# THE ROLE OF PARENTERAL PHENOBARBITONE IN THE TREATMENT OF NEONATAL HYPERBILIRUBINAEMIA

BY

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SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF MEDICINE
IN THE

DEPARTMENT OF PAEDIATRICS AND CHILD HEALTH

UNIVERSITY OF NATAL

SUPERVISOR

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DURBAN

1993

# DECLARATION

I, OMAR FAROUK ADAM, HEREBY DECLARE THAT THIS DISSERTATION IS MY OWN WORK AND HAS NOT BEEN PRESENTED FOR ANY DEGREE OF ANOTHER UNIVERSITY.

THE WORK REPORTED IN THIS DISSERTATION WAS PERFORMED IN THE DEPARTMENT OF PAEDIATRICS AND CHILD HEALTH, UNIVERSITY OF NATAL, DURBAN.

SIGNATURE

# DEDICATION

This is dedicated to all those children who, through sickness, suffering and even death, have contributed to our knowledge and understanding of disease processes.

## Abstract

A prospective, randomized controlled trial was conducted to determine the effect of phenobarbitone on neonatal jaundice and to derive pharmacokinetic parameters for South African Black African neonates. The study group comprised 22 term babies given phenobarbitone 12mg/kg intramuscularly as a single dose plus phototherapy. The 22 controls were given phototherapy only. There was no significant difference in the mean serum bilirubin levels for the 2 groups at 20 hours, 48 and 96 hours. It is concluded that phenobarbitone at this dose does not significantly decrease total serum bilirubin levels nor the duration of hospital stay in term babies with neonatal jaundice. NONMEM analysis of serum phenobarbitone data in this study resulted in clearance (Cl) of 0.008 l/hr and a volume of distribution (Vd) of 0.84 l/kg with an intra-individual variability of 8.8%. These pharmacokinetic values, which are the first to be reported for Black South African neonates, are consistent with those reported in the literature.

## Supporting Services

In this research the statistical analyses have been done in consultation with the Institute of Biostatistics of the Medical Research Council and the Academic Computer Services of the University of Durban-Westville.

# ACKNOWLEDGEMENTS

I, Dr O F Adam, wish to express my sincere gratitude to the following individuals for their assistance in the preparation of this dissertation.

1. Professor M Adhikari, my supervisor, Department of Paediatrics and Child Health, University of Natal, for her invaluable assistance and constructive criticism.

2. Professor H M Coovadia, Head of the Department of Paediatrics and Child Health, University of Natal, for his encouragement and expert guidance in research methods.

3. Mr V Rambiritch, Senior Lecturer - Department of Pharmacology, University of Durban-Westville, for his immense contribution - both academic and supportive - in the preparation of this dissertation. His continuing guidance, encouragement, and example are especially appreciated. Also to his wife, Shanta and son, Euvir for their patience and understanding.

4. My wife, Rizwana, who supplied the primary motivation for this endeavour; and for her encouragement and valuable academic input.

5. My children, Shabnam and Muhammad and my mother for their co-operation and sacrifice.

6. Ms Fathima Karrim for her patience and diligence in the typing of this manuscript.

7. Miss Eleanor Gouws for her assistance with the statistical analysis.

8. The staff of the Natal Blood Transfusion Service and the nursery, King Edward VIII Hospital, for their assistance.

9. The Department of Pharmacology, University of Durban-Westville for their analysis of the phenobarbitone specimens and the use of their computer software.

10. The Superintendent, King Edward VIII Hospital, Congella, for permission to conduct this trial at the hospital.

11. My friends and collegues, too numerous to mention individually, for the interest in my work and constant concern.

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# GLOSSARY

| | | |
|---|---|---|
| **AUC** | - | Area under the curve |
| **G-6-PD** | - | Glucose - 6 - phosphate dehydrogenase |
| **NADPH** | - | Nicotinamide - adenine dinucleotide phosphate |
| **NAUC** | - | Normalised area under the curve |
| **NONMEM** | - | Nonlinear mixed effects model |
| **SD** | - | Standard deviation |
| **SnMP** | - | Tin - mesoporphyrin |
| **SnPP** | - | Tin - protoporphyrin |
| **UDPGA** | - | Uridine diphosphoglucuronic acid |
| **UDP-GT** | - | Uridine - diphosphoglucuronyl transferase |
| **Vd** | - | Volume of distribution |

# CHAPTER 1

## Introduction

ALL SEEMS INFECTED THAT TH' INFECTED SPY,

AS ALL LOOKS YELLOW TO THE JAUNDICED EYE.

*- Alexander Pope*

An Essay on Criticism, 1711.

Two out of three full-term newborns are clinically jaundiced in the first few days after birth. Each year at least half of the newborns in the nursery have at the minimum one bilirubin determination performed *(Newman and Maisels, 1990)*.

Although clinicians devote most of their efforts to the salvage of tiny infants, a good deal of recent clinical research has explored the problem of jaundice in the healthy full-term infant.

The physician has to decide when to obtain a serum bilirubin measurement in a jaundiced infant; when to perform further investigations; when to initiate some form of intervention and how to reassure the family that "in spite of our meddling" the baby really is normal and will not suffer any long term consequences. Here, the mother can neither anticipate the need for numerous bilirubin determinations, nor can she be asked to leave her infant in the hospital, blindfolded and receiving phototherapy and other forms of intervention.

Jaundice, phototherapy, eye shields and exchange transfusion cause a great deal of maternal anxiety.

Hyperbilirubinaemia is of concern in neonates because of the risk of bilirubin toxicity to the central nervous system, that is, bilirubin encephalopathy and kernicterus. Bilirubin encephalopathy can be prevented but cannot be reversed once it has occurred. Thus the goal of treatment of neonatal jaundice is the prevention of kernicterus and other documented or suspected sequelae of bilirubin encephalopathy.

For practical purposes, a simplified version of the pathogenesis of bilirubin encephalopathy has to be accepted, according to which plasma bilirubin levels are of paramount importance, inspite of, other factors contributing to bilirubin toxicity. Thus, efficacy of treatment is judged by the ability to reduce the intensity and duration of hyperbilirubinaemia and, as a consequence, the frequency of exchange transfusion.

Drug treatment of neonatal jaundice presents both theoretical opportunities and difficulties. The opportunities arise from the fact that for most newborns, jaundice is the expression of a transient delay in the transition from intrauterine to extrauterine patterns of bilirubin elimination. The transient nature of the difficulty allows for treatment to be of short duration and effective treatment need not be anything more than the acceleration of a naturally occurring maturational process. The difficulty arises from the fact that, as with all other preventive measures, many subjects have to be treated in order to prevent the condition in a few.

However, the arguments in favour of the pharmacological management of neonatal jaundice is reduced to the evidence that this therapy decreases the complexity and costs of treatment of hyperbilirubinaemia without increasing immediate morbidity or long-term sequelae. Furthermore, maternal bonding and breast feeding is enhanced because there is no separation in this crucial phase. Another potential benefit of phenobarbitone treatment is a reduction in the duration of phototherapy and the need for hospitalization.

There is great difficulty in assessing the effectiveness of pharmacological management of neonatal jaundice in the post phototherapy era. As with exchange transfusion, phototherapy has to be included in the protocol of studies on drug treatment in neonatal jaundice.

This results in blunting of the differences in bilirubin values between the control and experimental groups. The additional difference between the groups becomes the parameter by which the efficacy of the drug treatment may be judged. There is a moral and ethical obligation not to deprive the experimental group of the proven benefits of phototherapy and yet demonstrate the effect of the new treatment.

Exchange transfusion is incorporated into all protocols, because it is the gold standard in the management of severe hyperbilirubinaemia and the prevention of bilirubin toxicity, especially jaundice within the first 24 hours and in neonates with blood group incompatibilities and haemolysis.

## Purpose Of Study

1. To assess the role of parenteral phenobarbitone in the management of neonatal hyperbilirubinaemia.

2. To make recommendations on the future use of phenobarbitone in neonatal jaundice.

3. To derive pharmacokinetic data for parenteral phenobarbitone in neonates.

## Importance Of Study

The investigation of phenobarbitone in neonatal jaundice is primarily directed towards:

1. A Third World population group with a high risk of severe neonatal jaundice and scarcity of resources for its management, especially exchange transfusion or immunoglobulins.

2. The reduction of the risks associated with exchange transfusion especially in this AIDS awareness era. The other risks associated with exchange transfusion are:
   - the transmission of potentially serious infections
   - mild graft versus host reactions
   - the difficulty of the procedure and
   - the associated morbidity and mortality ($<1\%$).

3. The determination of pharmacokinetic parameters of phenobarbitone which are important in dosage design.

# CHAPTER 2

## 2.1 Review Of Literature

Interest in using phenobarbitone to decrease neonatal hyperbilirubinaemia was generated following the retrospective study of Trolle (1968), who reported a diminished incidence of neonatal jaundice among the offsprings of epileptic and pre-eclamptic women treated with phenobarbitone during their pregnancies. This original report led to the assessment of the administration of phenobarbitone post-natally for hyperbilirubinaemia. The post-natal use offered the advantage of focusing the treatment to easily identifiable high risk groups.

A retrospective analysis of the problem of neonatal jaundice (NNJ) at King Edward VIII Hospital for the one year period April 1992 to March 1993 showed that there were 2799 cases of neonatal jaundice of which 195 (14.35%) required exchange transfusions (Appendix A).

In all studies for the treatment of neonatal hyperbilirubinaemia using phenobarbitone (Table I), the treatment group had lower mean bilirubin values than the control group starting from day 3, with the maximum difference being reached on day 5 or later *(Valaes and Harvey-Wilkes, 1990)*. The occurrence of jaundice and the need to perform an exchange transfusion was also decreased, particularly in the low-birth weight infants. In some of the studies, the small sample size prevented this effect from reaching statistical significance. However, the consistency of the trend, favours the conclusion that phenobarbitone at a dose of 5-

10mg/kg/per day given orally or intramuscularly for the first 4 to 5 days of life will decrease the incidence of jaundice.

