Section 2:

REVIEW OF

LITERATURE
BIOAVAILABILITY AND BIOEQUIVALENCE

1. Bioavailability

Bioavailability is defined by the US-FDA as the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. For drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action.

The EMEA guidance defines Bioavailability as the rate and extent to which the active substance or active moiety is absorbed from a pharmaceutical form and becomes available at the site of action.

The CDSCO, India, defines Bioavailability as the relative amount of drug from an administered dosage form which enters the systemic circulation and the rate at which the drug appears in the systemic circulation.

Bioavailability of any product varies with many factors, which are conveniently divided into drug factors and host factors (Brahmankar and Jaiswal, 1995). The drug factors include physicochemical properties of drug substance and dosage form characteristics. The host factors include age, blood flow to gastrointestinal tract (GIT), presence of food or other contents in GIT, GIT pH, gastric emptying, disease state and presystemic metabolism by enzymes in the gut wall or in the liver.

Factors affecting bioavailability

The various factors affecting bioavailability of drugs can be classified as shown in Table 1.

1.1. Physicochemical properties of drug substances

1.1.1. Drug solubility and dissolution rate

Dissolution is the rate-determining step (RDS) for hydrophobic, poorly aqueous soluble drugs like griseofulvin and spironolactone; absorption of such drugs is said to be dissolution rate-limited. If the drug is hydrophilic with high aqueous solubility e.g. cromolyn sodium or neomycin, then dissolution is rapid and the RDS in the absorption of such drugs is rate of permeation through the biomembrane. Adsorption of such drugs is said to be permeation rate limited or transmembrane rate limited. Fig.1. shows a schematic representation of this concept.
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Fig. 1. The two rate-determining steps in the absorption of the drugs from orally administered formulations.

Table 1: Factors affecting absorption of a drug from its dosage form (Brahmankar & Jaiswal, 1995).

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<thead>
<tr>
<th>PHARMACEUTICAL FACTORS</th>
<th>PATIENT RELATED</th>
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<tr>
<td><strong>Physicochemical properties of drug substances</strong></td>
<td>Disintegration time Age</td>
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<tr>
<td>Drug solubility and dissolution rate</td>
<td>Dissolution time Gastric emptying time</td>
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<tr>
<td>Particle size and effective surface area</td>
<td>Dissolution time Intestinal transit time</td>
</tr>
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<td>Polymorphism and amorphism</td>
<td>Manufacturing variables GIT pH</td>
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<tr>
<td>Hydrates / solvates</td>
<td>Pharmaceutical ingredients Disease states</td>
</tr>
<tr>
<td>Salt form of the drug</td>
<td>Nature and type of dosage form Blood flow through GIT</td>
</tr>
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<td>Lipophilicity of the drug</td>
<td>Product age and storage conditions</td>
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RDS for lipophilic drugs
RDS for hydrophilic drugs
1.1.1.1. Particle size and effective surface area

Particle size and surface area of a solid drug are inversely related to each other. Particle size is of importance for drugs of low solubility. The critical point seems to be if the solubility is less than 0.3 percent. With decreasing particle size, the surface area increases, thus increasing the area of solid matter being exposed to the dissolution media and, hence, dissolution rate increases. However, the actual solubility does not significantly change with particle size reduction (micronization) in the range used in pharmaceutical manufacture. The following eqn. describes the dissolution rate:

$$\frac{dc}{dt} = k \cdot a \cdot (C_s - C_t)$$

$dc/dt$ = dissolution rate (amount per unit time) (Noyes Whitney equation)
$k$ = constant depending on intensity of agitation, temperature, structure of solid surface, and diffusion coefficient
$a$ = surface area of undissolved solute
$C_s$ = solubility of drug in solvent
$C_t$ = concentration of dissolved drug at time $t$

Examples of drugs for which therapeutic differences have been found depending on particle size are: amphotericin, aspirin, bishydroxycoumarin, chloramphenicol, digoxin, acetonide, griseofulvin, meprobamate, nitrofurantoin, Phenobarbital, phenothiazine, prednisolone, procaine penicillin, reserpine, spironolactone, sulfadiazine and tolbutamide (Ritschel, 1999).
1.1.2. Polymorphism and amorphism

Polymorphism is the phenomenon that a drug may exist in different crystalline forms, polymorphs. Polymorphism exists only in solid state. The most stable form has highest stability but lowest dissolution rate. The least stable form usually has the most rapid dissolution rate. The unstable (metastable) forms convert more or less slowly into the more stable form. e.g. chloramphenicol palmitate appears in three different polymorphs, but only polymorph B is biologically active, since the other forms do not dissolve and are not hydrolysed. The polymorphs differ from each other with respect to their physical properties such as solubility, melting point, density, hardness and compression characteristics.

Some drugs can exist in amorphous form (i.e. having no internal crystal structure). In general, the amorphous state is more soluble and has a higher dissolution rate than the crystalline form. The crystalline form requires a higher amount of energy to free a molecule of drug from it than does the amorphous form. e.g. amorphous novobiocin and amorphous chloramphenicol esters are biologically active while their crystalline forms are inactive.

1.1.3. Salt form of a drug

Most drugs are either weak acids or weak bases. One of the easiest approaches to enhance the solubility and dissolution rate of such drugs is to convert them into their salt forms. At a given pH, the solubility of a drug, whether acidic/basic or its salt form, is a constant.

1.1.4. Drug pKa and lipophilicity and GI pH-pH Partition hypothesis

The pH Partition theory explains in simple terms, the process of drug absorption from the GIT and its distribution across all biologic membranes. The theory states that for a drug compound of molecular weight greater than 100, which are primarily transported across the biomembrane by passive diffusion, the process of absorption is governed by:

1. The dissociation constant (pKa) of the drug.
2. The lipid solubility of the unionised drug (a function of drug $K_{ow}$).
3. The pH at the absorption site.

**Lipophilicity and drug absorption**

The pKa of a drug determines the degree of ionisation at a particular pH and that only the unionised drug, if sufficiently lipid soluble, is absorbed into the systemic circulation. Thus, even if the drug exists in the unionised form, it will be poorly absorbed if it has poor lipid solubility.
Ideally, for optimum absorption, a drug should have sufficient aqueous solubility to dissolve in the fluids at the absorption site and lipid solubility ($K_{ow}$) high enough to facilitate the partitioning of the drug in the lipoidal membrane and into the systemic circulation. Hence, a perfect hydrophilic-lipophilic balance (HLB) should be there in the structure of the drug for optimum bioavailability.

1.2. Patient related factors

1.2.1. Age

In infants, the gastric pH is high and intestinal surface and blood flow to the GIT is low resulting in related absorption pattern in comparison to adults. In elderly persons, causes of impaired drug absorption include altered gastric emptying, decreased intestinal surface area and GI blood flow.

1.2.2. Gastric emptying

Apart from dissolution of a drug and its permeation through the biomembrane, the passage from stomach to the small intestine, called as gastric emptying can also be rate limiting step in drug absorption because the major site of drug absorption is intestine. Thus generally speaking, rapid gastric emptying increases bioavailability of a drug.

Rapid gastric emptying is desired where:

1. A rapid onset of action is desired e.g. sedatives
2. Dissolution of drug occurs in the intestine e.g. enteric coated dosage forms
3. The drugs are not stable in the gastric fluids e.g. penicillin G, and erythromycin
4. The drugs is best absorbed from the distal part of the small intestine e.g. vitamin $B_12$

1.2.3. Intestinal Transit

Since small intestine is the major site for absorption of most drugs, long intestinal transit time is desirable for complete drug absorption. The residence time depends upon the intestinal motility or contractions. The mixing movement of the intestine that occurs due to peristaltic contractions promote drug absorption, firstly, by increasing the drug-intestinal membrane contact, and secondly, by enhancing the drug dissolution especially of poorly soluble drugs, through induced agitation.
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1.2.4. Blood flow to the GIT

GIT is extensively supplied by blood capillary network and the lymphatic system. The absorbed drug can thus be taken by the blood or the lymph. Since the blood flow rate to the GIT (splanchnic circulation) is 500 to 1000 times (28% of cardiac output) more than the lymph flow, most drugs reach the systemic circulation via blood whereas only a few drugs, especially low molecular weight, lipid soluble compounds are removed by lymphatic system. The high perfusion rate of GIT ensures that once the drug has crossed the membrane, it is rapidly removed from the absorption site thus maintaining the sink conditions and concentration gradient for continued drug absorption.

2. Bioequivalence

Bioequivalence is a relative term. It is defined as the absence of significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose and under similar conditions in an appropriately designed study (CDER, 2003). In bioequivalence studies, the primary question is to compare measures of release of drug substance between the test and reference product. Hence bioequivalence is primarily a product quality question. Because product BA and BE are closely related, similar approaches for establishing BA and BE may be followed. Some of the approaches are as discussed further on.

2.1. Historical perspective of BE

Showing their mistrust in the profiteering tactics of the pharmaceutical industry, the society, by law, has removed much of the decisions making about new drug products from the manufacturers, investigators, and physicians and vested it in the government (the Drug Regulatory Agencies). The Drug Regulations require the Regulatory Agencies to assess safety, efficacy and quality of all new drug formulations, before they are marketed. The fundamental mission of the Drug Regulatory Agencies is protection of the consumers. The historical milestones of drug law are summarized in Table 2.
Table 2: Chronology of events that shaped our regulatory scenario (Truman, 1992, updated).

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
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<tbody>
<tr>
<td>1902</td>
<td>Biologies Control Act</td>
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<td>1906</td>
<td>Pure Food and Drugs Act</td>
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<td>1912</td>
<td>Shirley Amendment to Pure Food and Drugs Act</td>
</tr>
<tr>
<td>1938</td>
<td>Elixir Sulfanilamide Disaster. FDA control over safety of new drugs</td>
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<tr>
<td>1948</td>
<td>Miller Amendment</td>
</tr>
<tr>
<td>1951</td>
<td>Durham-Humphrey Amendments</td>
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<tr>
<td>1952</td>
<td>Hench: Brand substitution case report</td>
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<tr>
<td>1962</td>
<td>Thalidomide disaster in Europe FDA; Control over both safety and efficacy of drugs-Kefauver-Harris amendment</td>
</tr>
<tr>
<td>1963</td>
<td>Initial Good Manufacturing Practices (GMP) regulations</td>
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<tr>
<td>1974</td>
<td>World Health organization, recommendations for conduct of bioavailability studies</td>
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<tr>
<td>1974</td>
<td>Dissolution test adopted as standard for \textit{in vitro} comparison of bioavailability in UK</td>
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<tr>
<td>1977</td>
<td>US FDA regulations for approval of BE. The $\pm 20%$ rule with $p&lt;0.05$</td>
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<tr>
<td>1983</td>
<td>Orphan drug act</td>
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<tr>
<td>1984</td>
<td>ANDA for generics approval-Waxman-Hatch act (Drug price competition and patent term restoration act)</td>
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<tr>
<td>1985</td>
<td>New 80-125$%$ for CI law for approval of generic products</td>
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<tr>
<td>1987</td>
<td>Standard 2x2 crossover test design for BE studies</td>
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<td>1989</td>
<td>Generics scandal in USA. Concern for adequate documentation and validation of BE studies</td>
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<td>1992</td>
<td>90-111$%$ CI for narrow therapeutic index drugs: Canadian FDA</td>
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<tr>
<td>1995</td>
<td>EEC-70-143$%$ limit for Cmax only for drug with wide safety margin</td>
</tr>
<tr>
<td>1999</td>
<td>Draft regulations for BE studies: In India</td>
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</table>
2.2. BE for first entry products
BE studies may be useful during drug development and registration for a first entry product during the Investigational New Drug (IND) or New Drug Application (NDA) period to establish links between (i) early and late clinical trial formulations (ii) formulations used in clinical trial and stability studies, if different (iii) Clinical trial formulations and to be marketed drug products (iv) other comparisons as appropriate. In each comparison, the new formulation or new method of manufacture is the test product and the prior formulation or method of manufacture is the reference product.

2.3. BE for interchangeable multi-source products
BE studies are a critical component of Abbreviated New Drug Applications (ANDA). The purpose of these studies is to compare relative BA measures between a pharmaceutically equivalent multi-source test product and the corresponding reference pioneer product. The innovator product is termed as reference listed drug (RLD). Together with the determination of pharmaceutical equivalence, demonstrating BE allows a regulatory conclusion of therapeutic equivalence and interchangeability between the test and reference product (CDER, 1999).

2.4. BE for post approval changes
Generally specifications are adequate to assure product quality on the assumption that no important change occurs post-approval. In the presence of major changes in components and composition, and/or method of manufacture of a drug product after approval, BE may need to be re-demonstrated. For approved first-entry products, the drug product after the change should be compared to the drug product before change. For approved interchangeable multi-source products, the drug product after the change should be compared to the reference listed drug.

2.5. Types of bioavailability
Bioavailability can be classified into four different types (Ritschel, 1999), depending on the purpose of the study and scientific questions to be solved.

2.5.1. Absolute bioavailability
Absolute bioavailability is the ratio of the total area under the blood level time curve upon extra vascular route of administration to the area under the blood level time curve upon intravenous administration, corrected for the difference in the dose size.

Absolute bioavailability = \( \frac{\text{AUC}_{\text{extravascular}} \times \text{dose}_{\text{extravascular}}}{\text{AUC}_{\text{i.v.}} \times \text{dose}_{\text{extravascular}}} \)
2.5.2. Relative bioavailability

The relative bioavailability is the extent (EBA) and rate (RBA) of the bioavailability of a drug from two or more different dosage forms given by the same route of administration. For determination of EBA or RBA blood level or urinary excretion data upon single or multiple dosing can be used. According to the FDA regulation the standard used in this procedure is an approved marketed drug product, a solution of the drug or suspension of the micronized drug.

Relative bioavailability = \( \frac{\text{AUC of } A}{\text{AUC of } B} \)

Where B is the reference standard.

2.5.3. Bioavailability in presence of first-pass effect

Drugs showing a first-pass effect may result in considerably lower blood level time curves. Even though the entire parent drug was absorbed from the site of administration, it does not reach systemic circulation in unchanged form.

