may account for the lowering of phenytoin plasma levels observed by Pippenger (personal communication 1978), Kulshrestha et al. (1978), and Garnett et al. (1979). when antacids containing aluminium hydroxide gel, magnesium hydroxide, magnesium trisilicate and calcium carbonate were administered with phenytoin. It is to be noted that aluminium hydroxide gel was often used in combination with magnesium containing compounds. It may be postulated that the magnesium containing component contributed to the

decreased blood levels of phenytoin reported.

In the light of the present findings it would appear unwise for patients to take magnesium or calcium containing compounds concurrently with oral phenytoin preparations. Furthermore, the inclusion of the diluents calcium carbonate, magnesium carbonate or magnesium oxide in capsules containing phenytoin sodium should be viewed with circumspection. The Pharmaceutical Codex (1979) cautions against the use of calcium sulphate as a diluent for phenytoin sodium capsules but recommends the abovementioned antacids as suitable diluents for capsules provided that the medicament and the diluent are compatible. It would seem appropriate if this precautionary note were extended to cover all calcium and magnesium containing diluents in phenytoin sodium capsules.

SUMMARY

Phenytoin has been reviewed with respect to its physico-chemical properties, absorption after oral administration and interactions with other medicinal compounds. A detailed discussion of the recorded interactions of phenytoin with antacids, excipients, metal ions and pharmaceutical adsorbents is presented. The analytical methods available for phenytoin are assessed. The *in vitro* methodology for the assessment of drug-adsorbent, drug-antacid and drug-excipient interactions is reviewed.

Two methods for the determination of phenytoin by use of the benzophenone-extraction procedure according to Saitoh *et al*. (1973) have been developed. These are presented in order that concentrations of up to 40 $\mu g/cm^3$ and up to 8 $\mu g/cm^3$ of phenytoin in aqueous solution may be determined.

The Sartorius Absorption Simulator has been modified to accommodate a length of isolated guinea-pig ileum after the circulation technique of Fisher and Parsons (1949). The construction of the apparatus and the experimental procedure is detailed.

The loss of phenytoin from solution onto some common pharmaceutical antacids, excipients and adsorbents has been assessed. Of the compounds tested, activated charcoal only is capable of removing significant amounts of phenytoin from solution.

The modified Sartorius Absorption Simulator has been used to demonstrate the effect of pH on the trans-ileal diffusion of phenytoin according to the theory governing the absorption of orally administered phenytoin as proposed by Woodbury and Swinyard (1972).

The influence of calcium and magnesium containing antacids

and the presence of calcium and magnesium ions in solution on the trans-ileal diffusion of phenytoin is demonstrated.

Conclusions are drawn as to the usefulness of the circulation technique for the demonstration of drug interactions in vitro. The concurrent administration of calcium and magnesium containing antacids with phenytoin is assessed in terms of the present findings and published clinical data. The use of calcium and magnesium containing compounds as excipients for phenytoin sodium capsules is questioned.

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