Other retrospective trials (some of which not mentioned in Table I) also give credence to the use of phenobarbitone.

In 1969, Yeung and Field conducted a randomised controlled study on 210 Chinese jaundiced neonates. Of these 93 were given phenobarbitone and 117 were used as controls. In the phenobarbitone group, only 4 patients (4.3%) required exchange transfusion whereas 53 patients (45.3%) required an exchange transfusion in the control group. This difference in the results was statistically significant ($p = < 0.001$), especially in babies with ABO incompatibility, glucose-6-phosphate deficiency and cephalhaematoma. However, in a similar study in full term infants Cunningham, et al. (1969) showed no significant decrease in serum bilirubin level.

In 1970, Levin, et al. conducted a controlled study of 24 patients with neonatal jaundice. All the infants were given oral phenobarbitone at a dose of 5mg 8 hourly for patients greater than 2.5kg and 5mg 12 hourly for patients less than 2.5kg. They noted a significant decrease in bilirubin levels ($p < 0.05$) in both groups.

In a randomized study of 38 patients by Valdes, et al. in 1970, 23 patients were given phenobarbitone 5mg/kg/day orally for 3 days and 15 patients were used as controls. A significant decrease in serum bilirubin occurred on day 3 in the treatment group.

In 1973, Wong and Wood studied the effect of phenobarbitone in 3 groups of jaundiced neonates. Group 1 was given phototherapy only, group 2 was given phenobarbitone only and group 3 was given a combination of phototherapy and phenobarbitone.

The dose of phenobarbitone used in groups 2 and 3 was 8mg/kg/day administered intramuscularly as 3 injections daily for a total of 10 doses. Groups 1 and 3 yielded similar results, with a significant fall in bilirubin occurring from 24 hours onwards. Hence combined phototherapy and phenobarbitone treatment showed no difference from phototherapy alone. The phenobarbitone group (group 2) had significantly higher levels of bilirubin up to 60 hours and it was concluded that phenobarbitone was of no added benefit.

**TABLE I**

## POSTNATAL PHENOBARBITONE FOR PREVENTION OF HYPERBILIRUBINEMIA

| REFERENCES | NO. IN GROUP CONTROL/TREATMENT | DAILY DOSE (MG/KG) | LENGTH OF TREATMENT (DAYS) | MEAN BILIRUBIN (MG/DL) CONTROL/TREATMENT | DECREASE (%) |
|---|---|---|---|---|---|
| | *Term Infants* | | | | |
| Ramboer et al. 1969 | 23/23 | 10 po | 3 | 7.8 / 5.9 (3) | 25 |
| Stern et al. 1970 | 20/20 | 8 po | 4 | 5.9 / 5.2 (3) | 12 |
| | | | | 5.6 / 3.1 (5) | 45 |
| Vest et al. 1970 | 14/13 | 5 im | 3 | 10.4 / 6.5 (4) | 37 |
| Kintzel et al. 1971 | 106/62 | 10 po | 5 | 6.3 / 5.5 (3) | 13 |
| | | | | 5.7 / 3.7 (5) | 35 |
| Yeung 1972 | 24/22 | 5 po | 3 | 12.9 / 7.6 (5) | 41 |
| | *Low Birth-Weight Infants* | | | | |
| Ramboer et al. 1969 | 10/10 | 10 po | 7 | 10.4 / 9.6 (3) | 8 |
| | | | | 8.4 / 7.0 (7) | 17 |
| Valaes et al. 1970 | 10/30 | 8 po | 6 | 12.4 / 9.8 (4) | 21 |
| | | | | 11.7 / 8.0 (6) | 32 |
| Vest et al. 1970 | 46/43 | 3.5 po | 3 | 11.2 / 10.0 (5) | 11 |
| Valdes et al. 1971 | 15/23 | 5 po | 5 | 8.2 / 5.5 (4) | 37 |
| Zwacka et al. 1971 | 121/35 | 10 im | 2 | 12.6 / 8.1 (5) | 36 |
| Gagyi and Frank 1971 | 30/30 | 10 im | 4 | 12.1 / 8.9 (3) | 21 |
| | | | | 13.7 / 7.4 (5) | 47 |
| Carswell et al. 1972 | 29/28 | 8 im | 7 | 10.5 / 7.7 (5) | 27 |
| Dortman et al. 1972 | 44/38 | 2 im | 5 | 15.3 / 13.3 (5) | 13 |
| | | | | 12.9 / 10.2 (7) | 21 |
| Cao et al. 1973 | 120/120 (AGA) | 6 im or po | 6 | 13.9 / 11.6 (5) | 17 |
| | 64/59 (SGA) | 6 im or po | 6 | 9.4 / 7.6 (5) | 19 |

ABBREVIATIONS: po = orally; and im = intramuscularly

* In parentheses: the day of life the bilirubin values were obtained

(Modified from Clinics of Perinatology, June 1990)

# 2.2 Neonatal Hyperbilirubinaemia

## 2.2.1 INTRODUCTION

We have been writing, talking and worrying about neonatal jaundice for more than a century - since Orth, in 1875, first observed bilirubin pigment in the brain of infants dying from severe jaundice. Yet, in spite of great strides made in our understanding of bilirubin biochemistry and the development of an armamentarium for the evaluation and treatment of hyperbilirubinaemia, some of the most basic questions remain unanswered, namely :

- What is the mechanism of bilirubin encephalopathy?
- How are the risks of hyperbilirubinaemia evaluated?
- Does bilirubin damage the brain of healthy full term infants?

These are important issues as two out of three full-term newborns are clinically jaundiced in the first few days after birth. Thus, we can look forward to many more decades of research before we have all, or even most, of the answers.

## 2.2.2 STRUCTURE OF BILIRUBIN

The structure of bilirubin as shown in Figure 1 is essentially 4 pyrrole rings joined by 3 single carbon bridges. For the middle 2 pyrrole rings, the bridge is a single bond and for the outer two rings, the bridges are double bonds. Each double bond can exist in two different arrangements or configurations. Such configurations are now designated as Z and E, the equivalent of cis and trans respectively. In bilirubin IX alpha, the configuration of these two double bonds are set by configurations in the parent heme molecule where they are both Z. Native bilirubin is thus 4 Z, 15 Z - bilirubin IX alpha *(Bonnett, et al. 1976)*.