The fraction of a peroral (po) or in part, rectal dose reaching systemic circulation \( F \), under the assumption of other wise linear kinetics can be described by eqn.

\[
F = 1 - \frac{\text{Dose}_{iv} \times f_m \times \text{LBF} \times \text{AUC}_{iv} \times 60 \times \lambda}{\text{Dose}_{po} \times \text{X}}
\]

\( f_m \) - fraction of drug metabolised in liver
\( \text{LBF} \) - liver blood flow
\( \lambda \) - ratio of the concentration of the drug in whole blood to that in plasma

2.5.4. Relative optimal bioavailability

This term was suggested for optimizing extent and rate of bioavailability for a drug product during the development phase.

For determination of EBA rel. opt., the active drug is administered in aqueous solution without the addition of any further excipient by the same route which is intended for the drug product under development

\[
\text{EBA}_{\text{rel. opt.}} = \frac{\text{AUC}_{\text{(drug + vehicle; granules; tablets)}}}{\text{AUC}_{\text{solution}}} \times 100
\]

2.6. Different approaches used for measurement of bioavailability

There are several direct and indirect methods for the measurement of bioavailability in humans. The selection of method depends on the purpose of the study, analytical method and nature of the drug product. The methods useful in quantitative evaluation of bioavailability can be broadly divided into two categories: (a) Pharmacokinetic methods (b) Pharmacodynamic methods
2.6.1. Pharmacokinetic Methods
These are very widely used and are based on the assumption that the pharmacokinetic profile reflects the therapeutic effectiveness of a drug. Thus these are indirect methods. The two major pharmacokinetic methods are;

2.6.1.1. Plasma level-time studies
Unless determination of plasma drug concentration is difficult or impossible, it is the most reliable method and method of choice in comparison to urine data. This method is based on the assumption that two dosage forms that exhibit superimposable plasma level-time profiles in a group of subjects should result in identical therapeutic activity. The three parameters of plasma level-time studies, which are considered important for determining bioavailability, are:

1. \( C_{\text{max}} \): The peak plasma concentration that gives an indication whether the drug is sufficiently absorbed systemically to provide a therapeutic response.
2. \( t_{\text{max}} \): The time of peak plasma concentration corresponds to the time required to reach maximum drug concentration after drug administration. At \( t_{\text{max}} \), absorption is maximized and the rate of drug absorption equals the rate of drug elimination. When comparing drug products, \( t_{\text{max}} \) can be used as an approximate indication of the drug absorption rate.
3. \( \text{AUC} \): The area under the plasma level-time curve that gives a measure of the extent of absorption or the amount of drug that reaches the systemic circulation.

The extent of bioavailability can be determined by eqn.

\[ F = \frac{\text{AUC}_{\text{oral}} \cdot D_{\text{iv}}}{\text{AUC}_{\text{iv}} \cdot D_{\text{oral}}} \]

2.6.1.2. Urinary excretion studies
This method of assessing bioavailability is based on the principle that the urinary excretion of unchanged drug is directly proportional to the plasma concentration of drug. This method is particularly useful for drugs extensively excreted unchanged in the urine. The method involves collection of urine at regular intervals for a time span equal to 7 biological half-lives, analysis of unchanged drug in the collected sample and determination of the amount of drug excreted in each interval and cumulative amount excreted. The three major parameters examined in urinary excretion data obtained with a single dose study are:

1. \( (dx/dt)_{\text{max}} \): The maximum urinary excretion rate, is obtained from the peak of plot between rate of excretion versus midpoint time of urine collection period. It is
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analogous to $C_{\text{max}}$ derived from plasma level studies since the rate of appearance of drug in the urine is proportional to its concentration systemic circulation.

2. $(t_{\text{max}})$: The time for maximum excretion rate, is analogous to the $t_{\text{max}}$ of plasma level data. Its value decreases as the absorption rate increases.

3. $X_{\text{ur}}$: The cumulative amount of drug excreted in the urine, is related to the AUC of plasma level data and increases as the extent of absorption increases.

The extent of bioavailability can be calculated using eqn:

$$F = \frac{(X_u)_{\text{oral}}}{(X_u)_{\text{iv}}} \cdot \frac{D_{\text{oral}}}{D_{\text{oral}}}$$

2.6.2. Pharmacodynamic Methods

These methods are complimentary to pharmacokinetic approaches and involve direct measurement of drug effect on a physiologic process as a function of time. The two pharmacodynamic methods involve determination of bioavailability from: (a) Acute pharmacologic response (b) Therapeutic response

2.6.2.1. Acute pharmacologic response

In some cases quantitative measurement of a drug is difficult, inaccurate or non reproducible. In such cases an acute pharmacologic effect such as effect on pupil diameter, heart rate or blood pressure can be a useful index of drug bioavailability. Bioavailability can be determined by construction of pharmacologic effect-time curve as well as dose-response graphs. The method requires measurement of responses for at least 3 biological half-lives of drug in order to obtain a good estimate of AUC.

2.6.2.2. Therapeutic response

Theoretically the most definite, this method is based on observing the clinical response to a drug formulation given to patients suffering from disease for which it is intended to be used. Bioequivalent drug products should have the same systemic drug bioavailability and therefore the same predictable drug response. However variable clinical responses among individuals that are unrelated to bioavailability might be due to differences in the pharmacodynamics of the drug. Various factors affecting pharmacodynamic drug behaviour may include age, drug tolerance, drug interactions and unknown pathophysiologic factors.
2.6.3. In vitro Methods

Under certain circumstances, product quality BA and BE can be documented using in vitro approaches. For highly soluble, highly permeable, rapidly dissolving, orally administered drug products, documentation of BE using an in vitro approach (dissolution studies) is appropriate based on the biopharmaceutics classification system (BCS) (CDER®, 2000). The preferred dissolution apparatus is USP apparatus I (basket) or II (paddle), used at compendially recognized rotation speeds (e.g., 100 rpm for the basket and 50-75 rpm for the paddle). In other cases, the dissolution properties of some ER formulations may be determined with USP apparatus III (reciprocating cylinder) or IV (flow through cell).

2.7. Population and Individual bioequivalence

The bioequivalence study in current use, so called average bioequivalence approach, judges bioequivalence between the test formulation and reference formulation by verifying that the confidence interval for the ratio of average bioavailability values of the 2 formulations is in a given acceptance range. However the average bioequivalence approach has been indicated to be insufficient to warrant bioequivalence of the test formulation and the reference formulation, since it compares the average bioavailability values of the test and the reference formulations and does not consider differences in variance of test and reference formulation (Nakai et al, 2002). Due to these concerns raised over the years, on the use of average bioequivalence for evaluation of comparability between formulations, scientists from academia, industry and regulatory agencies, propose the use of concepts of individual and population bioequivalence (Chen et al, 2000). The FDA also has proposed replacing the 1992 average bioequivalence (ABE) approach with population and individual bioequivalence (PBE and IBE) (CDER®, 1997).

2.8.1. Individual bioequivalence

In the IBE criterion, replicate designs are required, in which at least the R, and commonly both R and T drug products, are each administered on two separate occasions. The individual criteria may be utilized for equivalence questions when some change occurs in a stable dosage form. Examples include substitution of a generic for a pioneer product and, for both a pioneer and interchangeable equivalent, when re-documentation of BE is needed in the presence of specified post-approval changes in component/composition and/or method of manufacture. A regulatory objective is to encourage bioequivalent formulations over an extended period of time that clearly relate, in terms of performance, to the pivotal clinical trial material on which safety and efficacy were based. The proposed new criteria include variance as well as mean terms (Williams et al,
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2000). The variance term for population BE is total variance, which is the sum of between and within-subject variances. For individual BE, a subject-by-formulation (S*F) interaction variance, and within-subject variance for both T and R products are estimated. Both PBE and IBE criteria allow scaling of the BE limit (goalpost) by R product variability.

A key concept underlying IBE criterion relates to the term **switchability**, which denotes the situation where a patient currently on one formulation switches to another with the expectation that the safety and efficacy of the drug will remain essentially unchanged. The criterion uses, in the aggregate, a distance concept that compares means and variances of T and R products. By expanding the variance terms, the proposed criterion offers many consumer and producer advantages, including: (i) assurance of switchability; (ii) rewards for reduction of variance in the T product; (iii) scaling for highly variable and/or narrow therapeutic range drugs.

The IBE criterion encourages BE studies in subjects more representative of the general population or even in patients for whom the drug is intended, as opposed to healthy young males where detection of S*F interaction is less likely. This feature addresses a frequently expressed concern that BE studies in healthy young males lack clinical relevance (Levy¹, 1995). The re-test characteristics of replicate study design allow scrutiny of outliers.

IBE can be calculated as:

\[
\sigma_D^2 = [(\sigma_{BT}^2 - \sigma_{BR}^2)^2 + 2(1-\sigma) \sigma_{BT} \sigma_{BR}]
\]

\[\theta_I = [(\mu_T - \mu_R)^2 + \sigma_D^2 + \sigma_{WT}^2 - \sigma_{WR}^2] / \sigma_{WR}^2 \text{ when } \sigma_{WR} > 0.2\]

\[\theta_I = [(\mu_T - \mu_R)^2 + \sigma_D^2 + \sigma_{WT}^2 - \sigma_{WR}^2] / 0.2^2 \text{ when } \sigma_{WR} \leq 0.2\]

Where,

\[\mu_T = \text{mean (test)}\]
\[\mu_R = \text{mean (reference)}\]
\[\sigma_{WT}^2 = \text{within subject variance (test)}\]
\[\sigma_{WR}^2 = \text{within subject variance (reference)}\]
\[\sigma_{BT}^2 = \text{between subject variance (test)}\]
\[\sigma_{BR}^2 = \text{between subject variance (reference)}\]

Again, \(\sigma_{WR}^2\) is set to 0.20 (that is, constant scaled versus reference standard) in the denominator of the formula \(\theta_I\) when the point estimate of the parameter based on the original data set falls below 0.20 (CDER¹, 1997).
Individual BE is demonstrated when; $\theta_1 (0.95) < 2.45$, where $\theta_p (0.95)$ is defined as the 95$^{th}$ quartile of $\theta_1$ based on the non-parametric percentile method using 2000 bootstrap samples. The bootstrap is used, as the exact distribution for the parameter $\theta_p$ has not yet been derived.

2.8.2. Population bioequivalence

Population bioequivalence approach, which evaluates the total bioavailability variances in addition to the average bioavailability values, has been proposed as a method to overcome the disadvantages of average bioequivalence approach (Hauschke and Steinijans, 2000). FDA has also proposed the use of population bioequivalence as a bioequivalence study which might guarantee prescribability and which is applicable in the development stages of novel drugs (CDER, 1997). Based on earlier published reports of bioequivalence in literature, it was concluded that population bioequivalence value was affected more extensively by the bioavailability variance rather than by the average bioavailability (Nakai et al, 2002). PBE criteria aggregate the difference between the population means and variances. Both IBE and PBE criteria allow for scaling of the regulatory limits based on the variability of the reference product. Both require the use of boot strapping methodology to derive empirical distributions of the criteria, as the exact statistical distribution has not yet been established.

The key motivation behind the proposed changes in BE criteria lie in answering more appropriate questions regarding bioequivalence. In the case of pre-marketing approval, one can formulate the bioequivalence question as "Can a patient begin their therapy with either formulation (commercial or clinical trial) and be assured of same results in terms of safety and efficacy?" This has been called the concept of prescribability (CDER, 1997) and is linked to PBE criteria. In case of post-marketing changes, the BE question becomes "Can I safely and effectively switch my patient from their current formulation to another?" This has been called the concept on switchability and is linked to the IBE criteria. PBE can be calculated as:

$\theta_p = [(\mu_T - \mu_R)^2 + \sigma^2_{TT} - \sigma^2_{TR}] / \sigma^2_{TR}$ when $\sigma_{TR} > 0.2$

$\theta_p = [(\mu_T - \mu_R)^2 + \sigma^2_{TT} - 0.2^2 \sigma^2_{TR}] / 0.2^2$ when $\sigma_{TR} \leq 0.2$

Where,

$\mu_T$ = mean (test)

$\mu_R$ = mean (reference)

$\sigma^2_{TT}$ = total variance (test)

$\sigma^2_{TR}$ = total variance (reference)
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θ₀ is calculated in one of the two ways depending on the point estimate for σ²TR based on the original data set. When this estimate falls below 0.20, a constant scaling procedure is used. Otherwise, the scaling is proportional to σ²TR. This has been referred to as 'constant scaled' and 'reference scaled' respectively (CDER, 1997).

Population BE is demonstrated when; θ₀ (0.95) < 1.75, where θ₀ (0.95) is defined as the 95th quantile of θ₀ based on the non-parametric percentile method using 2000 bootstrap samples. The bootstrap is used, as the exact distribution for the parameter θ₀ has not yet been derived.

2.9. Design and evaluation of BE study

The preferred approach is an in vivo study carried out in healthy volunteers to whom the 2 preparations (generic and innovator) are alternatively administered. The design and evaluation of well controlled bioequivalence studies require the cooperative input from pharmacokineticists, statisticians, clinicians, bio-analytical chemists, and others.

2.9.1. Design

The design of a bioavailability and/or bioequivalence study is dependent upon the drug, dosage form and study objectives. For BE studies, both the test and reference drug formulations contain the pharmaceutical equivalent drug in the same dose and are given by the same route of administration. A pilot study in small number of subjects can be carried out before proceeding with a full BE study. This study can be used to validate analytical methodology, assess variability, optimize sample collection time intervals or provide any other information. Non replicate crossover study designs are recommended by FDA (CDER, 2003) for immediate release and modified release dosage forms. However replicate designs can also be used. The recommended method for analysis to establish BE is average bioequivalence. The study should be of crossover designs and suitably randomized as far as possible. Some of the designs are discussed below

2.9.1.1. Two-Period Crossover Design

In case of two formulations, an even number of subjects should be randomly divided into two equal groups. In the first period, each member of one group will receive a single dose of the test formulation and each member of the other group will receive standard formulation. After a suitable washout period (generally 5 half lives), in the second period, each member of the
The design can be depicted as follows:

<table>
<thead>
<tr>
<th>Vol. No.</th>
<th>Period 1</th>
<th>Period 2</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
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<td>6</td>
<td>B</td>
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</tbody>
</table>

2.9.1.2. Latin Square Design
In case of more than two formulations, a latin square design should be used. For example in a bioequivalence study of 3 formulations, a group of volunteers will receive formulations in the sequence shown below:

<table>
<thead>
<tr>
<th>Vol. No.</th>
<th>Period 1</th>
<th>Period 2</th>
<th>Period 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>A</td>
<td>B</td>
</tr>
</tbody>
</table>

The next group of 3 volunteers will receive formulations in the same sequence as shown above.

2.9.1.3. Balance Incomplete Block Design (BIBD)
In case there are more than 3 formulations, the Latin square design will not be ethically advisable, mainly because each volunteer may require the drawing of too many blood samples. However, if each volunteer is expected to receive at least two formulations, then such a study can be carried out using Balanced Incomplete Block Design. As per this design, if there are four formulations, six possible pairs or formulations can be chosen from four formulations. Then, the first 6 volunteers will receive these six pairs of formulations and the next six volunteers will receive the same six pairs in reverse order. The design is depicted below:
The minimum acceptable number of volunteers will be 12.

\[ n > \left( \frac{\left[\sigma^2\right]}{2D^2} \left[ t_{\alpha} + t_{\beta} \right]^2 + 0.25t^2 \right) \]

Where,

- \( n \) = no. of volunteers
- \( \alpha \) = Required level of significance (0.05)
- \( \beta \) = Required power of test (0.80)
- \( \sigma^2 \) = Error mean sum of squares from ANOVA (estimated/guess)
- \( D \) = Minimum difference between the means which if present, ought to be detected

The bioequivalence studies are conducted according to a well-defined protocol.

Some elements of a bio-equivalence protocol are listed in Table 3:

Table 3: Elements of a Bioavailability Protocol

<table>
<thead>
<tr>
<th>INVESTIGATORS' DECLARATION</th>
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<td>2.1 Clinical Services &amp; Clinical Laboratory</td>
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<tr>
<td>2.2. Analytical, Pharmacokinetics &amp; Statistical Services</td>
</tr>
<tr>
<td>OBJECTIVE</td>
</tr>
<tr>
<td>PRODUCTS TO BE EVALUATED</td>
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</table>
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<table>
<thead>
<tr>
<th>4.1 REFERENCE (R)</th>
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<tr>
<td>4.2 TEST (A)</td>
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<td>4.3 TEST (B)</td>
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</table>

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**PHARMACOLOGY**

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<td>6.3 Dosage</td>
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**STUDY DESIGN**

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<td>7.2 Number of Subjects</td>
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<td>7.3 Admissions and Stay</td>
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<td>7.5 Sampling Schedule</td>
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<td>7.7 Washout Period</td>
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**REstrictions**

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<td>11.2 Dose</td>
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<td>11.3 Assignment to Treatment Sequences</td>
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**Haemodynamic Measurements**

**Pharmacokinetics**

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<th>13.1 Blood Sampling</th>
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<tr>
<td>13.2 Analytical Procedures</td>
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<tr>
<td>13.3 Pharmacokinetic Parameters</td>
</tr>
</tbody>
</table>
2.9.2. Statistical issues in BE studies

The pharmacokinetic parameters, $C_{\text{max}}$, $T_{\text{max}}$, and AUC should be subjected to a three-way analysis of variance (3-way ANOVA) in order to test differences due to formulations, period and subjects. A more complex ANOVA may be appropriate in some circumstances, e.g. if treatments are replicated. The standard parametric ANOVA assumes homogeneity of variances, normality and additivity of independent variables.

In order to ensure homogeneity of variances between treatments, Bartlett's test or a similar test should be carried out prior to performing the ANOVA (CDSCO 2005). The primary comparison of interest in a bioequivalence study is the ratio of average parameter data (AUC or $C_{\text{max}}$) from the test and reference formulations rather than the difference between them. Log transformation of the data allows the general linear statistical model to draw inferences about the ratio of the two
averages on the original scale. Log transformation thus achieves the general comparison based on the ratio rather than on the difference.

Moreover, plasma concentration data, including AUC and $C_{max}$ tend to be skewed and their variances tend to increase with the means. Log transformation corrects this situation and makes the variances independent of the mean.

Further, the frequency distribution skewed to the left, i.e., those with a log tail to the right is made symmetrical by log transformation.

In case no suitable transformation is available, the non-parametric method should be used. $T_{max}$ values being discrete, data on $T_{max}$ should be analysed using non-parametric methods.

2.9.3 Two one-sided tests procedures (TOST):

This procedure is also referred to as confidence interval approach. This method is used to demonstrate if the bioavailability of the drug from the test formulation is too high or low in comparison to the reference drug product. The 90% confidence limits are estimated for the sample means. In this test, presently required by the FDA, a 90% confidence interval about the ratio of means of the two drug products must be within $\pm 20\%$ for measurement of the rate and extent of drug bioavailability. The lower 90% CI for the ratio of means cannot be less than 0.8, and the upper 90% CI for the ratio of the means cannot be greater than 1.20. The 90% CI is a function of sample size and study variability, including inter and intra subject variability (CDER, 2003).

Current DCGI requirements for bio-equivalence approval is that 90% confidence interval should be within 80-125% for log transformed AUC and 70-143% for log transformed $C_{max}$ provided that the drug is safe otherwise 80-125% will be applicable.

The T/R ratio should be as close as possible to 95-105%. Intra subject CV should be as low as possible (<15%). Table 4 mentions the bioequivalence criteria followed by various regulatory agencies in the world.
**Review of Literature**

**Table 4: Criteria of bio-equivalence of various regulatory agencies**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CPMP (EU)</th>
<th>USFDA</th>
<th>CANADIAN FDA (CEC)</th>
<th>DCGI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log transformed AUC₀-₂₄ using 90% CI</td>
<td>-80-125% of reference for Narrow therapeutic index (NTI)* drugs.</td>
<td>-80-125% of reference for NTI drugs</td>
<td>-80-125% of reference for NTI drugs</td>
<td>-80-125% of reference for NTI drugs</td>
</tr>
<tr>
<td>Log transformed Cₜₘₐₓ using 90% CI</td>
<td>-80-125% of reference (NTI drugs) -70-143% of reference (if clinically acceptable)</td>
<td>-80-125% of reference (NTI drugs) -70-143% of reference (if clinically acceptable)</td>
<td>-80-125% of reference (NTI drugs) -70-143% of reference (if clinically acceptable)</td>
<td>-80-125% of reference (NTI drugs) -70-143% of reference (if clinically acceptable)</td>
</tr>
</tbody>
</table>

*NTI- Narrow Therapeutic Index*
PHENYTOIN

Phenytoin has been in clinical use for the better part of a century as an established and effective anti-epileptic agent. It has also occasionally been used as for cardiac arrhythmias, tic douloresux, certain types of central pain and some varieties of migraine.

3.1. Phenytoin - Chemistry

Phenytoin is a white crystalline bitter-tasting powder with a molecular weight of 252.26 Daltons. It is sparingly soluble in water and almost insoluble in acids, but dissolves in aqueous bases and in organic solvents such as ethanol, acetone and chloroform. It is a weak acid with a pKa value at first believed to be 9.2 (Dill et al, 1956), but later reported to be 8.3 (Agarwal and Blake, 1968) or 8.06 (Schwartz et al, 1977). Phenytoin is commonly administered orally in the form of its more water soluble sodium salt. In such cases, one has to be careful about making allowance for the difference in molecular weights between acid phenytoin (M. W. 252.26 Dal) and sodium phenytoin (M. W. 274.25 Dal) when a change is brand is being realized.

3.2. Mechanism of action

Phenytoin acts by limiting the repetitive firing of action potentials evoked by a sustained depolarization of spinal cord neurons. The effect is mediated by a slowing of the rate of recovery of voltage-activated Na⁺ channels from inactivation. These effects are evident at concentrations in the range of therapeutic levels in cerebrospinal fluid in humans, concentrations that correlated well with the free concentration of drug in the serum.

3.3. Pharmacological effects

3.3.1. Central Nervous System: Phenytoin exerts antiseizure effects without general depression of the CNS. At toxic levels, it may produce excitatory signs and at lethal levels a type of decerebrate rigidity. The most significant effect of phenytoin is its ability to modify the pattern of maximal electroshock seizures. In fact, phenytoin has been found to suppress any type of hyper-excitability, whether induced by electrical, calcium withdrawal, oxygen withdrawal or by poisons. The characteristic tonic phase can be abolished completely, but the residual clonic phase may be exaggerated and prolonged. Further, in concentrations that affect abnormal cell functions, it does not affect normal cell function. It also has the ability to modify post-tetanic discharge. This may account for Phenytoin’s therapeutic effect on persistent and repetitive thinking and on unnecessary repetitive thinking.

Phenytoin is known to bind to specific receptors in the brain. There is known to be an interaction between the binding site for phenytoin and the GABA-receptor-Cl⁻ ionophore.
Review of Literature

- benzodiazepine receptor complex (Spero, 1982). It is also known that chronic phenytoin intake increases the number of benzodiazepine binding sites in the rat brain (Gallagher et al, 1980) and that phenytoin competitively inhibits both the binding of diazepam to brain benzodiazepine receptors (Tunnicliff et al, 1979) and the binding of certain ligands to membrane Na⁺ ion channels (Willow and Caterall, 1982, Willow et al, 1985). At therapeutically relevant concentrations, phenytoin also binds to Ca²⁺ ion channels in brain membrane (Greenberg et al, 1984). All of these actions contribute to the anti-convulsant effect of the drug.

3.3.2. Cardiac Effects: Phenytoin has been classified as a Subclass IB antiarrhythmic drug according to Vaughn Williams system of classification due to its capability to block sodium channels. But due to very low potency, it produces little if any change in action potential duration (no effect on QRS interval) in normal tissue, and shorten repolarization (decrease QT interval).

3.3.3. Cutaneous Effects: The observation that use of phenytoin triggers swelling of gums led to further research into the cutaneous effects of phenytoin. Researchers have investigated the effects of phenytoin on a cellular level. Phenytoin inhibits collagenase in-vitro (Eisenberg et al, 1978). Phenytoin modulates connective tissue metabolism and cell proliferation in human skin fibroblast cultures (Moy et al, 1985). Patients with epidermolysis bullosa treated with phenytoin had lower levels of inflammatory mediators such as arachidonic acid in plasma and erythrocyte phospholipids than did untreated epidermolysis bullosa patients (Cunnane et al, 1987). Phenytoin increases gene expression of the platelet-derived growth factor B chain in macrophages and monocytes (Dillb et al, 1993). Healthy granulation tissue appears earlier with phenytoin than with conventional saline dressings (Pendse et al, 1993). Phenytoin may promote wound healing through multiple mechanisms, including stimulation of fibroblast proliferation, facilitation of collagen deposition, glucocorticoid antagonism, and antibacterial activity (Anstead et al, 1996).

3.3.4. Immune function and Phenytoin: Phenytoin has complex effects on the immune system. Phenytoin suppresses cytotoxic activities of cells such as natural killer (NK) cells and cytotoxic T lymphocytes (CTL), but not lymphokine-activated killer (LAK) cells (Okamoto et al, 1989). Phenytoin treatment preferentially induces a Th2-type response (Okada et al, 2001). Phenytoin also significantly depresses interferon augmentation of NK cell cytotoxicity in a dose-dependent manner (Margetten et al, 1987). Phenytoin suppresses the production of cortisol.
Specifically, Phenytoin induces the liver cytochrome P450 enzyme system and stimulates steroid clearance (Putignano et al, 1998). It can also induce adrenal suppression. These effects may partially explain the side effects of Phenytoin, which are forms of immune dysfunction.

3.3.5. Endocrine effects and neurotransmitters: Phenytoin inhibits Anti-Diuretic Hormone (ADH) release in patients with inappropriate ADH secretion. It also inhibits insulin release. It also inhibits gastrointestinal absorption of Ca\(^{2+}\) and alters metabolism of Vit D. It also causes increased metabolism of Vit K and reduces concentration of Vit K dependent proteins that are important for normal Ca\(^{2+}\) metabolism in bone. Phenytoin also affects thyroid function. It increases thyroid function by reducing thyroid hormone concentration directly by inducing UDP-Glucuronyl Transferases (UGTs).

a. GABA: Many studies have been carried out to study effect of phenytoin on GABA. It has been suggested that phenytoin may increase concentrations of the inhibitory neurotransmitter Gamma- Amino Butyric Acid (GABA) in certain brain regions (Vernadakis and Woodbury, 1965, Saad et al 1972). It also reduces the brain concentrations of glutamate, the metabolic precursor of GABA (Mori, 1974).

b. Acetylcholine: Phenytoin is known to alter Acetylcholine (ACH) levels, an excitatory neurotransmitter in brain (Woodbury 1969). Low drug doses increase, and high doses or chronic administration, decrease the release of ACH.

c. Serotonin: it is known that raising Serotonin levels corresponds with protection against epileptic seizures. Phenytoin raises brain Serotonin levels, probably because the drug increases Serotonin synthesis (Green, Graeme and Smith, 1975).

3.3.6. Effect of Lipid Metabolism: Phenytoin increases HDL-C levels (Reddy et al, 1985).

3.3.7. Effect on folate metabolism: Phenytoin is well known for its propensity to cause folate depletion (Klipstein 1964, Reynolds et al 1975). It probably does so by interfering with absorption of dietary folate (Hoffbrand and Necheles 1968; Rosenberg et al 1968; Ariel et al 1982).

Thus, the effects of phenytoin on several second messenger systems, such as the cyclic nucleotides and calcium systems, might explain the widespread action of this compound on numerous cells and physiologic functions.
3.4. Pharmacokinetics
Phenytoin is an extensively studied molecule with respect to its pharmacokinetics. The pharmacokinetics of phenytoin is markedly affected by its binding to serum proteins, the non-linearity of its elimination kinetics, and by its metabolism by the CYP450 enzymes. Phenytoin is one of the few drugs for which the rate of elimination varies as a function of its concentration (Non-Linear Pharmacokinetics). The plasma half-life of phenytoin ranges between 6 and 24 hours at plasma concentrations below 10 μg/ml, but increases disproportionately as dosage is increased even with small adjustments for levels near the therapeutic range. This also implies that half-life and clearance of the drug is a function of the serum concentration. The time required to steady state varies with individual Kd and Vmax values.
Some of the key experiments to determine pharmacokinetic profile of phenytoin were carried out in the 1970s.

3.4.1. Dosage Regimen (Product Information, Dilantin Kapseals)
❖ Adults:

Loading dose:
❖ Oral loading regimen
   • Dose 1: 400mg
   • Dose 2: 300 mg at 2hrs post dose 1
   • Dose 3: 300 mg at 4 hrs post dose 1

Maintenance Dose:
   • Initial: 5mg/kg to 300 mg/day divided qd to tid.