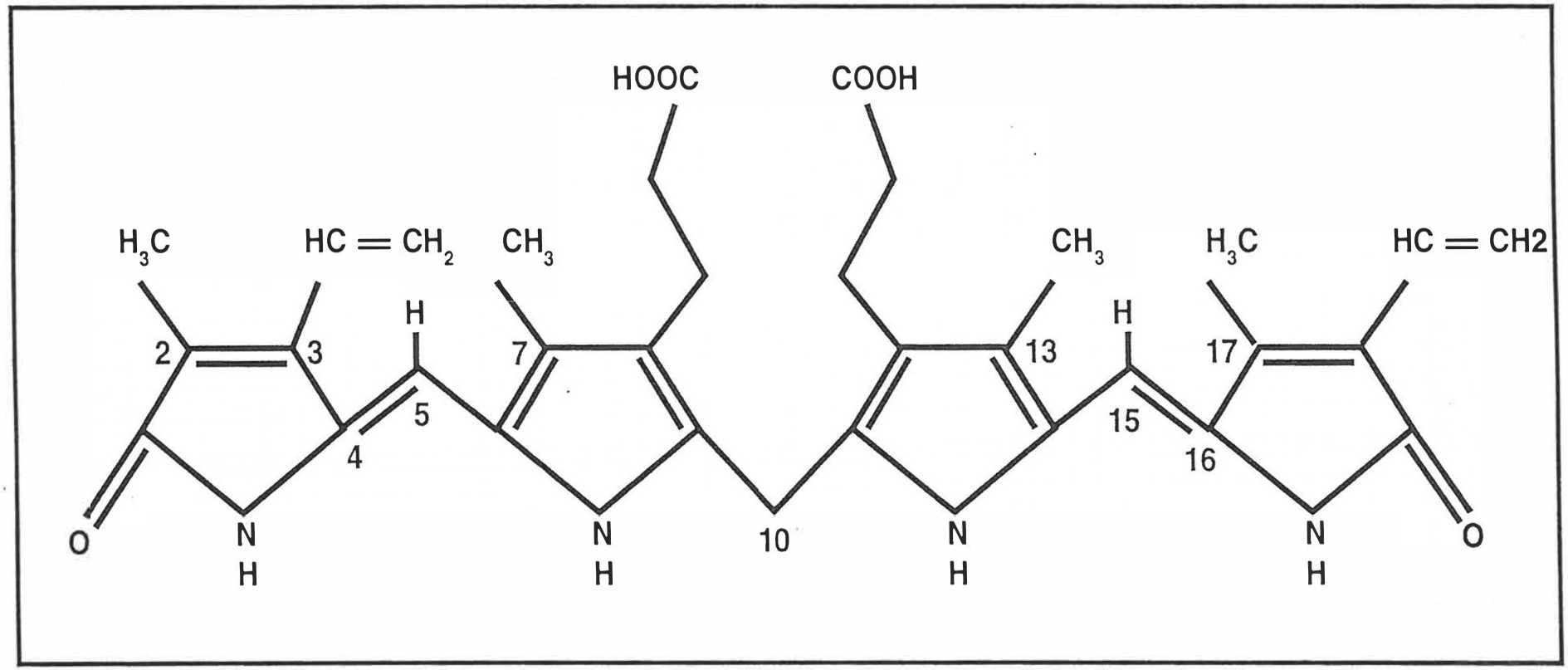



**FIG 1**

## STRUCTURE OF BILIRUBIN

MOLECULAR FORMULA OF 4Z, 15Z – BILIRUBIN IX $\alpha$

THE CARBON ATOMS ARE NUMBERED ACCORDING TO STANDARD NOMENCLATURE

Modified from Clinics in Perinatology – June 1990

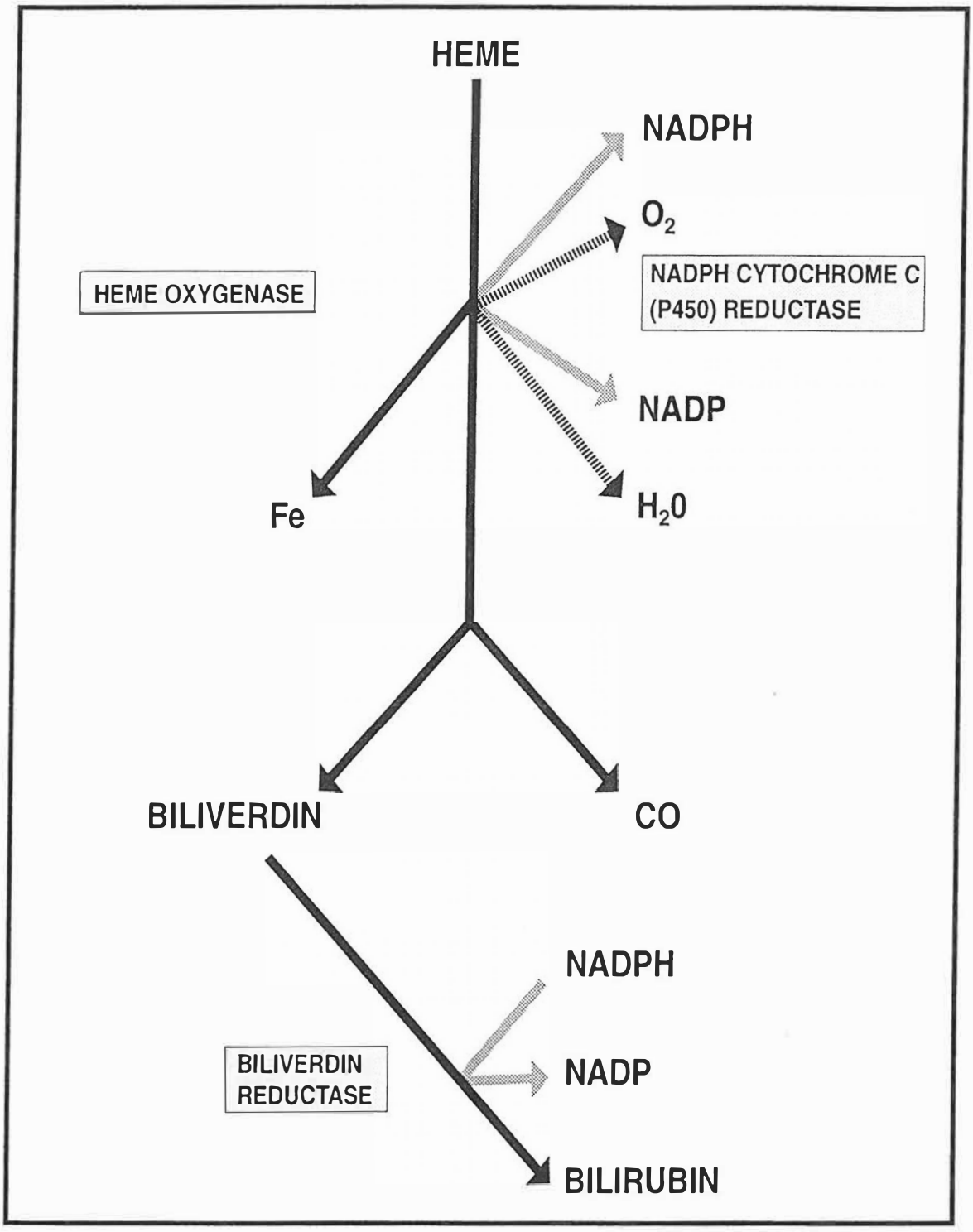



FIG 2

# BIOSYNTHESIS OF BILIRUBIN – HEME DEGRADATION PATHWAY

Modified from Clinics of Perinatology, June 1990

### 2.2.3 BIOSYNTHESIS (FIGURE 2)

Most of the bilirubin formed in the tissue by the breakdown of haemoglobin is bound to albumin in the circulation. Heme is degraded to biliverdin through the concerted action of the microsomal enzymes heme oxygenase and nicotinamide - adenine dinucleotide phosphate (NADPH) cytochrome cP450 reductase and via the cytosolic enzyme biliverdin reductase to bilirubin *(Yoshinaga, et al. 1982)*.

The current hypothesis proposes that the enzyme complex promotes the auto-catalysis of heme. This hypothesis is supported by recent studies of the heme oxygenase gene that identified inducer element binding sites responsive to metal administration, heat shock and nutrient availability. Several metalloporphyrins, such as tin and zinc porphyrin complexes, inhibit heme oxygenase activity and thus have therapeutic potential for the treatment of neonatal jaundice *(Drummond, et al. 1981)*.

### 2.2.4 METABOLISM AND EXCRETION

Figure 3 describe the metabolism and excretion of bilirubin. The free bilirubin enters the liver cell where it is bound to cytoplasmic proteins. It is conjugated to glucuronic acid in a reaction catalysed by the enzyme glucuronyl transferase. This enzyme is located primarily in the smooth endoplasmic reticulum. Each bilirubin molecule binds two glucuronic acid molecules. The source of glucuronic acid is uridine diphosphoglucuronic acid (UDPGA). The bilirubin glucuronide, which is more water soluble than free bilirubin, is then transported against a concentration gradient by a presumably active process into the bile canaliculi. Most of the bilirubin glucuronide passes via the bile ducts to the intestine.

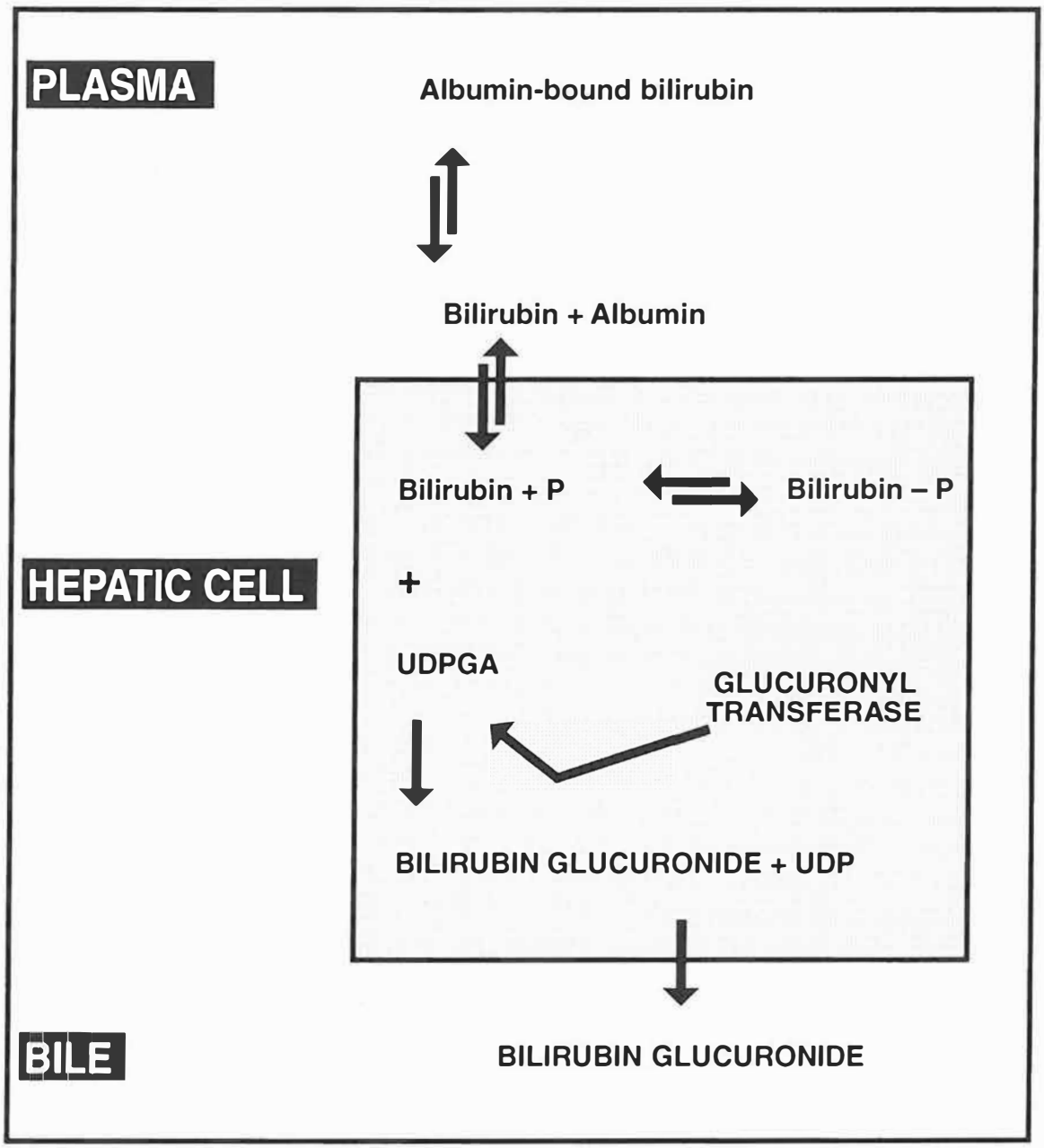



FIG 3

# METABOLISM AND EXCRETION OF BILIRUBIN

Modified from Ganong, Textbook of Medical Physiology, 11th Ed., 1983

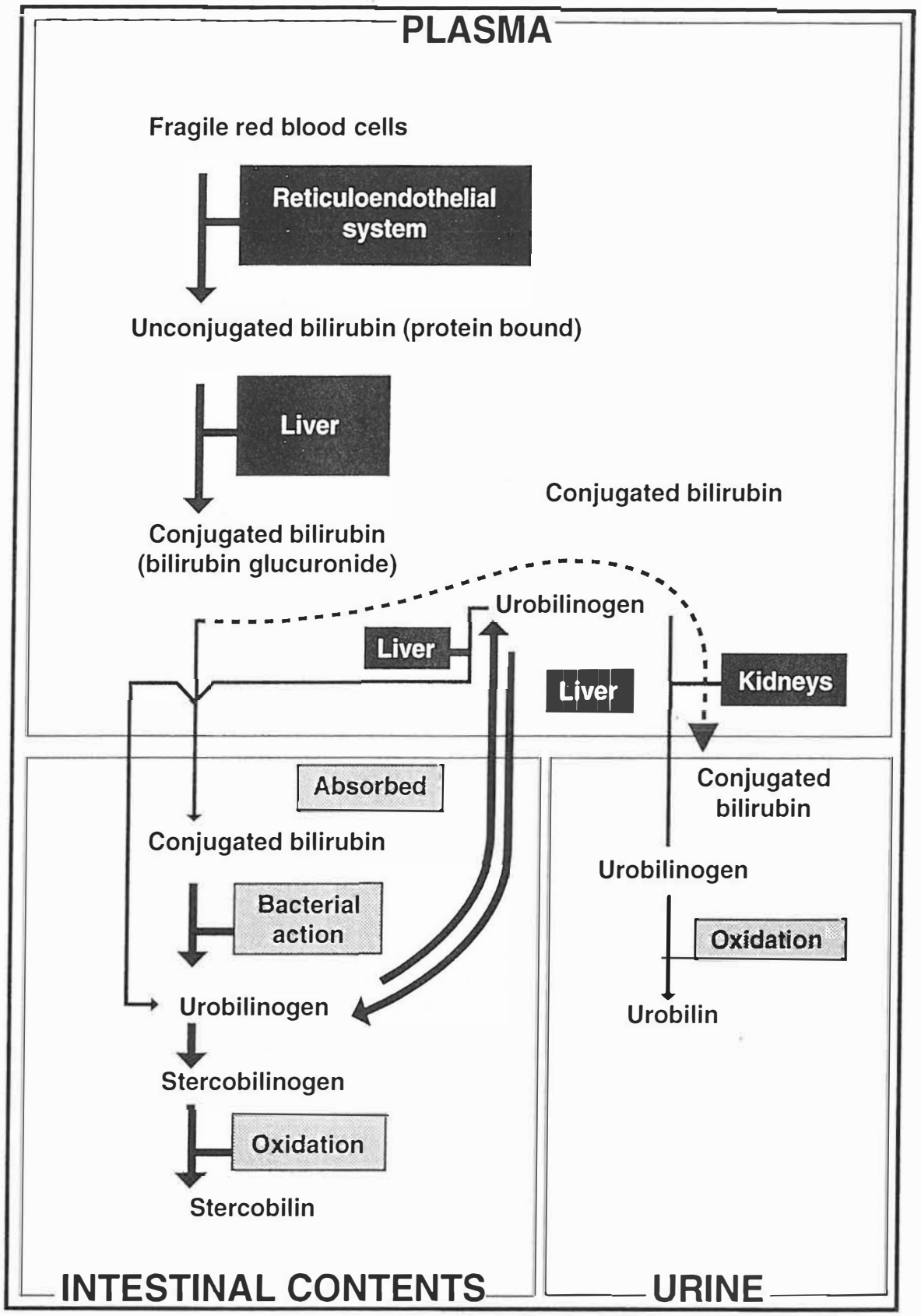



FIG 4

# LIFE CYCLE OF BILIRUBIN

Modified from Guyton, A.C.: Textbook of Medical Phsyiology, 6th ed. Philadelphia. W.B. Saunders Company, 1981.

The intestinal mucosa is relatively impermeable to conjugated bilirubin but is permeable to unconjugated (indirect) bilirubin and to urobilinogens, a series of colourless derivatives of bilirubin formed by the action of bacteria in the intestine. Consequently some of the bile pigments and urobilinogens are reabsorbed into the portal circulation. Some of the reabsorbed substances are again excreted by the liver (enterohepatic circulation), but small amounts of urobilinogen enter the general circulation and are excreted in the urine. In conclusion plasma bilirubin normally includes free bilirubin plus conjugated (direct) bilirubin. The life-cycle of bilirubin is summarised in Figure 4.

The free bilirubin is measured as the indirect-reacting bilirubin and the conjugated bilirubin is measured as the direct reacting bilirubin *(Guyton, Textbook of Medical Physiology, 6th edition, 1981).*

## 2.2.5 ETIOLOGY OF NEONATAL JAUNDICE (TABLE II)

The etiology of neonatal jaundice as described in *Nelson, Textbook of Paediatrics, 13th edition, (1987)* is summarised below. The metabolism of bilirubin in the newborns is in transition from the foetal stage, during which the placenta is the principal route of elimination of the lipid soluble bilirubin, to the adult stage, during which the water soluble conjugated form is excreted from the hepatic cell, into the biliary system and then into the gastrointestinal tract.

Jaundice may be due to any factor that:

1. Increases the load of bilirubin to be metabolised by the liver, e.g.
    a. haemolytic anaemias
    b. shortened red cell life owing to immaturity or to transfused cells
    c. increased enterohepatic circulation
    d. infection

2. May damage or reduce the activity of the enzyme glucuronyl transferase, e.g.
    a. hypoxia
    b. infection
    c. possible hypothermia
    d. thyroid deficiency

3. May compete for or block the enzyme, e.g.
    a. drugs
    b. other substances requiring glucuronic acid conjugation for excretion

TABLE II

## DIAGNOSTIC FEATURES OF THE VARIOUS TYPES OF NEONATAL JAUNDICE

| DIAGNOSIS | NATURE OF VAN BERGH REACTION | PEAK BILIRUBIN CONC. | | PEAK BILIRUBIN CONC. | | BILIRUBIN RATE OF ACCUMULATION | REMARKS |
|---|---|---|---|---|---|---|---|
| | | *APPEARS* | *DISAPPEARS* | *mg/dL* | *AGE IN DAYS* | | |
| 1. "Physiologic jaundice": | | | | | | | 1. Usually relates to degree of maturity |
| Full-term | Indirect | 2-3 days | 4-5 days | 10-12 | 2-3 | <5 | |
| Premature | Indirect | 3-4 days | 7-9 days | 15 | 6-8 | <5 | |
| 2. Hyperbilirubinemia due to metabolic factors, etc: | | | | | | | 2. **Metabolic factors:** Hypoxia, respiratory distress, lack of carbohydrate. |
| Full-term | Indirect | 2-3 days | Variable | >12 | 1st week | <5 | **Hormonal Influences:** cretinism, hormones. |
| Premature | Indirect | 3-4 days | Variable | >15 | 1st week | <5 | **Genetic factors:** Crigler-Najjar syndrome, transient familial hyperbilirubinemia. **Drugs:** Vitamin K. Novobiocin |
| 3. Hemolytic states and hematoma | Indirect | May appear in 1st 24 hrs | Variable | Unlimited | Variable | Usually >5 | 3. **Erythroblastosis:** Rh, ABO. **Congenital hemolytic states:** spherocytic, nonspherocytic, Infantile pyknocytosis. **Drugs:** Vitamin K. Enclosed hemorrage - hematoma. |
| 4. Mixed hemolytic and hepatotoxic factors | Indirect and direct | May appear in 1st 24 hrs | Variable | Unlimited | Variable | Usually >5 | 4. **Infection:** bacterial sepsis, pyelonephritis, hepatis, toxoplasmosis, cytomegalic inclusion disease, rubella **Drugs:** vitamin K. |
| 5. Hepatocellular damage | Indrect and direct | Usually 2-3 days | Variable | Unlimited | Variable | Variable can be >5 | 5. Biliary atresia; galactosemia; hepatitis and infection as in (4). |

Modified from Nelson Textbook of Paediatrics 13th Edition - Behrman, Vaughan

4. Leads to an absence of or decreased amount of the enzyme or to the reduction of bilirubin uptake by the liver cells, e.g.
   a. genetic defect
   b. prematurity

The risks of toxic effects from elevated levels of bilirubin in the serum is increased by factors that :

1. Decrease the retention of bilirubin in the circulation e.g. hypoproteinaemia, displacement of bilirubin from its binding sites on albumin by competitive binding of drugs such as sulfisoxazole, acidosis, hyperosmolality, increased free fatty acid concentration secondary to hypoglycaemia, starvation or hypothermia;

2. Increase the permeability of the blood-brain barrier or nerve cell membranes to bilirubin or the susceptibility of brain cells to its toxicity such as asphyxia, prematurity and infection.

The differential diagnosis of neonatal jaundice is summarised in Table III.

TABLE III

## DIFFERENTIAL DIAGNOSIS OF NEONATAL JAUNDICE

### A. Present at birth or <24 hours

1. Erythroblastosis foetalis
2. Concealed haemorrhage or extensive ecchymosis/ haematoma
3. Sepsis
4. Cytomegalic inclusion disease, rubella, congenital toxoplasmosis
5. Intrauterine transfusions (high portion of direct reaction bilirubin)

### B. Present Day 2 – 3

1. Physiologic (commonest)
2. Hyperbilirubinaemia of the newborn (more severe form)
3. Familial nonhaemolytic icterus viz. Criggler-Najjar syndrome

### C. Present Day 4 – 7

1. Septicaemia
2. Other infections eg. syphilis, toxoplasmosis, CMV inclusion disease

### D. Present after 1st week

1. Breast milk jaundice
2. Acute bacterial infections – Septicaemia, UT.I.
3. Congenital Biliary Atresia
4. Hepatitis
5. Rubella, Chronic Herpetic Hepatitis
6. Galactosaemia
7. i) Congenital Haemolytic anaemia (Spherocytosis)
   ii) Crises of other haemolytic anaemias eg. pyruvate kinase and other glycolytic enzyme deficiencies, thalassaemia, sickle cell disease, hereditary nonspherocytic anaemia.
   iii) Haemolytic anaemia due to drugs eg. in congenital deficiencies of glucose–6–phosphate dehydrogenase, glutathione synthetase, reductase or peroxidase.