Titrating dose:
   • Avoid increasing dose by >25 to 30 mg per trial
   • Wait for steady state (7 days) before level check.

3.4.2. Onset and Duration:
Initial response:
   • Seizure disorder, oral without a loading dose: 7 to 10 days
   • Seizure disorder, oral with a 1 gm loading dose: 8 to 12 hours
   • Seizure disorder, intravenous with a 1 gram loading dose: immediate onset

3.4.3. Drug Concentration Levels:
Therapeutic Drug Concentration:
   • Seizure disorder, 10 to 20 μg/ml
Review of Literature

- Total concentrations of 6 to 14 μg/ml may be more appropriate in the neonate since they have a higher unbound fraction.
- The clinical effectiveness of phenytoin were reported to correlate better with unbound drug as compared to total plasma concentrations, with usual unbound drug concentrations associated with optimal therapy being 1 to 2 μg/ml.

3.4.4. Time to Peak Concentration:

- Oral: Immediate release Phenytoin: 1.5 to 3 hrs
- Extended Release Phenytoin Sodium Capsules: 4 to 12 hrs
- Intravenous with loading dose: 20 to 25 minutes

3.5. ADME

3.5.1. Absorption

Bioavailability (F)

1. Oral, Phenytoin Sodium Capsules: 70 to 100%
   a. The bioavailability of Phenytoin varies from different manufacturers from 20% to 90%.
Phenytoin is almost always given orally, though it can be given by intramuscular or intravenous injection as well. Absorption of phenytoin from the alimentary canal is reasonably complete. Reports of inadequate absorption are rare. Orally administered phenytoin probably absorbs mainly at small gut level.

Effect of Dosage Form and Formulation

Significant variations in plasma levels of phenytoin at steady state after multiple oral doses of different dosage forms and commercial products of phenytoin have been reported by many researchers.

Phenytoin is available in both acid and salt form. The two differ with respect to solubility, the salt form being more soluble in water than the acid form, the rationale being that though it is macrocrystalline and precipitates into phenytoin acid in the stomach, it does so in a form that is more readily absorbed (Neuvonen 1979, Lund 1974). The acid form was thought to be absorbed faster, at that time thought to be due to particle size differences only. However, since the speed of absorption is probably not as important as extent of absorption in long term use of the drug, this factor may safely be discounted. Salt form is available as tablet and capsule while acid form is available as tablet and suspension. Kohda et al (1982) showed lower phenytoin absorption from
powder form, and a concomitant decrease in absorption upon increase in dose range in children. A comparison of the tablet and powder acid form in healthy volunteers in 1983 by the same authors confirmed the above results.

The wide variations in phenytoin preparations allows for flexibility of dosage regimens. Any studies have compared generic versions with Dilantin, the innovator's product, and most results showed difference in bioavailability parameters between the innovator and test. Hence change from innovator's product to a generic version warrants careful attention to avoid fluctuations in plasma levels. While bioavailability studies show similar absorption characteristics of tablets and capsules (Smith and Kinkel 1976), they must be interpreted cautiously. Tablets, especially chewable ones give higher plasma levels than capsules. In this case, the scarcely wettable surface of drug crystals are covered with hydrophilic binders, and thus higher dissolution and absorption rate can be expected (Kohda et al 1983).

Also, while acid tablets contain 100% phenytoin, salt tablets contain only 92% drug. Kirshner et al (1983) reported a doubling of plasma levels when the same dose was administered as tablet form and instead of capsule.

Following the Australian Phenytoin Intoxication outbreak in 1968-69, many reports of phenytoin bioavailability studies have been carried out. Investigations in Brisbane revealed that the outbreak occurred due to replacement of Calcium Sulphate Dihydrate (an excipient) with Lactose (Tyrer et al, 1970, Bochner et al, 1972). The drug, when combined with Lactose was much better absorbed than with Calcium Sulphate (Bochner et al 1972). While various manufacturing techniques are able to influence phenytoin bioavailability, the physician has control of only two factors- salt form and dosage form. Further, there are differences in dissolution behaviour between various capsule formulations. A definite bioinequivalence exists between prompt release phenytoin and extended release products (United States Pharmacopeia: national formulary. United States Pharmacopeial Convention 1980). The manufacturers of the only extended release product claimed in a study that 3 * 100mg extended release preparations were bioequivalent to 300mg extended release capsule (Rantinides et al 1990).

Effect of Food

Food helps in speeding up the process of absorption (Melander et al 1979), as is mostly found for poorly soluble drugs.
**Effect of Antacids**

While a general consensus on the effects of antacids on phenytoin is not known, antacids in general have been found to decrease the absorption of Phenotin, putatively by a combination of various mechanisms - chelation of the drug by metal salts, adsorption of phenytoin into the preparation, altered solubility, and accelerated gastrointestinal motility due to laxative effect of antacids. Antacid dose seems to affect bioavailability of phenytoin. Thus, it is generally recommended that antacids and phenytoin must be administered at a gap of about 2-4 hours (O'Brien 1978, Kulshrestha et al 1978).

**Effect of Gender**

Bioavailability differences between treated population groups have been documented. Lower AUC levels were observed in females than males (Meyer et al 2001), and menstrual cycles did not affect bioavailability parameters.

**Kinetics of Absorption**

Since then, many studies have been published that estimated phenytoin bioavailability from different dosage forms. It has been felt that since in most of these studies, extent of bioavailability has been estimated by area under the plasma-level-time curve method, bioavailability differences have probably been magnified. This is because phenytoin does not follow normal first-order kinetics to allow such estimates (Jusko et al, 1976). The same authors compared oral and intravenous routes of bioavailability by both linear and non-linear methods of pharmacokinetic fitting. They found that the linear method underestimated the bioavailability from the preparations. They opined that the use of AUC values to estimate bioavailability is valid only when the dose is below the critical point when profile becomes non-linear. In their opinion, this critical absorption rate constant for phenytoin was $2 \text{day}^{-1}$, which gave a half-life of 8hrs.

In a study to elucidate phenytoin absolute and relative bioavailability, Gugler et al (1977) administered oral and intravenous doses to healthy subjects. The absolute bioavailability was found to be in the range of 58-86%, average of 70%. The $T_{\text{max}}$, although was quite large (2.9 - 8.9hrs), indicating slow absorption from the oral dosage form. Moreover, the dose of 300 mg did not produce the therapeutic concentration above 10μg/ml save in one volunteer. They found a bioavailability of 0.87 and 0.98 with linear and non-linear fit respectively.

Metabolite excretion ratios is a popular method used to assess bioavailability of phenytoin. Where this method could not be done directly, tracer doses of phenytoin were given to directly follow metabolite formation. An alternative method, also recommended by the FDA relatively recently,
to assess bioavailability of phenytoin, is using steady state parameters. This method can be used where pharmacokinetics of the drug is unknown, and also reflects the true clinical use of the drug. Though one fear is that the differences in the amount absorbed from the two preparations may be overestimated when steady state ratios are compared (Jusko et al 1976).

3.5.2. Distribution

Total Protein Binding: 88 to 93%

- Good in brain and placenta

Phenytoin is extensively plasma-protein bound and even small variations in the pH can cause major changes in protein binding capability. In experimental animals, brain phenytoin levels were found to be similar to, or al little higher than, simultaneous phenytoin levels in whole plasma or serum. (Lee and Bass, 1970). In humans, these values have been found to be roughly 1:1 with respect to plasma. Houghton et al (1975) estimated Phenytoin levels in brain tissue, CSF and muscle in patients who had undergone unilateral temporal lobe resection. These patients were receiving any one of the drugs, Phenytoin, Phenobarbitone or Primidone a day prior to surgery. The authors found that only one out of 24 had phenytoin brain levels in the therapeutic range, though brain tissue concentrations correlated well with plasma concentration. The ratio of brain: plasma was found to be 1.04. The authors admit that patients may not have taken the drugs for long enough to reach steady state. These results roughly coincide with those obtained by other workers as well (Sherwin et al, 1973, Vajda et al, 1974).

Drug levels are also similar in neonatal umbilical cord plasma and in maternal plasma (Mirkin et al 1971, Ishizaki et al 1981).

Small variations in the percentage of Phenytoin that is bound dramatically affect the absolute amount of free (active) drug. Some agents, such as Valproate, can compete for binding sites on plasma proteins, hence increased free phenytoin can be observed when valproate is added to therapy. Therefore, measurement of free phenytoin rather than total drug content has been mooted to be a more useful practice in actual clinical situation to elicit better results. However, certain researchers feel that adjustment of dosage according to serum-albumin levels (which take plasma albumin content into account) may be better indicators of clinical status (Tandon et al, 2004, Fedler and Stewart 1999, Mlynarek et al, 1996). Such estimates were suggested to be more useful when free fraction of phenytoin was altered by various states such as hypoalbuminemia (Mlynarek et al 1996), Uremia (Reidenberg et al, 1971), Pregnancy (Perucca and McLemore, 1998) and drugs like NSAIDs (Dasgupta and Timmerman 1996). But the more recent publications advocate...
estimations of free drug levels rather than total drug. Berg et al (1987) felt that knowledge of all three concentrations (total, free and unbound) would be useful in patients whose seizure control was not optimum. Martin et al (1977) found very little interindividual variation in protein binding.

Distribution Kinetics

Volume of Distribution (Vd): 0.5 to 1. L/kg. Vd for the unbound fraction is about 0.5 to 0.64 l/kg.

3.5.3. Elimination

Elimination rate constant

The drug, as discussed earlier, is a Narrow Therapeutic Index Molecule, which means that it complies with any of the following criteria compiled by the FDA:

a. There is less than a 2-fold difference in median lethal dose (LD50) and median effective dose (ED50) values, or

b. There is less than a 2-fold difference in the minimum toxic concentrations and minimum effective concentrations in the blood, and

c. Safe and effective use of the drug products require careful titration and patient monitoring.

The range of plasma concentration that is therapeutically beneficial without eliciting excessive unwanted effects is quite narrow (10 to 20 μg/ml or 40 to 80 μmol/L). This is also the range which shows maximum therapeutic range for the majority of patients (Kutt et al, 1964, Kutt and McDowell, 1968). This relationship, as can be seen, is quite steep; hence the potential for considerable interindividual variation in the plasma concentration achieved with a given dose exists. Regular and accurate methods for therapeutic Drug Monitoring helps to arrive at the optimum daily dose for a patient that elicits minimum toxic effects.

Phenytoin exhibits non-linear kinetics. This type of kinetics is generally demonstrated where a saturable mechanism for metabolism exists. The Michaelis-Menten Equation:

\[
\frac{-dC}{dt} = \frac{V_m}{K_m} C/K_m + C
\]

where,

\[-dC/dt\] rate of decline of drug at time t

\[V_m\] Theoretical maximum rate of the process

\[K_m\] Michaelis constant

It can be seen that determining “C” when \(-dC/dt = \frac{1}{2} (V_m)\) shows that \(K_m\) is in reality the drug concentration at which the rate of the process is equal to one-half of its theoretical maximum rate.

There are two limiting cases of the Michaelis-Menten equation:
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1. If $K_m \gg C$, then (1) reduces to:
   
   \[-\frac{dC}{dt} = \frac{V_m}{K_m} C,\]

   which has the same form as a first order rate equation. This order is seen after intravenous administration in a one-compartment model or in the post-absorptive phase in a multi-compartment model. This implies that the first order rate constant “$K$” will be denoted by $V_m/K_m$ in this case.

2. If $C \gg K_m$, then (1) becomes:

\[-\frac{dC}{dt} = V_m.\]

The rate here is independent of drug concentration and mimics a typical zero-order reaction.

If $-\frac{dC}{dt}$ is plotted as a function of plasma concentration, $-\frac{dC}{dt}$ would increase linearly with concentration, indicating first order kinetics. As the concentration increases further, $-\frac{dC}{dt}$ would increase at a rate less than proportional to concentration, and eventually asymptote at a rate equal to $V_m$ which would be independent of concentration, i.e., zero-order kinetics.

To assess whether a drug follows non-linear pharmacokinetic processes, a series of doses of varying size is administered. If a plot of the resulting plasma concentration divided by the administered dose are superimposable, the drug has linear kinetics over the tested dose range. If the curves are not superimposable, the drug follows non-linear pharmacokinetics.

To determine the apparent in-vivo $K_m$ and $V_m$ values from plasma concentration in the post-absorptive or distributive phase, the rate of change of plasma concentration from one sampling point to next, $\Delta C/\Delta t$ is estimated as a function of the plasma concentration at the mid-point of the sampling interval. The data are usually plotted according to one of the linearised forms of the Michaelis-Menten equation, such as the Lineweaver-Burke plot:

\[
\frac{1}{\Delta C/\Delta t} = \frac{K_m}{V_m} C_m + \frac{1}{V_m}
\]

A plot of the reciprocal of $\Delta C/\Delta t$ vs the reciprocal of $C_m$ gives a straight line with intercept $1/V_m$ and slope $K_m/V_m$.

Phenytoin Michaelis-Menten elimination parameters have been estimated by many authors. On the whole, the $V_{max}$ values tend to be fairly consistent in the different studies, but the $K_m$ values show more variation. Previous studies indicate that $K_m$ values of phenytoin should reach at plasma concentration of 4-7mg/L, corresponding to a dose of 250 mg. However, the degree of curvature of the plasma curve at this point is too slight to be easily seen or may be obscured by the data. This is why data from single dose studies are unable to use non-linear pharmacokinetics. (Jusko et al, 1976).
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It is widely recognized that major errors may occur in fitting Michaelis-Menten parameters to sets of plasma concentration-time data obtained from single patients at one drug dose (Metzler and Tong 1981).

Several values of $K_m$ and $V_{max}$ have been reported for phenytoin. But coherence among these values has not been observed possibly because of the variable design of experiments conducted. Dose, duration of treatment, type of subject (healthy vs patient, aged vs young etc), method used for analysis all affect the outcome of the experiment.