### E. Persistent jaundice During 1st Month

1. Inspissated bile syndrome (which may follow Haemolytic disease of the newborn)
2. Hyperalimentation associated cholestasis
3. Hepatitis
4. CMV Inclusion disease, Toxoplasmosis, Syphilis
5. Familial non haemolytic icterus
6. Congenital Biliary Atresia
7. Galactosaemia
8. Physiological (very rare – especially in hypothyroidism and pyloric stenosis)

## 2.2.6 CLINICAL CONSEQUENCES OF MODERATE HYPERBILIRUBINAEMIA

*Newman and Maisels, (1992)* in their analysis of various studies, demonstrated that no evidence exists to support the view that bilirubin levels of 20mg/dl (340umols/l) are hazardous to healthy term infants nor does any evidence suggest that phototherapy has affected cognitive or neurologic outcome in any way.

### 1. Term Infants

While minor delays in motor development have been demonstrated during the first year, this delay was not apparent with long term follow up. Associated conditions such as sepsis, anoxia and acidosis may increase the likelihood of neurotoxicity of bilirubin in these infants *(Kim, et al. 1980)*.

### 2. Pre-term Infants

In this sub group, the neurotoxic consequences are unclear. No acute clinical syndrome is recognisable during the first weeks. The commonest consequence is hearing loss *(Perlman, et al. 1983)*.

## 2.2.7 CLINICAL FEATURES OF BILIRUBIN ENCEPHALOPATHY

The features of bilirubin encephalopathy vary depending on the age of the infant and the degree of hyperbilirubinaemia.

### 1. Neonatal Period

In term infants, the clinical features are well documented by Van Praagh (1961) in a study

of 31 kernicteric infants with haemolytic disease. He described the progression through three distinct clinical phases in 18 children who survived the neonatal period.

### First Phase

Stupor, hypotonia and poor sucking were present in the first few days. In Van Praagh's series, 3 infants died during this 1st phase. Although these signs are non-specific, their presence is associated with hyperbilirubinaemia encephalopathy. Infants who survive and progress to the 2nd phase have a worse prognosis.

### Second Phase

Hypertonia and fever develop after the first several days. Hypertonia involves extensor muscle groups, with most infants exhibiting retrocollis (backward arching of the neck) and opisthotonos (backward arching of the trunk). Fever which occurred in 80% of the infants studied by Van Praagh (1961) did not relate to any recognised cause and may have been related to the diencephalic involvement of kernicterus.

### Third Phase

This phase is characterised by diminution or disappearance of hypertonia which usually occurs after the first week. Although the apparent normalisation of tone is occasionally interpreted as evidence against kernicterus, in fact all infants who develop hypertonia of the second phase ultimately develop chronic postkernicteric bilirubin encephalopathy *(Johnston, 1967)*.

## 2. Post-Neonatal Period

The period following the acute phase is referred to as chronic postkernicteric bilirubin encephalopathy with the following consequences:

### Extrapyramidal Disturbances

Choreoathetosis in particular, may develop as early as 18 months but occasionally is delayed until as late as 8 or 9 years. In several affected children, choreoathetosis may prevent useful limb function and these unfortunate children may also have severe dysarthria, facial grimacing, drooling, difficulty in chewing and swallowing *(Byers, et al. 1955)*.

### Auditory Abnormalities

Hearing loss may be the only clinical manifestation due primarily to injury to the brainstem cochlear nuclei. This is the commonest neurological abnormality in children with chronic bilirubin encephalopathy *(Johnston, 1967)*.

### Ocular Abnormalities

These are characterised by:

1. Limitation of upward gaze (common).
2. Paralytic gaze palsies.

### Intellectual Deficits

Only the minority of affected neonates have major deficits. This relative sparing of intellect in these term infants is consistent with the pathologic observation of relative sparing of the cerebral cortex *(Byers, et al. 1955)*.

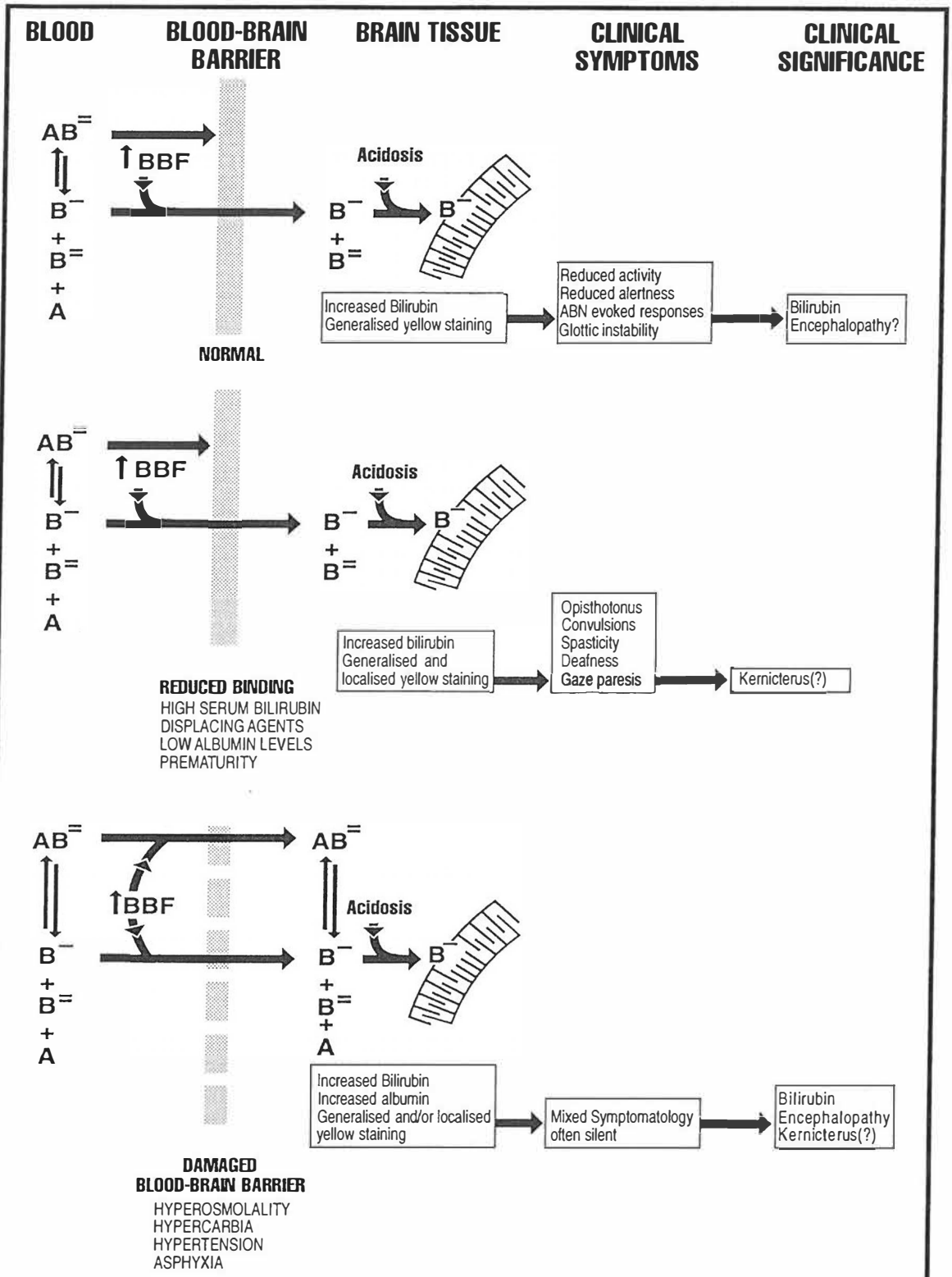
## 2.2.8 THE PASSAGE OF BILIRUBIN INTO THE BRAIN

Bilirubin can enter the brain via 3 different mechanisms (Figure 5). Firstly, a steady passage of unbound bilirubin crosses the blood brain barrier even during physiologic hyper-bilirubinaemia. This probably colours the brain as well as the CSF yellow. General and regional increases in brain blood flow will increase passage of bilirubin across the blood brain barrier, owing to increased bilirubin delivery.

The pathologic significance of this normal brain bilirubin deposition is uncertain. Under normal circumstances, this low level of bilirubin in the brain is probably not harmful. However, significant behaviourial and neurophysiologic effects of the physiological "brain icterus" has been demonstrated in jaundiced neonates. The response to external stimuli, visual orientation, attention, alertness and motor performance have been found to be lower in jaundiced infants *(Ebbesen and Brodersen, 1982)*.

Although these changes have usually been found to be reversible, it is not known if this also holds true for prolonged hyperbilirubinaemia. Thus it has been suggested that not only the peak serum bilirubin concentration but also the duration of hyperbilirubinaemia in the infant correlates with neurodevelopmental outcome *(Rye, 1981)*.

Secondly, increased levels of unbound bilirubin in the blood can be seen in different pathologic conditions. These conditions will therefore dramatically increase the entry of bilirubin into the brain, similarly increasing binding to the nerve cells. The high concentrations of unbound bilirubin will finally saturate the membranes and cause precipitation of bilirubin acid *(Wennberg, 1988; Vazquez, 1988)*.




Schematic representation of possible mechanisms for bilirubin entry into the brain and binding to neuronal cell membranes. The different factors affecting this process are also indicated. Abbreviations: A=albumin; $AB^-$=albumin-bilirubin complex; $B^-$=bilirubin monoanion; $B^=$=bilirubin dianion; BBF=brain blood flow

**FIG 5**

# MECHANISMS FOR THE PASSAGE OF BILIRUBIN INTO THE BRAIN

Modified from Clinics of Perinatology, June 1990

This condition represents the classical picture of kernicterus, seen mainly in rhesus isoimmunisation in which the serum bilirubin concentrations exceeds the binding capacity of the albumin molecule.

Such increases in free bilirubin concentrations are also seen in the following situations:

1. The sick infant whose albumin binds less bilirubin.
2. The premature infant due to low serum albumin.
3. Bilirubin displacement by drugs and free fatty acids (FFA).

Finally, bilirubin can enter the brain through a disrupted blood-brain barrier. In the newborn, this is probably most often caused by hypoxaemia, hypercarbia and hyperosmolar conditions, but it is also possible that hyperthermia and septicaemia have similar effects.

The last few decades of bilirubin research seem to have clarified the basic mechanisms by which bilirubin enters the brain. Clinical studies have so far failed to show any value of the presently known risk factors in predicting bilirubin toxicity in the newborn infant. This indicates that bilirubin entry into the brain does not necessarily equal bilirubin toxicity *(Kim, et al. 1980)*. Future research must therefore concentrate on further clarifying the molecular basis for the interaction between the nerve cell membrane and bilirubin as well as bilirubin effects on nerve cell metabolism.

### 2.2.9 DOES HYPERBILIRUBINAEMIA DAMAGE THE BRAIN OF HEALTHY, FULL-TERM INFANTS?

In the 1950's, exchange transfusion to keep the total serum bilirubin (TSB) level below 20mg/dL (340 umol/l) was shown to be an effective way of preventing kernicterus in babies with erythroblastosis. Subsequently, several investigators showed that babies without Rhesus disease were less likely to develop kernicterus and could be managed more conservatively *(Mollison, et al. 1952)*.

Thereafter, several post-mortem observations of bilirubin staining of the brain in premature infants with low bilirubin levels raised new concerns about possible "brain damage" in surviving infants who had low levels of bilirubin in the newborn period.

This information was erroneously extrapolated to healthy full-term infants and undoubtedly spurred on by medicolegal pressures, the exchange-transfusion threshold for these infants crept downwards. With the introduction of phototherapy, serum bilirubin was lowered painlessly, and paediatricians could not be blamed for assuming that it thus reduced the risk of bilirubin induced brain damage.

However, *Newman and Maisels (1992)* after careful analysis of numerous studies, concluded that no evidence exists to support the view that bilirubin levels of 20mg/dl is hazardous to healthy term infants nor does any evidence suggest that phototherapy has affected cognitive or neurologic outcome in any way.

The implications of confounding or effect modification by prematurity and haemolysis have in the past either not been recognised or have received insufficient attention. Premature babies and infants with haemolytic disease have to be considered separately. If they are not, the relationship between bilirubin and adverse outcome in well babies is likely to be overestimated.

Thus, the aggressive treatment of jaundice in term babies without haemolysis is now being questioned. *Newman and Maisels (1992)* have shown that good evidence exists that such babies are not at risk of mental or physical impairment until serum bilirubin levels rise well above 20mg/dl.

Hence, before we treat the thousands of babies in our nurseries who daily manifest this benign form of neonatal jaundice, we should be sure that the potential benefits of our interventions outweigh the risks. Current evidence suggests that this is not the case.

## 2.3 Pharmacologic Treatment Of Neonatal Jaundice

### 2.3.1 MATERNAL PHENOBARBITONE TREATMENT FOR THE PREVENTION OF NEONATAL JAUNDICE

#### 2.3.1.1 Antenatal Phenobarbitone

The practical impact of antenatal phenobarbitone prophylaxis of neonatal jaundice was studied in Greek neonates, a population characterised by a high incidence of marked jaundice and kernicterus due to a high incidence of glucose-6-phosphate dehydrogenase deficiency (G-6-PD)

*(Valaes, et al. 1969)*. The large sample size (total 3075) allowed confident conclusions to be drawn regarding the effectiveness of the treatment in reducing the occurrence of dangerous hyperbilirubinaemia from all causes in term infants in the pre-phototherapy era *(Valaes, et al. 1980)*.

In the infants of mothers who received at least ten nightly doses of 100mg phenobarbitone, marked jaundice and exchange transfusion was reduced six fold in comparison to the control group. On the other hand the frequency of jaundice was not significantly reduced among the 212 infants of the mothers who received less than 1g phenobarbitone in total over the 10 day study period *(Valaes et al.1980)*.

For most western countries, the advent of phototherapy removed the emphasis for the prevention of neonatal jaundice and thus interest in antenatal phenobarbitone has waned. Whether antenatal phenobarbitone should be recommended for general use; restricted to predictable high risk groups or rejected depends on:

1. The immediate and long term safety of the treatment.
2. The availability, costs, and relative merits of alternative methods of preventing or treating neonatal hyperbilirubinaemia.

For the Third World countries the low cost and simplicity of antenatal phenobarbitone for the prevention of neonatal jaundice make it extremely attractive in comparison to the alternative of developing facilities for adequate monitoring of bilirubin levels and providing the necessary phototherapy units.

Evidence supports the hypothesis that antenatal phenobarbitone is effective in decreasing the severity of periventricular - intraventricular haemorrhage (PIVH) in low birthweight neonates *(Barnes and Thompson, 1993)*. However, the decision whether antenatal phenobarbitone has a role in the management of neonatal jaundice depends on reaching a firm conclusion regarding its safety. In this regard, the authors caution that further data are necessary regarding the incidence of low Apgar scores and respiratory depression in low birthweight neonates given antenatal phenobarbitone.

#### 2.3.1.2 Postnatal Phenobarbitone

The postnatal use of phenobarbitone offers the advantage of focusing the treatment to easily identifiable high risk groups and particularly the pre-term infants who are not covered by the schedules for antenatal prophylaxis. One disadvantage, however, is a time lag between the administration of phenobarbitone and the expression of its effect on bilirubin elimination.

In contrast, phototherapy accelerates bilirubin elimination immediately, and for this reason has practical advantages over the induction of bilirubin clearance by phenobarbitone or other agents. The extent of individual variation in bilirubin values coupled with the paucity of pharmacokinetic data in newborns make it impossible to correlate bilirubin and phenobarbitone levels meaningfully.

Thus no recommendation can be made regarding the dose and schedule of administration that will maximise the effect on bilirubin clearance from previous studies.

The assumption that the dose should be the same as that used for sedation or control of seizures is unlikely to be correct.

From the available data it appears that the method of administration, orally or intramuscularly, does not influence the effect. The dosages of 5-10mg/kg/day are probably equally effective and a short treatment of 10mg/kg/day for two days may be equally effective as the more prolonged treatment with lower dosage (Table I).

Thus it appears that both plasma phenobarbitone levels and the length of exposure to high levels are important in determining the effect on bilirubin clearance. The effect of a single dose of 12mg/kg phenobarbitone given intramuscularly is still unknown. However, doses of 15-20mg/kg was required to achieve a plasma concentration in the range of 9-25mg/l *(Painter, et al. 1989).*

The choice of 12mg/kg dose of phenobarbitone in the present study is within the range used clinically without producing adverse effects *(Painter, et al. 1989).*

Another question not adequately answered is the earliest gestational age at which a good response to inducing agents can be obtained. It has been shown that the uridine-diphoshpo-glucuronyl transferase (UDP-GT) activity increases after birth irrespective of gestational age, indicating that the ability to respond to the endogenous agents is not related to maturity. This conclusion is blurred by the simultaneous use of phototherapy, which has gained general acceptance as its efficacy is firmly established.

### 2.3.1.3 Combination Therapy

The combination of antenatal and postnatal phenobarbitone was found to be the most effective schedule in infants without haemolytic disease and greater than 2.5kg at birth *(Trolle, 1968)*. From the practical viewpoint, such a schedule is suitable for the management of pre-term labour and threatened delivery in combination with tocolysis and steroids. This schedule was found to reduce the frequency and severity of intraventricular haemorrhage in comparison to any other postnatal treatment. If these results are confirmed by other studies, the combined benefits of the treatment in reducing intraventricular haemorrhage and in accelerating bilirubin clearance may outweigh any possible risks.

## 2.3.2 RECENT THERAPEUTIC MODALITIES

A number of agents modify bilirubin metabolism indirectly (Table IV) such as barbiturates, agents acting by interference with enterohepatic circulation of bilirubin, sequestrating agents, inhibitors of heme oxygenase and intravenous immune globulin therapy.

1. The combination of diethylnicotinamide (coramine) and phenobarbitone proved effective and for some time became the standard treatment for hyperbilirubinaemia in several centres in Europe *(Ertel, et al. 1969)*. The combination of nicotinamide with phenobarbitone offered the advantages of the additive effect of two inducing agents, while each of the agents was expected to neutralise the side effects of the other. The sedative effect of phenobarbitone was countered by the stimulant effect of nicotinamide and vice versa.

TABLE IV

## PHARMACOLOGIC AGENTS MODIFYING BILIRUBIN METABOLISM AND ELIMINATION

| METABOLIC STEP | AGENTS | EFFECT |
|---|---|---|
| **Bilirubin production** | Synthetic metalloporphyrins | |
| Heme degradation | Tin-protoporphyrin | Inhibition of heme oxygenase |
| Alternative pathway of heme elimination | Tin-mesoporphyrin | |
| | Zinc-protoporphyrin | Excretion of heme in bile |
| **Hepatic bilirubin transportation** | Inducers of hepatic endoplasmic reticulum enzymes | Accelerated |
| Bilirubin uptake | eg. Phenobarbital, Nicotinamide, etc. | – Uptake |
| Bilirubin conjugation | | – Conjugation |
| Excretion of conjugated bilirubin | | – Excretion of bilirubin |
| Alternative pathways of bilirubin disposition | Phototherapy | Photoisomerization and excretion of bilirubin |
| **Enterohepatic circulation of bilirubin** | | |
| | Agar, cholestyramine, and charcoal | Sequestration of bilirubin in the bowel |
| | Bilirubin oxidase | Degradation of bilirubin in the bowel |

(Modified from Clinics of Perinatology, June 1990)

2. Charcoal was the first of the adsorptive agents that was tested in human neonates *(Ulstrom, et al. 1964)*. In normal term infants a depression of peak plasma bilirubin by 30% was observed in the infants given activated charcoal at 4 hours of life, whereas no effect was seen in those fed charcoal at 12 hours.