Jusko et al (1976) gave a value of 4.7 mg/l as the value of $K_m$ at a dose of 250mg. $K_m$ of 5.8$\mu$g/ml and $V_{max}$ of 8.1mg/day was reported by Eadie et al (1976). Gerber and Wagner (1972) reported $K_m$ to be 6.77$\mu$g/ml and $V_{max}$ to be 6.1mg/day. Atkinson and Shaw (1973) derived a value of 16.3 $\mu$g/ml for $K_m$ and 14.4mg/day for $V_{max}$. Martin et al (1977) reported mean values of 11.5$\mu$g/ml and 10.3mg/day respectively. Jung et al (1980) gave values of 9.43$\mu$g/ml and 8.25mg/day. Odani et al (1997) gave values between the range of 10.3 and 8.52$\mu$g/ml for $K_m$ and 6.37 and 10.7mg/day for $V_{max}$. Mamiya et al (2000) reported values between 4 and 6.16$\mu$g/ml for $K_m$ and 3.53 and 6.07mg/day for $V_{max}$, to cite a few examples. The value of $K_m$ and $V_{max}$, the critical parameters in the Michaelis-Menten pharmacokinetics have also recently been suggested to be affected by the genetic makeup of the individual, as these parameters are a direct measure of the hydroxylating capacity of an individual which is known to be encoded by mutable genes.

Clearance

In the case of a drug whose elimination follows Michaelis-Menten kinetics, clearance does not have a constant value in the individual. For such a drug, the calculated clearance value depends on the prevailing plasma drug concentrations. Different values have been observed for different classes of patients: Cranford (1978) reported a mean value of $0.0157 \pm 0.0132$ l/kg/h, while Gugler (1976) reported a value of $0.022 \pm 0.0021$ l/kg/h. Abernathy and Greenblatt (1985) showed that clearance of phenytoin was higher in the obese (59 vs 39 ml/min), while Shavit et al (1984) found the value to be higher during menstruation (23.1$\pm$5.5 ml/min).

Biotransformation (Pharmacogenetics and Phenytoin)

The importance of genetics in phenytoin metabolism was recognized very early on. Stewart et al (1975) suggested that the wide range of metabolic rates seen with phenytoin is the result of genetic factors and saturable kinetics. In a different vein, Arnold and Gerber (1970) mentioned a difference in $t_{1/2}$ between Negro and Caucasian patients. Kutt et al (1971) attributed genetic factors to wide variations in elimination kinetics.
Review of Literature

The majority of phenytoin is metabolized principally in the hepatic endoplasmic reticulum and mainly by the CYP450 isoforms CYP2C9/10 (80-90%, Hung et al, 2004) and to a lesser extent, by CYP2C19. The CYP group of enzymes is now known to be affected by genotype of individual. Besides, Phenyltoin is also a potent inducer of CYP2C9, CYP3A4 and the UGT enzymes (Anderson, 2004). This raises the potential for many clinically important drug-interactions, which will be discussed later in detail. Reports of abnormally fast or abnormally slow metabolisers of phenytoin have also appeared. This phenomenon appears to have a genetic component. Studies in this area have been few. Anderson (2004) reported auto-induction of CYP2C9 and CYP3A4 along with UGT upon phenytoin usage. The activity of these enzymes was dependent on genetic, physiological and environmental factors. Genetic polymorphism in the genetic expression of CYP2C9, 2C19 and 2D6 has been identified. Poor metabolisers are homozygous for the mutant gene. Extensive metabolisers are either homozygous or heterozygous for the wild-type gene, with heterozygous carriers having intermediate activity. Ultrametabolisers have multiple copies of the gene. It has been estimated that approximately 40% of the White and 5% of the Asian or Black Populations are heterozygous for either CYP2C9*1/*1. The proportion of poor metabolisers with respect to CYP2C19 is 15 to 25% in Asians and about 2 to 5% in the white and black populations. Another study found that the ratio of steady state phenytoin concentration to dose was 35% higher in heterozygous patients than in 52 with the homozygous wild-type. Odani et al (1997) found that the maximal elimination rate ($V_{max}$) was 33% lower in patients who were heterozygous for CYP2C9*1/*2 compared with homozygous wild-type CYP2C*1/*1. Such genotyping may putatively be useful in predicting the most effective dosage regimen for phenytoin. However, the scope for such interventions are currently limited, mostly because the calculated Michaelis-Menten parameters vary greatly over various ethnic groups. Hung et al (2004) and Taguchi et al (2005) made an attempt at arriving at such results using data from Japanese population. Both groups divided their patients into sub-groups according to their genotype and estimated the Michaelis-Menten parameters. Taguchi et al observed that the Michaelis-Menten parameters varied largely even within groups. Allabi et al (2005) made a similar study in Black (Beninese) population. They concluded that not all variants of the CYP2C9 gene contribute to the metabolism of phenytoin.

Phenytoin is hydroxylated in the liver by a saturable enzyme system. It is also a low-hepatic-extraction drug. There is some evidence that phenytoin enhances its own elimination through enzyme induction. In a study with healthy volunteers by Chetty et al (1998), three phases of Phenyltoin administration were conducted. It was found that the AUC$_{0-\infty}$ and AUC$_{0-t}$ measured
after the third phase was statistically significantly smaller than the value at first phase of the study.

The principle metabolite of Phenytoin is 5-(para-hydroxypheny)-5-phenyl hydantoin (HPPH), which is inactive. The metabolite is conjugated with glucuronide in the liver and excreted in bile. Measurable concentrations of the metabolite can be found plasma between 30 to 60 minutes after the drug is first taken by mouth. After intravenous administration, the metabolite can be discerned within 20-40 minutes (Loeser et al 1961).

3.5.4. Excretion

Small amounts of Phenytoin leave the body in faeces, unmetabolised or as p-HPPH. Most of the phenytoin dose is finally excreted in the urine as the glucuronide conjugate. Phenytoin is handled by the kidney as a lipid-soluble substance which is filtered through the glomeruli and then passively resorbed across the renal tubules when water is reabsorbed. pHPPH accounts for 70-80% of the administered dose (Glazsko et al, 1969, Wilder et al, 1974). Excretion study of p-HPPH is a widely used method for phenytoin experiments, providing ease of measurement as an attraction to researchers.

3.6. Clinical Pharmacokinetics

Many study designs have been designed to determine pharmacokinetics of phenytoin while varying influential parameters. To achieve therapeutic concentrations, the rate of administration of the drug must approach the maximum rate at which phenytoin can be eliminated. Because of non-linear pharmacokinetic pattern of this drug, simple extrapolations are insufficient to calculate and predict optimum dosage regimen for individual patients. Even though a standard dose of 300mg has been used clinically to produce seizure control, literature indicates that this dose produces a rather wide range (1-50 mg/liter) of phenytoin concentrations in patients. Yet, it is widely agreed that therapeutic concentrations of 10-20 µg/ml are sufficient to produce therapeutic effects without eliciting toxic effects in most clinical situations.

3.6.1. Time course of Plasma drug levels

Single dose

After a single oral dose, plasma phenytoin levels reach peak in 2-8 hours, thereafter, declining slowly.

In one of the earliest studies that determined pharmacokinetics of phenytoin after single oral dose, time required to reach maximum plasma levels ranged from 4-12 hrs, and the half-life was found
to be 18.24 hrs, which is in agreement with observations made later on (Dill et al, 1956). A
similar experiment by Glaszko et al (1969) using Intravenous dose of 250 mg of phenytoin,
resulted in half-life of 10.5 to 28.7 hrs, averaging about 15 hrs. This study looked at phenytoin
concentrations beyond 24 hrs post dose.
Another study by Kutt et al (1964) studied plasma levels and associated toxicity of phenytoin.
They used dosage increments in intact individuals and individuals with defective metabolic
processes. They postulated that the observed large differences in intrasubject plasma levels were a
result of three factors- use of body mass rather than “metabolizing mass” to calculate individual
dosage, genetic variations in metabolizing properties of enzymes and attainment of appropriate
blood levels that can “stimulate” liver to metabolise the drug.
Albert et al (1974) used a different design of experiment that used multiples of 100mg dose to
assess bioavailability. The area under the curve following administration of 3 * 100 mg capsules
was 3 times the area following administration of a single 100mg capsule. The differences in size
of dose were reflected in the rate of absorption or efficiency of absorption. The authors felt that
rapid absorption was probably less important, or even undesirable, because of side effects due to
either high initial blood levels of DPH or adverse reaction to the vehicle itself. In fact, a delayed
release preparation of phenytoin that was introduced into the market did not gain much
popularity.
The above authors cited a lag time in absorption, bile cycling and saturation kinetics as the factors
affecting pharmacokinetics of phenytoin in man. The study elucidated a clearly linear relationship
between area and the dose of phenytoin in the dose range of 100 and 200 mg. Glaszko et al
(1972) reported similar results with a dose range of 200 to 400 mg. Glaszko et al (1969) attributed
the erratic absorption to enterohepatic circulation.
A similar experiment was carried out by Gugler et al (1977). The design of the study was similar
to the one by Gugler et al (1976) as well. They found the plasma concentration- time curve
following Intravenous administration best described by a 2-compartment open model with a ta1/2
of 0.63 ±0.14 hr and tβ ½ of 16.8± 1.3 hr. They found no significant difference in between t1/2 vs
between single dose intravenous and oral experiments. They reported a slow absorption from the
oral dosage form, and subject to intrasubject variation. While Glaszko et al (1972) reported a
longer t1/2 with oral administration than after intravenous administration, Gugler et al do not
confirm this observation in their study. It appears as if t1/2 may be dependent upon the time point
was done after 24 hrs post dose. Steady state levels obtained were below 10 μg/ml.
Jusko et al (1976) carried out studies that compared single oral and intravenous administration of 4.6 mg phenytoin. The authors used Michaelis-Menten parameters to describe phenytoin pharmacokinetics, to which they found good correlation, as evidenced by extremely small standard deviations for $K_m$ and $V_{max}$. The absorption profile was irregular, as observed by Hamdely et al as well in 1970. The authors noted a secondary absorption peak which they attributed to the fact that the drug was insoluble and food aided dissolution possibly gave rise to the secondary peak. The authors admit that the secondary peak may be a result of circadian factors. Such secondary peaks have not been commonly observed in the case of phenytoin.

Loeser et al (1961) reported blood levels of 7.6 μg/ml (range 1.5 to 3.8 μg/ml) in 24 hrs with a t1/2 of 14 hrs. Bigger et al (1968) used single intravenous doses of 300 mg and observed a rapid fall to 4-8 μg/ml 20-40 min after injection. In general, intravenous trials demonstrate a high rate of removal of DPH from the blood during the 1st 20-30 min after dosing.

However, it has been noted that single dose studies were not satisfactory to elucidate bioavailability differences or to calculate pharmacokinetic parameters (Lund et al 1974, Albert et al 1974, Halkin et al 1985, Jusko et al 1976). This is because linear kinetics are observed only at low plasma concentrations below the value of $K_m$ for drug that follow Michaelis-Menten pharmacokinetics (Gibaldi and Perrier 1975). Steady state concentrations of 10-20 μg/ml are above the value of $K_m$ in most patients, which result in major changes in area under the curve with a small change bioavailability. Therefore, a meaningful difference in bioavailability becomes apparent only at higher levels of the therapeutic range.

Hence, most studies that addressed bioavailability issues were done with a multiple dose design. Lund et al (1974) carried out single and multiple dose studies in healthy volunteers. They found a $C_{max}$ between 2.6 and 5.0 μg/ml and a half-life of 14.5±1.2 and 16.6±3.8 hrs after oral and intravenous doses respectively. Maximum plasma levels were maintained for several hours post dose. First order kinetics were observed on intravenous dosing and the post steady state elimination phase. As observed by Arnold and Berger in 1969, at higher doses, elimination followed zero-order process.

**Repeated doses**

While many ranges have been quoted, it is generally agreed by researchers that phenytoin levels reach steady state in about a week's time from start of therapy. While Svensmark et al (1960) reported 2-7 days, Friedman et al (1981) reported 4-8 days, Buchanan et al (1972) reported 7-8 days, Ludden et al reported 1-2 weeks, Jung et al (1980) mentioned that it may take up to a month,
Gugler et al (1977) reported a value not less than 12 days and Arnold and Gerber (1969) reported a period of 7 days for phenytoin to reach steady state levels.

Loeser et al (1961) noted that the same daily dose gave similar steady state blood levels of phenytoin whether the drug was taken once or twice daily. In a slightly different experiment, Jung et al (1980) compared a single dose oral dose of 400 mg with a divided dose regimen i.e., 400 mg every 3 hrs. The divided dose was absorbed as well (91%) as the single dose. The tmax was longer for the 1600mg dose than 400 mg dose, though Cmax levels were maintained upto 72 hrs after dosing, suggesting that dosing may not be necessary for upto 3 days after the first maintenance dose. Therapeutic serum concentration were rapidly attained by an initial oral dose, administered as a divided rather than a single dose. Jusko et al (1976) found similar results; they found absorption processes for upto 2 days post dose.

Serrano et al (1973) showed that steady state plasma levels fluctuated through a ±10% range throughout the day with twice daily intake of drug. Buchanan et al (1972) compared the effects of giving the drug once daily and 3 times a day; and concluded that the different dosage intervals had no effect on the fluctuations in the plasma phenytoin levels. These results were concurred by Haerer and Buchanan (1972), Strandjord and Johannessen (1972) and Wilder and Serrano (1973). Buchanan et al (1972) showed no deterioration in the control of seizures in children when multiple dosing was changed to single dosing. Houghton and Richens (1974) studied elimination kinetics of phenytoin in epileptic patients who were in a steady state condition. Their observations coincided with those of Gerber and Wagner (1972) that rate of decline of serum phenytoin concentration on stopping maintenance therapy was log-linear with time in such patients. A linear relationship was found between serum phenytoin concentration and t1/2. The authors opined that intrasubject variability in response to a given dose of phenytoin depended on the genetic make-up of the individual and auto-induction properties of phenytoin. They also concurred with Bochner et al (1972) and Mawer et al (1974) recommendation that dose increment must be undertaken cautiously.

Martin et al, (1977) designed a study to obtain multiple steady-state concentrations in each subject over a certain concentration range (2-20 mg/liter). The study was designed to increase the dose in a stepwise manner over a time period of 6-14 days. They found that the ratio of the daily dose to the steady-state concentration decreased as the dose was increased.