3. A barbiturate derivative, bucolome was used for the prevention of hyperbilirubinaemia in term and pre-term infants *(Baba, 1972)*. However, in kernicteric rats, bucolome produced an immediate decrease in plasma bilirubin levels. This was coupled with an increase in plasma unbound bilirubin, cerebellar bilirubin content and hence subsequent increase in mortality. Hence its use is now not currently recommended because of these potential adverse effects.

4. Agar, a polysaccharide extracted from seaweed, has been studied extensively in neonatal jaundice *(Blum and Etienne, 1973)*. It binds unconjugated bilirubin with a high affinity in aqueous solutions. However, clinical studies have not shown any benefit *(Odell, et al. 1983)*.

5. Cholestyramine, a cationic resin, was also studied as an adjuvant to phototherapy in neonatal jaundice *(Nicolopoulos, et al. 1984)*. However, it produced hyperchloraemic acidosis and these infants required intravenous sodium bicarbonate.

6. Bilirubin oxidase, an enzyme isolated from the fungus *myrothecium verrucarea* and from orange peels degrades bilirubin and is resistant to proteolytic enzymes *(Murao and Tanaka, 1981)*.

Further studies are required to determine the potential clinical application of bilirubin oxidase in the management of hyperbilirubinaemia.

7. The use of specific competitive inhibitors of heme oxygenase, the rate limiting enzyme in the catabolism of heme, to decrease the rate of heme degradation to bilirubin in the neonatal period, when bilirubin production exceeds the capacity for its disposition, represents a new therapeutic modality in the clinical management of neonatal hyperbilirubinaemia *(Drummond and Kappas, 1981)*. Tin-protoporphyrin (SnPP), tin-mesoporphyrin (SnMP), and other synthetic analogues of the natural metalloporphyrin (ferroporphyrin - heme) are potent competitive inhibitors of heme oxygenase.

In contrast to other methods of controlling dangerously elevated serum levels of bilirubin, such as exchange transfusion, phototherapy and phenobarbitone, which are directed towards disposing of the excessive preformed bilirubin, competitive inhibition of heme oxygenase acts by suppressing the formation of bilirubin. SnPP and SnMP bind more avidly than heme to the catalytic site of heme oxygenase, but because the central metal atom, tin, cannot bind molecular oxygen, the enzymatic degradation of the metalloporphyrin to bilirubin does not occur. The inhibition of heme degradation to bilirubin does not result in the accumulation of heme. Instead, SnPP increased the bilirubin excretion of heme in amounts that fully compensated for the decreased excretion of bilirubin.

The ability of SnPP to ameliorate the course of hyperbilirubinaemia in neonates maybe considered to be the net result of complex events that most likely include:

a. intensification and prolongation of the alternative pathway of heme excretion in the bile with commensurate reduction in bilirubin formation
b. increased elimination of heme in faeces
c. enterohepatic circulation of heme
d. heme degradation by the developing bacterial flora.

Recently it has been reported that the prolonged use of a heme oxygenase inhibitor resulted in haematological and iron metabolic indices consistent with the development of reversible iron deficiency state *(Attalah, et al. 1993)*.

Thus in conclusion, the competitive inhibition of heme oxygenase by synthetic metalloporphyrins offers a simple solution, but the efficacy achieved with the doses and compounds used thus far are not comparable with that of phenobarbitone.

8. More recently, a multicentre controlled trial evaluated the use of high dose intravenous human-derived immunoglobulin (HDivIg) therapy *(Jochen, et al. 1992)* for the treatment of hyperbilirubinaemia caused by rhesus haemolytic disease. It was concluded, that immunoglobulin therapy, by a yet unknown mechanism, reduces serum bilirubin levels and consequently, the need for blood exchange transfusions, in children with rhesus haemolytic disease. No side effects were observed. The optimal dose of HDivIg therapy, the most efficacious number of infusions, cost effectiveness, and the best preparation remains to be determined. Presently, a similar study is underway at the University of Natal, Medical School on the use of HDivIg for neonatal hyperbilirubinaemia.

### 2.3.3 CONCLUSION

In conclusion, we cannot assume that 1 or 2 days of phototherapy, the interruption of nursing for 48 hours or merely an expression of concern about jaundice are benign events that have no adverse consequences for mothers and infants in the early postnatal period.

In fact the mere presence of neonatal jaundice (and the aura of alarm that it engenders) has negative consequences for maternal behaviour and attitude in the early crucial months of infant development. Laboratory investigations are initiated and often repeated in normal infants with physiological jaundice. These tests are painful for the infant, distressing to the parents, costly and rarely enlightening. Consequently, a simple effective regimen with a single dose of phenobarbitone will obviate these traumatic invasive investigations.

If parenteral phenobarbitone is shown to effectively decrease bilirubin levels, the potential benefits would be a decrease in exchange transfusion rates, decrease duration of hospital stay, fewer days of phototherapy with its associated side-effects. If the research shows that there is a reduction in serum bilirubin levels and or duration of hospital stay, it would be quite justifiable to use phenobarbitone rather than phototherapy in this common situation where bilirubin is regarded as being benign.

## 2.4 Phenobarbitone

### 2.4.1 HISTORY

Phenobarbitone was the first effective organic anti-epileptic agent *(Hauptmann, 1909)*. It has a relatively low toxicity, is inexpensive, and is still an effective and widely used drug for this purpose.

### 2.4.2 CHEMISTRY

Phenobarbitone is also known as phenobarbital (Luminal®, Gardenal®). The structural formula of phenobarbitone (5-phenyl-5-ethylbarbituric acid) is shown in Figure 6.

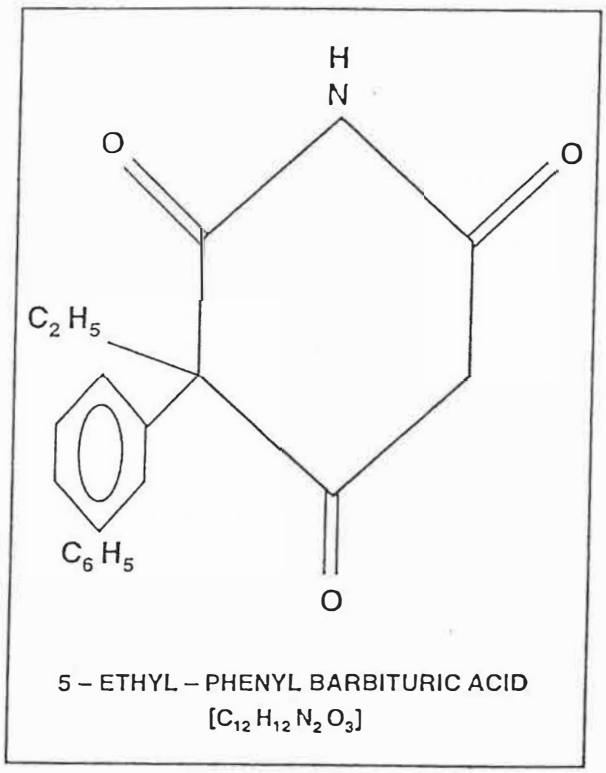



FIG 6

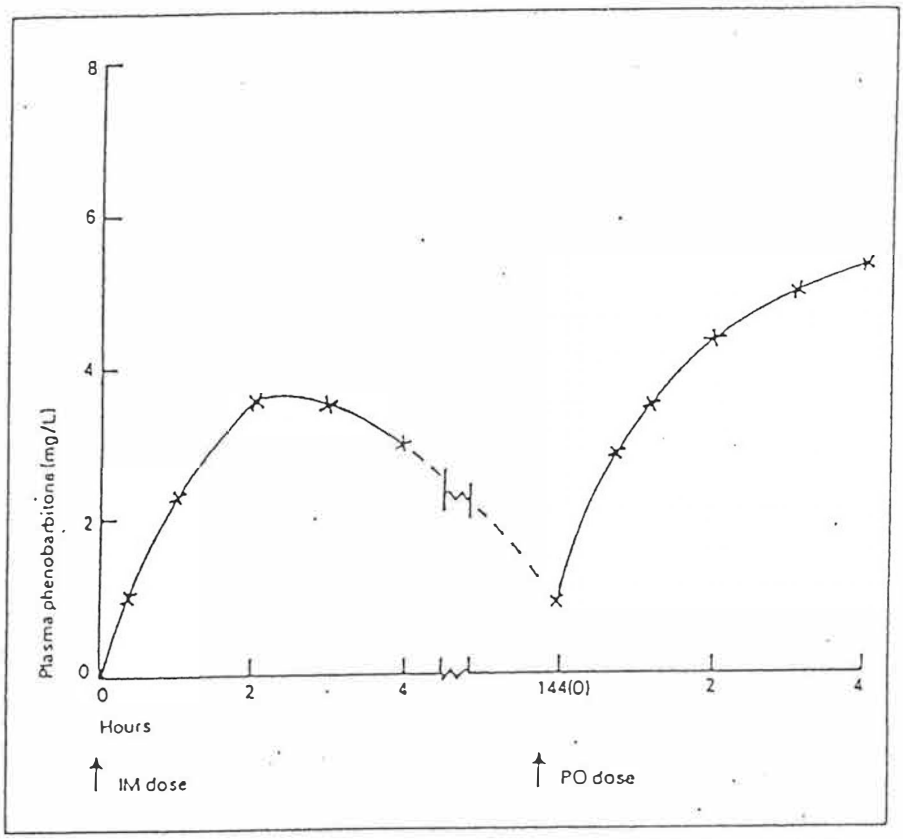
STRUCTURE OF PHENOBARBITONE

### 2.4.3 PHARMACOKINETICS

Table V describes the pharmacokinetic parameters of phenobarbitone *(Miller and McFadyen, 1992)*.