Since phenytoin is generally given orally, a delay in achieving steady state indicates need for oral loading dose for rapid effect. Wilder et al (1974) fond that a loading dose of 1 gm produced plasma phenytoin levels above 7.5 µg/ml in most subjects within 8-24 hrs after administration. Serrano et al (1973) used intravenous loading doses of 500 to 1000mg, followed by
or oral administration of 300-400mg as required. They found a lowering of plasma phenytoin levels to below therapeutic range upon a switch to intramuscular and postulated that precipitation of the insoluble drug at injection site may be the reason for slow release of drug. Lund et al (1974) showed that steady state levels after 12 days dosing was 5.3 – 12.2 µg/ml and negligible day to day variation in all subjects. Gugler et al (1977) studied pharmacokinetics of single and multiple doses of phenytoin in man. A T\text{max} of 5.4±0.9hrs was observed. Mean steady state levels between 4.2 and 8.7µg/ml was observed at least 12 days post administration.

This seemingly satisfactory situation seems to arise because in normal routine patients take the medicine along with food, which is proven to improve phenytoin absorption. This results in the rate of absorption close to rate of elimination. This phenomenon is observed markedly at levels above 10 µg/ml, since then, according to Michaelis-Menten kinetics, first-order kinetics change to zero-order kinetics. Thus, once a day dosing seems appropriate to maintain optimum plasma levels if once steady state is attained above 10 µg/ml.

While the above studies seem to indicate that once steady state is attained, little, if any, changes in dosage regimen may need to be made to maintain therapeutic plasma levels, Ludden et al (1978) seem to differ. They feel that phenytoin concentration in the body may reach toxic levels if the same dosage regimen is maintained over prolonged periods of time.

**Correlation of plasma drug concentrations and dose**

It is generally agreed that plasma concentrations of 10-20 µg/ml is optimum for producing anti-convulsant effect in majority of patients; levels below 10 µg/ml are sub-therapeutic and above 20 µg/ml are toxic. However, due to Michaelis-Menten kinetics of phenytoin, dosage regimens are not easily calculated, and hence there has been widespread interest in assessing daily levels of phenytoin in clinical practice to keep track of patients’ progress.

Early studies incorporated varied dosage regimens to arrive at a single formula to calculate daily dose of phenytoin. Nomograms have been generated to aid in this mission. Jung et al (1980) carried out a landmark experiment to study effect of dose on phenytoin absorption, using 400, 800 and 1600 mg in single and divided doses. They found that C\text{max} after 800 and 1600mg doses were not simple multiples of 400 mg dose. T\text{max} increased with increase in dose. Average T\text{max} increased from 8.4 to 13.2 to 31.5 hrs as the dose was increased progressively.

Little or no change was seen in serum concentrations from 10-72 hrs, obviating the need for maintenance dose upto 72 hrs after first loading dose. Because the drug is essentially insoluble at physiological pH, a general decrease in rate of dissolution with increased dose explains sustained
serum levels after the higher doses. The authors found it difficult to fit all the oral data with either a zero-order, first-order or a dose-dependent absorption model due to irregular intersubject absorption patterns.

Arnold and Gerber (1969) conducted a study where loading doses were followed by maintenance doses of 300mg to maintain plasma levels of phenytoin above 10μg/ml. As the dose (in mg/kg) and initial plasma concentration increase so did the plasma t1/2. When an initial low and high loading dose was correlated with corresponding plasma levels, it was found that at the lower (100mg) dose, the decline is not a zero-order process, but at higher dose (1000mg) the decline is a zero-order process, suggesting saturation of the drug-metabolic processes. Plasma half-life was also found to be dependent on plasma concentration, and ranged from 7 to 42 hrs (avg 22hrs). The plasma fall-off curves were not log-linear, especially at higher doses. The saturation dose may vary widely in different individuals. The authors also noted no correlation between plasma t1/2 and dose in mg/kg.

Other studies were designed to use dosage increments to estimate blood levels. Bochner et al (1972) found that at oral doses that achieved blood level of 6-9 μg/ml, any further increment (in 100mg) tended to produce a more rapid rise in phenytoin concentration. A small jump of 30mg from 500mg caused blood levels concentrations to escalate from 11.7μg/ml to 26μg/ml, but was not accompanied by a concurrent increase in HPPH excretion, indicating no more increase in metabolism. The authors recommended a cautious approach to dosage increments once plasma levels reached 6-9μg/ml.

The significance of saturation kinetics in phenytoin therapy is easily discerned in a study by Mawer et al (1974). Maintenance dose was calculated for each subject based on K_m, V_max and V_d estimated from previous observations. The predicted dose was found to be accurate for 15 out of 18 subjects, as evidenced by seizure control. The authors felt that increments of 100mg were too large and could result in under- or over-treatment. Fine adjustments to the tune of 25 mg were sufficient to adjust serum levels between 10 and 20 μg/ml. They also confirmed Bocher et al’s (1972) observation that relationship between dose and steady state level of phenytoin was non-linear.

Eadie et al (1976) carried out a study with different doses of drug. They found that the plasma phenytoin concentration rose after each increment of dose, the increase being much more prominent at higher doses. This critical dosing increment varied from subject to subject. But the rise in plasma phenytoin concentration was not correlated with a rise in p-HPPH excretion. This observation underlined the postulate that saturable elimination processes were active in phenytoin kinetics.
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Dosage Prediction techniques

Many methods have been used to predict optimum phenytoin dosage regimen - nomograms, graphic-based, Michaelis-Menten computations, Bayesian pharmacokinetic forecasting, dosage regimen based on genotype of individual.

Ludden et al (1977) used at least 2-steady state concentrations of phenytoin to arrive at an optimum dose for individual dose with good results. They used a graphical method in their study. Richens and Dunlop (1975) gave a nomograph to calculate dose, as did Martin et al (1977), Richens et al (1975).

Martin et al (1977) designed a study with a starting dose of 100 mg, aimed to achieve plasma levels of 2-20 mg/litre by stepwise increments of dose. Steady state levels at one dose were used to estimate the next dose using a nomogram. The authors confirmed previous findings that relationship between dose and plasma concentration was non-linear. They noted a high intra-individual variability in pharmacokinetic variables but not inter-individually, which reinforced the view that each patient must be monitored while on phenytoin therapy. The authors also gave a nomogram which could be used to predict the optimum dose. The authors cautioned against factors that may affect such predictions. Patient compliance, bioavailability of product, duration of therapy (time to steady state), drug interactions, hepatic disease, altered plasma protein binding in renal failure can increase the error of prediction.

Vozeh et al (1981) used a general method given by Sheiner et al (1979) to predict dose and compared their observations with those of Ludden et al and Richens and Dunlop and the "no-feedback" methods.

Nakashima et al (1995) used a one-point steady state method to predict dosage regimen in Japanese population, and found their method superior to Bayesian method in terms of prediction of dosage.


Recognising the impact of genetics in the metabolism of phenytoin, in recent years, with advance of technology, some studies have been carried out that attempted to correlate phenytoin dosage with genotype of individual. Although this method is not popular yet due to wide ethnic variations in Michaelis-Menten parameters, the method seems promising. Since phenytoin is mostly metabolized by the CYP group of enzymes, which are very much prone to genetic influences, researchers have targeted on variations in this gene to study phenytoin pharmacokinetics.
Hung et al (2004) carried out such studies in Taiwanese patients. Genetic polymorphisms in the CYP family coding genes were determined, patients grouped according to their genotype, and their pharmacokinetic parameters estimated. Thereafter, doses were predicted to give best response. Although the $K_m$ values were fairly uniform across all genotypes, $V_{\text{max}}$ seemed to have more influence upon metabolism.

Odani et al (1997) and Mamiya et al (2000) carried out similar studies in Japanese patients. Their observations differ much the reported $K_m$ and $V_{\text{max}}$ values, underlining the nascent stage of this technology to predict dose.

Phenytoin is ~90% plasma protein bound. As such, the fraction of phenytoin unbound in the plasma may have a drastic impact on phenytoin efficacy. Tandon et al (2004) recommended a correction for albumin binding according to the formula of Sheiner and Tozer and found an improvement in clinical status of patients after adjustment of dose.

Fedler and Stewart (1999) carried out similar studies in rural Black population. Their results were encouraging enough for them to incorporate this correction in laboratories in their institute.

Accuracy of any of these methods is varied, and application in uncontrolled ambulatory environment is unclear (Spruill et al 2001). Many confounding variables affect the outcome of these predictions, which is why most physicians prefer to rely on clinical intuitions to define dosage regimen. At the most, single-point observations and dosage-pairs are used to fine-tune individual dosing. Moreover, none of these methods are without inherent disadvantages. Nomograms are developed using healthy volunteer data, and hence may not prove useful in clinical setting (Privitera et al 1989). Population-based methods are not believed to truly represent patients with epilepsy (Spruill et al 2001). Bayesian techniques were shown to be unreliable at initial drug concentrations below $8 \mu g/ml$ (Toscano and Jameson 1986). Spruill et al (2001) suggested patient-specific information as best predictors of performance. Vozeh et al (1980) agreed with Spruill et al. Welty et al (1986) concluded that single-point methods were best. Privitera et al (1989) favored Bayesian methods over physician forecasting. The study recognized applicability of the method to determine serum drug levels and make dosing adjustments from both steady state and non-steady state concentration data.

3.6.2. Half-life

*Parent Compound*

Elimination half-life: 22hrs (ranges from 7 to 42 hrs), the variability is mainly due to saturation kinetics). After intravenous administration, half-life ranges from 10 to 15 hrs. It has been
observed that the value of calculated half-life depends on the concentration range over which it has been measured. Early researchers calculated values ranging from 14 to 51 hrs for phenytoin half-life. However, it was soon realized that the half-life depended heavily on the concentration range over which it was calculated. It was Dayton et al (1967) who showed that the half-life of phenytoin in man was dose-dependent. It is now generally known that the elimination of phenytoin is more appropriately described in terms of the Michaelis Menten kinetics, also known as Non-linear or Saturable Pharmacokinetics. The plasma concentration rate of inactivation is not proportionate to plasma concentration. This is why the plasma half-life, normally, in the range of 22 hrs, increases with dose, and also the steady state mean plasma concentration achieved with a certain fixed daily dose is not proportionate with dose. Houghton and Richens (1975) correlated an increase in dose with increase in $t_{1/2}$ and the reverse upon lowering the dose.

3.7. Toxicology

The toxicity of antiepileptic drugs can be a limiting factor in the long term management of the patient. Toxic effects depend upon route of administration, duration of exposure and dosage. Central Nervous System toxicity is the most commonly observed and is usually dose related. Toxic effects may appear in other systems as well- hematopoietic, gastrointestinal, immune, endocrine, skeletal and skin. Intravenous administration can precipitate cardiac arrhythmias.

3.7.1. Acute toxicity

Acute toxicity with phenytoin is usually the result of overdosage and is less often secondary to an allergic or idiosyncratic reaction, and occurs due to direct drug action on receptor sites as therapeutic concentrations are exceeded. The CNS signs can be usually be predicted on the basis of drug dose-weight or drug dose-plasma concentration relationships.

A key study that has till this date remained a reference for elucidating such relationships was carried out by Kutt et al in 1964. They attributed the various signs of toxicity to either an inborn enzymic defect or liver disease. Nystagmus developed when blood levels reached 20μg/ml, with a range of 15-25μg/ml; ataxia was noted with blood levels of 30μg/ml and above. Mental changes occurred with blood levels of 40μg/ml and above. Other data is also available. Hærær and Grace (1969) stated that 90% of patients with plasma levels above 25μg/ml showed nystagmus. Livingston et al (1975) commented that most patients tolerate plasma concentrations below 25μg/ml, that levels above 30μg/ml are related with nystagmus, diplopia and ataxia, and
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concentrations above 50µg/ml caused extreme lethargy and coma. Depression of mood has been shown to be associated with plasma levels above 30µg/ml (Eadie and Tyrer 1980).

Gannaway and Mawer (1981) showed variations in the intensity of signs and symptoms with similar blood levels can be seen among individuals, suggesting interplay of factors that affect toxicity. While intact individuals can maintain a metabolic balance by increasing their capacity to metabolise phenytoin with dosages up to 10mg/kg or more, amounts as low as 1-3mg/kg will cause continuous accumulation of unmetabolised drug and appearance of signs of toxicity in individuals who are limited in this capability due to various factors.

Undesirable effects of phenytoin may develop even with plasma phenytoin levels in the conventional therapeutic range. These effects become more obvious as the plasma drug levels rise further. Reynolds and Travers (1974) showed a correlation between increasing plasma phenytoin levels within the conventional therapeutic range and psychomotor slowing, intellectual deterioration, personality change and the development of psychiatric illness in persons treated with the drug.

As toxicity increases, higher cortical functions (judgment, behaviour, mood, concentration, speech) are affected. Epileptic seizures may be exacerbated. When toxicity is retained for a long time, autonomic dysfunction and altered levels of consciousness may occur. As such, phenytoin intoxication is not easily recognized and plasma level monitoring can be useful in detecting subtle toxicity.

Cerebellar Syndrome

Cerebellar dysfunction with nystagmus and ataxia is well recognized as a manifestation of acute phenytoin toxicity, and is usually dose-related (Ghatak et al 1976). Neurologically handicapped patients who are on polytherapy generally experience adverse effects at lower plasma concentrations, and a wide interindividual difference exists regarding what plasma concentrations will be associated with toxicity, which could be due to interindividual differences in metabolism of phenytoin.

Prolonged treatment with phenytoin may lead to irreversible cerebellar deficits, which may be difficult to distinguish from chronic ataxia (Munoz-Garcia et al 1982). Long term phenytoin therapy has been associated with cerebellar degeneration (Ghatak et al 1976, Haberland 1962, Hoffbrand et al 1968), although such cases are uncommon in clinical practice.
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*Extrapyramidal and pyramidal signs*

Dyskinesia, chorea, ballismus and dystonia are observed with phenytoin therapy (Chadwick et al 1976). The dyskinetic movements may involve the extremities, trunk and face and generally occur early in the course of therapy. The dyskinesias are more frequently with high dose therapy, and usually improve with lowering of dose.

Spasticity and transient hemiparesis have also been reported with high phenytoin concentrations (Findler and Lavy 1979, Stark 1979).

Occasionally, a Parkinsonian syndrome or aggravation of Parkinson’s disease can occur.

*Acute Encephalopathy*

Glaser et al 1972 and Levy et al (1965) showed that phenytoin therapy can cause encephalopathy that can exacerbate seizure, and may even lead to stupor and coma.

*Allergic and idiosyncratic reactions*

Phenytoin hypersensitivity reactions are well documented. Idiosyncratic drug reactions generally occur early in the course of therapy and represent genetically determined abnormal response to drugs. Skin rashes, mostly morbilliform, represent the most frequently observed reactions. Exfoliative dermatitis with systemic symptoms or toxic epidermal necrolysis may be observed, which may be potentially lethal.

Cutaneous vaculitis, a lupuslike syndrome, lichenoid eruptions and purpuric rashes are less frequently observed manifestations (Chang et al 1992).

Other less common reactions include pancytopenia, thrombocytopenia, erythema multiformae, pseudolymphoma, interstitial nephritis, myositis, polyarteritis nodosa, disseminated intravascular coagulation, and red cell aplasia (Reynolds 1975, Haruda 1979, Pelekanos 1991). With intravenous phenytoin, extravasation of the drug into interstitial tissues can cause limb ischemia and skin necrosis. Mild elevation of hepatic enzymes may be seen in 10-25% patients.

Lymphadenopathy may be associated with systemic symptoms such as fever, rash, arthralgias, and hepatosplenomegaly. This is a benign condition and is reversible with discontinuation of phenytoin therapy. Depressed immunologic functions can be seen in phenytoin patients. IgA production is reduced, and IgG and IgM production may be altered (Slavin et al 1974).

### 3.7.2. Chronic Toxicity

Chronic toxicity with phenytoin therapy involves the following systems: Neurologic, Connective Tissue and Dermatologic effects, endocrine disturbances and metabolic effects, hematologic effects and deficiency states and immunologic effects.
Evidence of peripheral neuropathy as a result of phenytoin therapy alone has not been received. Phenytoin may impair motor speed, concentration, and memory. These disturbances appear to be dose related (Dodrill and Troupin 1991, Aman et al 1994). A predominantly sensory peripheral polyneuropathy has been observed in patients receiving long-term phenytoin therapy.

Even though it has been believed that patients stabilized on therapeutic concentrations of phenytoin rarely have adverse effects on functioning of the nervous system, this is not strictly true. Some patients complain of intellectual clouding while on phenytoin therapy. Thompson et al (1981) and Thompson and Trimble (1981) carried out some psychomotor tests and showed that impairment of memory and slow mental and motor responses could be observed in the first few weeks of phenytoin therapy. There are reports of total external ophthalmoplegia and widespread fasciculation (Spector et al 1976 and Direkze and Fernando 1977) that recovered when phenytoin intake was stopped. Some patients may feel vaguely unwell or depressed when overdosed with phenytoin, while others may feel no ill-effects at concentrations as large as 35 mg/litre. Nevertheless, the overall incidences of neurological ill-effects rise as plasma phenytoin levels rise.

Even though chronic phenytoin therapy may not result in neuropathy, it has been associated with a slowing of motor conduction in peripheral nerves (Lovelace and Horowitz 1968, Birket-Smith and Krogh 1971, Eisen et al 1974, Encinoza 1974, Chokroverty and Sayeed 1975).

Gastrointestinal
Nausea, vomiting, and constipation may be caused by phenytoin therapy.

Integumentary
Thickening of subcutaneous tissue, enlargement of lips and nose, coarsening of facial features and subcutaneous fibrous deposits are often recognised in patients who receive phenytoin therapy. These features have been collectively termed "Hydantoin facies" (Falconer and Davidson, 1973). Such changes have been related to long term therapy and high doses of phenytoin. Other dermatological changes like hirsutism, acne and hyperpigmentation may also contribute to the characteristic facies.

By its influence on collagen synthesis, phenytoin may increase the risk of Dupuytren's contracture (Houck et al, 1972).
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Gingival Hyperplasia is by far the most commonly observed condition in patients on long term phenytoin therapy, mostly, in children below the age of 21, probably mediated by its propensity to increase collagen production. Poor oral hygiene (Angepoulos and Goaz 1972) and a deficiency of IgA in saliva (Aarli et al 1976), genetic factors and high phenytoin concentrations may also contribute to the condition. It disappears within a few months of ceasing phenytoin intake, and can be minimized by careful oral hygiene.

Hematopoietic

Hematopoietic complications with phenytoin therapy can be classified under 3 categories:

1. Neonatal Coagulation Defects: Neonatal coagulation defect that causes disturbances in coagulation processes but rarely manifested clinically as haemorrhage has been reported. This condition is now known to be caused by Vit K-dependent clotting factors and may be circumvented by administration of Vit K to the mother before delivery and to the infant at delivery.

2. Bone Marrow Suppression: Agranulocytosis, Pancytopenia, neutropenia, thrombocytopenia and aplastic anemia occur very rarely with phenytoin use (Pisciotta 1972). Megaloblastic anemia, caused by depletion of Folate in the body during phenytoin therapy, can be treated by folate therapy. Mild macrocytosis can be observed in ≤50% of patients. Folate deficiency can result in decreased folate content in CSF, marrow megaloblastosis, decreased serum lactoc dehydrogenase, and hypersegmentation of peripheral neutrophils. Phenytoin can drastically alter the folate balance in the body, putatively by altering folate absorption, folate coenzyme metabolism and tissue use of folate.

3. Immunologic effects: some phenytoin treated patients have depressed cellular and humoral immunity (Sorel et al 1971). Production of IgA and alteration in production of IgG and IgM have been reported.

Endocrine Disturbances and Metabolic Effects

At least four systems are affected profoundly during therapy with phenytoin.

1. Metabolic Bone Disease: Although biochemical abnormalities such as elevated alkaline phosphatase, reduced serum calcium and decreased serum 2-hydroxycholecalciferol are
seen in many patients, clinically significant metabolic bone disease with osteomalacia and rickets is uncommon. Dietary factors and exposure to sunlight may be contributory factors. It must be pointed out here that low serum calcium levels have certainly been reported in patients taking phenytoin (Hahn et al 1972, Varkey et al 1973). Metabolic bone disease may be secondary to decreased intestinal absorption of calcium, altered hepatic vitamin D hydroxylation, and inhibition of parathyroid hormone-induced release of calcium from bone. Metabolic bone disease may lead to fractures, postural changes, and muscle weakness, including a specific myopathy.

Mosenkilde and Melsen (1976) suggested low dietary Vitamin D intake, a low exposure to sunlight, a high hepatic clearance of phenytoin, anticonvulsant therapy and male sex as the prominent risk factors that may contribute to anticonvulsant osteomalacia.

2. **Thyroid function**: Phenytoin can induce changes in protein binding of the thyroid hormones and increase their clearance thereby causing a decline in serum protein iodine (Yeo et al 1989). Triiodothyronine and thyroid-Stimulating hormone levels are usually normal. The symptoms of phenytoin toxicity may mimic those of hypothyroidism.

3. **Pancreatic β cells**: Though most patients on phenytoin therapy have normal carbohydrate metabolism, insulin secretion may be impaired in some patients, especially those with prediabetes or diabetes. This effect may be manifested by an abnormal glucose tolerance test, and acute phenytoin intoxication may be associated with high blood glucose levels.

4. **Pituitary-Adrenal-Gonadal Axis**: Phenytoin short term administration in large doses may initially increase circulating adrenocorticotropic hormone and cortisol levels. Long term administration may lead to shift in steroid metabolic, with an increase in urinary excretion of 6-hydroxycortisol. Phenytoin may also depress the release of antidiuretic hormone (Gharib and Munoz 1974)

Testosterone and estradiol metabolism may also be enhanced by phenytoin. Long term therapy may be associated with elevated plasma concentrations of sex hormone-binding globulin in male and female patients (Cramer and Jones 1991). A higher degree of hyposexuality and serum abnormalities has been observed in men with epilepsy (Herzog et al 1991, Taneja et al 1994), maybe as a result of altered pituitary hormones or a direct result of
an effect on testes. Phenytoin has been known to stimulate secretion of luteinising hormone, follicle-stimulating hormone and prolactin.

3.8. Drug Interactions

3.8.1. Mechanisms of pharmacokinetic interactions

Absorption

Absorption of phenytoin is slow but complete. In clinical studies using low doses of antacids, little or no effect was seen (O'Brien et al. 1978), but at higher doses, bioavailability was reduced (Cacek et al. 1986, D'Arcy and McElnay 1987), more so if both were administered at the same time. Therefore, it has been advised that phenytoin and antacids be administered at a gap of about 2 hrs.

Food has been found to have variable but modest, usually enhancing effects on phenytoin absorption (O'Brien et al. 1978). While lipid rich diet increased phenytoin bioavailability (Sekikawa et al. 1980), protein rich diet improved absorption of the sodium salt (Kennedy and Wade 1982). A constant relationship in time between food intake and ingestion is recommended (Kennedy and Wade 1982, Melander et al. 1979).

Protein binding

Since 90% of phenytoin is plasma protein bound, there is a strong potential for drug interactions with other drugs that exhibit same properties. Thus, drugs such as tolbutamide (Wesseling and Molstthurkow 1975), Salicylates (Fraser et al. 1980), Valproate (Monks and Richens 1980) and phenylbutazone (Skovsted et al. 1976) can displace phenytoin from its binding sites, thus precipitating therapeutic and toxic effects at lower serum concentrations than usual.

Biotransformation

Phenytoin undergoes extensive metabolism, primarily mediated by the CYP group of enzymes, which are known to be genetically coded by polymorphic alleles in different populations.
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Phenytoin and other antiepileptic drugs

The therapy of epilepsy usually involves polypharmacy. Seldom is a patient treated with a single anti-epileptic drug. However, other classes of antiepileptic have the potential to interact with phenytoin.

Chlordiazepoxide: can cause elevated blood levels of phenytoin in some patients (Kutt et al 1975)

Diazepam: may have both increasing (Vajda et al 1971), or decreasing (Houghton and Richens 1975) phenytoin levels, though mostly, no effect is seen. Same phenomenon is seen with Topiramate (Perrucca 1997).

Felbamate: Sachdeo et al (1999) showed that increasing doses of felbamate slowed down the metabolism of phenytoin.

Oxcarbazepine: is an inhibitor of CYP enzymes and so may cause a moderate increase in plasma phenytoin concentration (Barcs et al 2000).

Phenobarbital: is a potent inducer of CYP2C enzymes and also a substrate. The net effect of this phenomenon mostly is no effect on phenytoin levels.

Vigabatrin: may cause up to 40% decline in phenytoin levels in some patients.

The concurrent use of phenytoin with patients on mexiletine resulted in a decrease in the half-life (17 to 8 hours) and a 55% decrease in the AUC (Begg et al, 1982).

Other drugs like Diazepam, Lamotrigine, Levetiracetam, Tiagabine, Valproate, Zonisamide may not cause alterations in plasma phenytoin levels.

Other drugs

Activated charcoal: Inhibits the absorption of phenytoin from the GI track (Welling 1984).

Phenybutazone: May displace Phenyoitn from the plasma binding sites and inhibit Phenyoitn metabolism causing an increase in plasma Phenyoitn levels, prolong half life and cause Phenyoitn intoxication, as can Salicylates (Frazer et al 1980)
Propoxyphene, Ticlopidine (Klaassen 1998), Fluoxetine (Jalil 1992) and Fluoxamine all inhibit CYP family, causing Phenytoin toxicity. Sertraline, though a known inhibitor of CYP2C family, was not shown to elicit symptoms of toxicity though a rise in plasma concentration was seen (Haselberger 1997).

Concomitant phenytoin and aspirin therapy has been reported to result in decrease in total serum phenytoin concentrations with high doses of aspirin (975 mg every four hours) but not with lower doses (325 mg or 650 mg every four hours), indicating that protein binding displacement of phenytoin by aspirin occurs only at the highest salicylate dose level. Although total serum phenytoin concentrations may decrease during aspirin therapy, free serum phenytoin concentrations appear to be unaffected, thus the phenytoin dose need not be altered in most patients (Leonard et al, 1981).

Trazodone: caused an elevation of plasma levels of phenytoin (Dorn 1986)

Shankhapushpi is an Ayurvedic preparation containing the following six herbs: Nepeta elliptica, Onosma bracteatum, Convolvulus pluricaulis, Centella asiatica, Nardostachys jatamansi, and Nepeta hinostana and recommended within the Ayurvedic tradition for epilepsy. Two patients taking both this preparation and phenytoin experienced decreased plasma phenytoin levels and loss of seizure control (Dandekar et al, 1992).

Phenytoin and Antifungal agents

Fluconazole: is a known potent inhibitor of CYP2C9 and CYP2C19 and as such has been shown to increase plasma levels of phenytoin to toxic proportions (Cadle et al 1994, Howitt and Oziemski 1989, Mitchell and Holland 1989).

Antimicrobial agents

Chloramphenicol can increase the plasma levels of phenytoin in various proportions (Koup et al 1978, Nation et al 1990). Isoniazid: can cause an accumulation of phenytoin in some patients, primarily those who are "slow acetylators" of phenytoin (Brennan et al 1970, de Wolff et al 1983, Walubo and Aboo 1995). Rifampicin: can lower phenytoin levels and increase its clearance by a factor of two (Kay et al 1985). Sulphonamides: may reduce phenytoin clearance and prolong its half life (Molholm et al 1979), mediated by an inhibition of CYP enzymes. Elevations of serum phenytoin levels may occur with the concomitant use of azithromycin (Prod Info Zithromax(R),
Review of Literature

2001) and Clarithromycin (Prod Info Biaxin(R), 2000). Concurrent treatment with cotrimoxazole or trimethoprim may result in decreased phenytoin clearance and possibly phenytoin toxicity.

**Antineoplastic agents**

Low plasma phenytoin levels have been observed in several patients on Vinblastine, Ciplatin, or Bleomycin and Adriamycin (Bollini et al 1983). Some patients receiving capecitabine and phenytoin may experience phenytoin toxicity as a result of increased phenytoin plasma levels. (Prod Info Xeloda(R), 2001).

**Antidulcer agents**

Antacids are associated with reduced plasma levels of phenytoin. Cimetidine: inhibits metabolism and hence may increase phenytoin concentration (Levine et al 1985, Phillips and Hansky 1984). Omeprazole: causes a small but significant rise in plasma levels of phenytoin (Gugler and Jensen 1985, Prichard et al 1987)

**Cardiovascular Agents**

Amiodarone caused a twofold to fourfold increase in phenytoin plasma levels (McGovern et al 1984, Nolan et al 1989). Calcium Channel Blockers: Phenytoin Sodium caused elevations and intoxication on patients (Bahls et al 1991). But Nifedipine and Phenacetin seem to be safe in this respect.

**Lipid Lowering Agents**

Coadministered clofibrate may displace serum phenytoin from protein binding sites (Prod Info Atromid-S(R), 1995).

**Hypoglycaemic agents**

Tolbutamide: displaces Phenytoin from plasma binding sites and lower plasma phenytoin levels in patients (Wesseling et al 1975)
3.8.2. Effect of Phenytoin on Kinetics of Antiepileptic Agents

Phenytoin is an inducer of metabolizing enzymes, and also is extensively plasma protein bound, which may have an effect on kinetics of other drugs coadministered with it. Thus, it causes reduction in plasma levels of Carbamazepine (Duncan et al 1991, Lander et al 1975), via induction of enzymes, Clobazepam (Sennoune et al 1992), Flunarizine (Treiman et al 1993), Lamotrigine (Peck 1991), Tiagabine (Richens et al 1991), Topiramate (Perucca et al 1997), Valproate (May and Rambeck 1985) and Zonisamide (Henry and Sackellares 1992).

Phenytoin does not seem to produce clinically important alterations in the concentrations of Phenobarbital, Levetiracetam, and Primidone.

Other Drugs

Analgesics and Antipyretics

Phenytoin accelerates the elimination of Acetaminophen, possibly by induction of its biotransformation (Nation et al 1990). It also increased the elimination on Meperidine (Pond and Kretschmar 1981) and Methadone (Tong et al 1981).

Antihistaminics

Half life and blood levels of theophylline were reduced and clearance increased upon coadministration of both drugs (Jonkman and Upton 1984).

Anticoagulants

Phenytoin can reduce the blood levels of Dicoumarol (Hansen et al 1971). The effect on Warfarin has been observed to be opposite to that expected; plasma levels of Warfarin rose after Phenytoin administration (Nappi 1979). Phenytoin may induce acenocoumarol metabolism resulting in reduced acenocoumarol serum concentrations and possible loss of efficaciy of that agent (Prod Info Cerebyx(R), 1999).

Antifungals

Phenytoin drastically increased the AUC and half-life of Itraconazole, possibly by the protein displacement mechanism (Ducharme et al 1995). Case reports and pharmacokinetic studies have
shown that concurrent fluconazole and phenytoin administration may produce increased plasma levels of phenytoin resulting in toxicity (Mitchell & Holland, 1989; Lazar & Wilner, 1990; Blum et al, 1990).

**Antimicrobials**
Plasma levels of Chloramphenicol (Krasinski et al 1982), Doxycycline (Neuvonen et al 1975) and Praziquantel (Bittencourt 1992) were found to be reduced during concomitant use with phenytoin, possibly caused by the enzyme induction caused by Phenyl toin.

**Antineoplastic agents**
Phenytoin has been reported to increase the clearance of cyclophosphamide by up to 150% in patients (Williams et al 1999). Concurrent administration of busulfan and phenytoin has resulted in decreased plasma concentrations of busulfan by 15% or more, possibly due to induction of glutathione-S-transferase (Prod Info Myleran(R), 2003).

**Cardiovascular Agents**
While Phenytoin caused only a mild reduction in Digitoxin levels (Solomon et al 1971), it caused a significant decrease in half life of Digoxin (Rameis et al 1985). Though Diltiazem is also subjected to effects of enzyme induction, the therapeutic loss is minimal because of the active metabolite of the drug (Aitio et al 1981). Phenytoin may also reduce the absorption of Furosemide and interfere with its action in the kidney (Ahmad 1974, Williamson 1986).
Phenytoin may increase the metabolism of Mexiletine (Begg et al 1982) and Nisoldipine (Michelucci 1996). It can also reduce the half-life of Quinidine (Nation et al 1990).

**Lipid Lowering Agents**
Phenytoin may decrease the efficacy of Atorvastatin and Simvastatin, based on a patient with familial hypercholesterolemia who was treated with concurrent medication. Phenyltoin is known to induce cytochrome P450 3A4 enzymes, which are partially responsible for atorvastatin metabolism. Total cholesterol values improved considerably when phenytoin was discontinued, and elevated gamma-glutamyl transpeptidase levels returned to normal (Murphy & Dominiczak, 1999).
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Immunosuppressants
Phenytoin reduced the half-life, AUC and Cmax of Cyclosporine (Freeman et al 1984). Phenytoin has been shown to decrease cyclosporine levels (Freeman et al, 1984).

CNS Agents
Phenytoin may induce the metabolism of bupropion, resulting in decreased efficacy of bupropion (Prod Info Wellbutrin SR(R), 2002).

Steroids
Phenytoin has been demonstrated to increase the hepatic metabolism of corticosteroids. Those steroids with a pronounced effect include dexamethasone, fludrocortisone, methylprednisolone, and prednisone (Petrerit & Meikle, 1977; McLelland & Jack, 1978).
Failure of Oral contraceptives has been reported in some epileptic patients (Hemphel and Klinger 1976). Breakthrough bleeding, spotting, and pregnancy have been reported in women taking both phenytoin and hormonal contraceptives (Prod Info Ortho Evra(TM), 2001).

3.9. CLINICAL USES OF PHENYTOIN
According to an unofficial source ("www.Medhunters.com"), Phenytoin has the largest number of off-label uses in the US. It has been named as one of those drugs that "refuse to die". The US-FDA has approved it for only two indications- treatment of Epilepsy and some forms of Neuralgia. Phenytoin is indicated for the initial treatment of generalized tonic-clonic seizures (grand mal), complex partial (psychomotor, temporal lobe), and seizures occurring during or following neurosurgery. Additionally, intravenous phenytoin is indicated for the control of grand mal status epilepticus. It is ineffective in the treatment of absence seizures.
Phenytoin acts via a wide range of second messengers and has actions on many types of tissues. It is this ubiquitous property that becomes useful in a variety of disorders other than epilepsy.
Its Sodium channel-blocking capability renders it useful in some forms of arrhythmias. The property of inhibiting repetitive firing from neurons makes it effective in some forms of pain and also some types of CNS disorders. It has a positive effect of tissue regeneration and so is useful in topical treatments.
Other uses for phenytoin are emerging in the literature.
1. Alcohol Withdrawal: It has been suggested to be useful in Alcohol withdrawal syndrome (Lowenstein and Allredge 1993, Sampliner and Iber 1974, though as an adjunct to other drugs and more useful if patient is epileptic.
Review of Literature

2. **Cardiac Arrhythmias:** Phenytoin has been used in cardiac arrhythmias since 1958 and has been demonstrated effective in the treatment of atrial and ventricular arrhythmias and in arrhythmias secondary to digitalis intoxication (Atkinson & Davison, 1974, Wit et al, 1975). Phenytoin is not a first-line antiarrhythmic agent. Phenytoin has also been effective in the treatment of ventricular arrhythmias occurring after open heart surgery and acute myocardial infarction; however, lidocaine is generally preferred over phenytoin due to its equivalent efficacy and because it is easier to use. The drug has been effective in the treatment of postoperative ventricular arrhythmias in children, and in young adults after congenital heart disease surgery (Garson et al, 1980). Efficacy in the treatment of arrhythmias associated with prolonged QT intervals has been observed.

3. **Bipolar Disorder:** In a small, double-blind, controlled trial (n=25), a 5-week course of phenytoin added to haloperidol brought symptomatic improvement in some patients with bipolar disorder (Mishory et al 2000).

4. **Chronic Pain:** Phenytoin has been found useful in some forms of chronic pain. Neuropathic Pain has been found to respond to phenytoin. *Tic Douloureux,* a form of Neuralgia, has also been found to respond to phenytoin (McCleane 1999, Swerdlow & Cundill, 1981; Swerdlow, 1980)

5. **Cocaine Abuse:** In a study of sixty active cocaine users, a fixed dose of phenytoin (300 mg/day) resulted in a reduction of cocaine use by 20% compared to those patients who received placebo. Serum phenytoin levels above 6μg/mL were associated with the greatest improvement (Crosby et al, 1996). Further trials are needed to substantiate these results and to identify an optimal duration of therapy.

6. **Epidermolysis Bullosa:** Some studies have shown that phenytoin is useful in treating recessive dystrophic epidermolysis bullosa and in reducing its blister count (Abahussein et al 1992). It has been used in short- and long-term management of dystrophic epidermolysis bullosa (Cooper and Bauer 1984).

7. **Ulcers:** Phenytoin has been studied (mostly with inadequate controls) in the healing of pressure ulcers, venous stasis ulcers, diabetic ulcers, traumatic wounds, and burns (Muthukumarasamy et al 1991) Used topically, it appears to enhance healing without side effects. Phenytoin increases gene expression of the platelet-derived growth factor B chain in macrophages and monocytes (Dillb et al 1993). Healthy granulation tissue appears earlier with phenytoin than with conventional saline dressings (Pendse et al 1993). Phenytoin seems promising in enhancing the healing of decubitus ulcers (Margolis et al 1995). Phenytoin has been used to treat ulcers that result from mycobacterial infections.
8. Rheumatoid Arthritis: Three patients with inflammatory arthritis developed seizure disorders requiring phenytoin therapy (Bobrove, 1983). Within 6 months of initiating phenytoin, all 3 patients noted a definite and sustained improvement in their joint disease, which was attributed to inhibition of collagenase synthesis by Phenytoin.

9. Wound Healing: Studies have shown phenytoin to be effective in wound healing; however, these studies have been performed in close proximity to the active war front, making control of study conditions more difficult. Further clinical trials are required to evaluate the healing properties of topical phenytoin (El Zayat, 1989).
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3.10. BIOEQUIVALENCE STUDIES CONDUCTED WITH PHENYTOIN

Since the reported bioinequivalence of phenytoin highlighted by the Australian Outbreak of Phenytoin Intoxication, many studies have been undertaken that compared different phenytoin products.

The major factors that have affected the outcome in such studies have been the choice of innovator product, choice of study population (Patient vs Healthy volunteers) and Dosing Regimen (Single vs Multiple).

Most studies have been titled “Comparative Bioavailability” studies because such studies compared a liquid formulation (such as a suspension or intravenous formulation) with other oral products. While this serves to assess absolute bioavailability of each product, it does not test the oral product vis-à-vis the popular method of dosing, viz., oral dosing.

Another issue has been the choice of subjects. Due to potential toxicity problems inherent with phenytoin that becomes manifest, at times, at low blood levels, researchers have been hesitant to use Healthy Volunteers in bioequivalence studies for purely ethical reasons. Nor can this population generally be used for lengthy studies for the same reasons, although they have been used (Rambeck et al 1977, Hirji et al 1985). Invariably, healthy volunteers have been given lower doses of phenytoin in such studies, which generally underestimates bioavailability of each product because non linear pharmacokinetics enhances bioavailability differences at high doses only (Arnold and Gerber 1970, Gerber and Wagner 1972, Lund et al 1974, Richens 1975, Eadie et al 1976). On the other hand, healthy volunteers provide a convenient population in which evaluation of bioavailability is uncomplicated by presence of other drugs (Rambeck et al 1977).

Whether phenytoin bioequivalence studies should be done with single or multiple doses is yet a matter of debate. The core of the issue is whether reliable bioavailability estimates are obtained with simple AUC method or steady state. It has been a tacit understanding among researchers that studies with healthy volunteers are, perhaps, safely carried out with single doses, and multiple dose studies can be carried out in patients (Stewart et al 1975, Hirji et al 1985, Soryal and Richens 1992). A pharmacodynamic end-point study with phenytoin is rather difficult to execute, and hence most researchers have used pharmacokinetic methods only. Many authors agree that true bioavailability differences are elicited only at steady state to rule out effects of enzyme induction (Rambeck et al 1977, Gogtay et al 2003, Lund et al 1974, Albert et al 1974, Halkin et al 1985, Jusko et al 1976).

Because of the non-linear pharmacokinetics of phenytoin, a high intrasubject variability is observed in the study population. Therefore, an adequate number of subjects should ideally be recruited into such studies, and a crossover design incorporated.
Review of Literature

Thus, many designs of bioequivalence studies have been conducted with phenytoin. A listing of some such studies is given in Table 5:

Table 5: Examples of bioequivalence studies carried out with phenytoin

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Year</th>
<th>Authors</th>
<th>N</th>
<th>Single/Multiple Dose</th>
<th>Healthy Patients</th>
<th>BE- Y/N?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1975</td>
<td>Pentikainen et al</td>
<td>6-F*</td>
<td>S</td>
<td>H</td>
<td>1-BE; 3- failed</td>
</tr>
<tr>
<td>2</td>
<td>1975</td>
<td>Stewart et al</td>
<td>60</td>
<td>M</td>
<td>P</td>
<td>N</td>
</tr>
<tr>
<td>3</td>
<td>1975</td>
<td>Manson et al</td>
<td>18-C†</td>
<td>M</td>
<td>P</td>
<td>N</td>
</tr>
<tr>
<td>4</td>
<td>1977</td>
<td>Rambeck et al</td>
<td>6</td>
<td>M</td>
<td>H and P</td>
<td>Y</td>
</tr>
<tr>
<td>5</td>
<td>1980</td>
<td>Baichwal et al</td>
<td>6</td>
<td>S</td>
<td>H</td>
<td>4-BE; 2-Failed</td>
</tr>
<tr>
<td>6</td>
<td>1982</td>
<td>Chen et al</td>
<td>20</td>
<td>M</td>
<td>P</td>
<td>Y</td>
</tr>
<tr>
<td>7</td>
<td>1985</td>
<td>Kitzes et al</td>
<td>13</td>
<td>S</td>
<td>H</td>
<td>Y</td>
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<tr>
<td>8</td>
<td>1985</td>
<td>Hirji et al</td>
<td>8</td>
<td>M</td>
<td>H</td>
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<tr>
<td>9</td>
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<td>Halkin et al</td>
<td>20</td>
<td>M</td>
<td>P</td>
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<tr>
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<td>1992</td>
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<td>17</td>
<td>M</td>
<td>P</td>
<td>N</td>
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<tr>
<td>11</td>
<td>1994</td>
<td>Rosenbaum et al</td>
<td>36</td>
<td>M</td>
<td>P</td>
<td>N</td>
</tr>
<tr>
<td>12</td>
<td>2003</td>
<td>Gogtay et al</td>
<td>12</td>
<td>S</td>
<td>H</td>
<td>N</td>
</tr>
</tbody>
</table>

*F-Females; †C- Children; BE- Bioequivalent; H-Healthy volunteers; M- Multiple Dose studies; N-Power of study; P-Patients; S-Single Dose studies