### 2.4.3.1 Absorption

Phenobarbitone is well absorbed after oral, rectal and intramuscular administration. Peak plasma concentration of phenobarbitone usually occurs 6-18 hours after oral dosing though shorter times to peak plasma level have been reported *(Lous, 1954 and Wilensky, et al. 1982)*. After intramuscular administration peak plasma concentration occurs 0.5-3 hours after dosing *(Boreus, et al. 1978; Graham, 1978)* (see illustration below).




Time course of plasma phenobarbitone levels after 180mg of the drug given by the intramuscular and oral routes, 144 hours apart.

Serum half-life, may be up to about 75 hours in children. After an intramuscular dose of 10mg per kg, plasma concentration of 9, 12 and 13ug/ml are obtained at 30, 90 and 120 minutes respectively *(Gal, 1986)*. Extensive literature searches were unsuccessful in providing pharmacokinetic data on South African Black neonates.

TABLE V

## PHARMACOKINETIC PARAMETERS OF PHENOBARBITONE

| | |
|---|---|
| **Therapeutic Range** | |
| Mg/L | 10 – 30 |
| ummo1/L | 40 – 130 |
| Conversion factor | 4.3 |
| Bioavailability (F) | 1 |
| Salt factor | 0.91 |
| **Volume of distribution (L/kg)** | |
| Children | 0.70 ± 1.0 |
| Neonates | 0.97 ± 0.20 |
| **Clearance (L/hr/kg)** | |
| Adults | 0.00372 |
| Children | 0.0082 |
| Free fraction | 0.5 |
| **Half-life (hrs)** | |
| Adults | 96 |
| Children | 62 |
| Neonates | 103 – 115 |
| Time to steady state (days) | 10 – 16<br>(3 – 4 weeks) |
| Time to peak (hrs) | 1 – 3 |
| **Concentrations (%)** | |
| in CSF | 43 – 52 |
| in saliva | 30 – 38 |
| Elimination in urine (%) | 20 – 40 |

Modified from: A Practitioners guide to
Optimum use of Anti-Epileptic drugs, 1992 (Miller & McFadyen)

### 2.4.3.2 Distribution

Phenobarbitone distributes readily into most body tissues and approximately 50% is bound to plasma proteins. The distribution is sensitive to variations in the pH of plasma with acidosis and alkalosis causing increased tissue and serum concentrations respectively *(Gal, 1986)*. The concentration of phenobarbitone in the cerebrospinal (CSF) averages between 43 and 52% of that in the plasma, and salivary concentrations are between 30 and 38% of the plasma concentrations *(Eadie, 1968)*. The apparent volume of distribution (Vd) of phenobarbitone is 0.7 to 1.0 l/kg *(Hvidberg and Dam, 1976)* with studies in neonates providing a mean value of 0.97 $\pm$ 0.20 l/kg *(Pitlick, et al. 1978)*.

### 2.4.3.3 Metabolism

The metabolism of phenobarbitone has been reviewed by Whyte, et al. (1977). Most of the drug is probably eliminated by metabolism as little appears in the urine unchanged. The main oxidised metabolite is a phenolic derivative. About 30% of a dose may appear as the 1-N-glucoside metabolite.

### 2.4.3.4 Elimination

Phenobarbitone elimination follows linear pharmacokinetics. Approximately 20-40% of administered phenobarbitone is excreted in the urine while the remainder is metabolised in the liver to inactive metabolites. In general, clearance varies with age. It is fastest in children (8.2ml/hr/kg) and slower in adults (3.2ml/hr/kg). Clearance is highly variable in neonates and is on average similar to adults. This is reflected in the half-life which is 96 hours in adults, 103 to 115 hours in neonates and 62 hours in children *(Pitlick, et al. 1978)*. Due to long half-life and small fluctuations in plasma concentrations, phenobarbitone can be administered as a single dose at night in most children *(Davis, et al. 1981, Walson, et al. 1980)*.

### 2.4.4 ENZYME INDUCTION AND MODE OF ACTION

Many enzymes are able to increase in amount and activity in response to certain substances known as inducers. The liver microsomal cytochrome P-450 system is readily induced by many drugs and other exogenous compounds. Phenobarbitone is an example of an efficient enzyme inducer. Other examples are phenylbutazone, phenytoin and polycyclic hydrocarbon insecticides, such as DDT and chlordane.

Hydrocarbons in tobacco smoke are very strong inducers of the microsomal drug-metabolising system *(Goodman and Gilman, 1985)*. Several studies have shown that smokers have a more rapid biotransformation of drugs (shorter plasma half life) than do non-smokers.

Most of the hepatic drug metabolising enzymes are induced by phenobarbitone and thus the metabolism of a wide range of drugs is enhanced by phenobarbitone pre-treatment. The common drugs involved are paracetamol, phenytoin and warfarin. Enzyme induction by phenobarbitone has been applied to the treatment of hyperbilirubinaemia in which phenobarbitone induces the activity of glucuronyl transferase which converts bilirubin to the glucuronide *(Levi, et al. 1970)*. ***It is this enzyme inducing property of phenobarbitone which forms the basis of this study.***

### 2.4.5 ADVERSE EFFECTS

Potentially life threatening effects although rare include exfoliative dermatitis, agranulocytosis, aplastic anaemia and hepatitis *(Schmidt, 1985)*. Adverse effects of barbiturates include respiratory depression, sedation, and occasional allergic reactions particularly affecting the skin (1-3%). With excessive doses, irritability and hyperexcitability, nystagmus, ataxia have been reported particulary in children. Hypoprothrombinaemia has occurred in infants of mothers who had received phenobarbitone in pregnancy *(Mountain, et al. 1970)*.

### 2.4.6 SAFETY OF PERINATAL PHENOBARBITONE

Barbiturates, being among the most frequently used drugs during pregnancy and the perinatal period, have been studied extensively regarding their potential for immediate and long term untoward effects. In humans, few immediate and no long term undesirable effects have been described after many years of therapeutic use of phenobarbitone in the perinatal period. Moreover, the use of phenobarbitone for the prevention of neonatal jaundice constitutes, in many ways, a different type of perinatal exposure that results from its use in epileptic mothers, or for neonatal seizures or for intra-partum sedation and anaesthesia *(Goodman and Gilman, 1985)*. No sedation or withdrawal effects were observed after antenatal phenobarbitone for the prevention of neonatal jaundice. The absence of sedation was consistent with cord blood levels of 2-10mcg/ml. However, a sedative effect was observed with postnatal phenobarbitone particularly when high doses (20mg/kg) were used *(Guys Paediatric formulary, 1990)*.

Severe haemorrhagic disease of the newborn owing to depression of the vitamin K-dependant clotting factors is a well defined complication in infants of epileptic mothers treated with phenobarbitone and other anti-convulsants that have hepatic enzyme inducing properties. This may be due to hypoprothrombinaemia induced by phenobarbitone, leading to a bleeding tendency in the neonate.

The clotting abnormalities are rapidly corrected by the administration of 1mg of vitamin K given at birth. This still leaves the possibility of minor obstetric trauma producing life-threatening haemorrhage and administration of 10mg vitamin K to the mother is recommended *(Mountain, et al. 1970)*.

### 2.4.7 POPULATION PHARMACOKINETIC DATA ANALYSIS - THE NONMEM APPROACH

Population pharmacokinetics describes the variability a drug exhibits in terms of a number of factors called fixed and random effects.

The fixed effects are the population average values of pharmacokinetic parameters which may in turn be a function of various patient characteristics such as:

a. age, weight, height and sex
b. underlying pathology such as renal or hepatic impairment
c. other influences on drug disposition such as concomitant drug therapy, smoking and alcohol intake.

The random effects quantify the amount of pharmacokinetic variability which is not explained by the fixed effects i.e. inter- and intra-subject variability. Estimation of these fixed and random effects allows :

1. The design of dosage regimens which will, in general, suit patient groups who are at particular risk, e.g. the elderly or those with impaired renal or hepatic function.

2. The design of individual dosage regimens and their optimisation by means of Bayesian feedback techniques.

In order to determine the influence of fixed and random effects, the Nonlinear Mixed Effects Model (NONMEM) computer programme was developed by Beal and Sheiner in 1980. This programme treats the population as the unit of analysis, rather than the individual, and in general requires fewer data points per individual (but many more individuals) than are normally required in a standard pharmacokinetic study. In this way a more representative sample of the target population can be obtained and quantitative relationships between pharmacokinetic parameters and pathophysiologic features can be investigated in a single step. These relationships may then explain a considerable amount of inter-subject variability present in the population *(Sheiner, Rosenberg B and Marathe, 1977)*.

Pharmacokinetic studies are often not representative of the patient population in whom the drug is used therapeutically. The experimental protocol, for various reasons, has to exclude many of the clinical cases who are later treated with the drug. Consequently, investigations are performed in a more homogenous group of individuals, commonly healthy volunteers. Evaluation of the simplest pharmacokinetic model, the one-

compartment open model and i.v. bolus injection, requires at least 5 to 6 serum concentration measurements in each subject if reliable estimates of the two parameters, clearance and volume of distribution, are to be obtained. The NONMEM approach permits the use of unsystematically sampled serum concentrations and few measurements per individual to determine the mean population parameters and their inter-individual variability *(Sheiner, Rosenberg and Melmon, 1972)*.
The statistical model used in this system is based on the reasonable premise that the individual pharmacokinetic parameters of a patient population arise from a distribution which can be described by the population mean and the inter-individual variance. Thus, each individual pharmacokinetic parameter can be expressed as a population mean and a deviation, typical for that individual. The deviation, i.e. the difference between the population mean and the individual parameter, is assumed to be a random variable and is referred to as the inter-individual variation *(Vozeh, et al. 1982)*.

The variability that occurs between patients (inter-individual) and within a patient (intra-individual) causes the average pharmacokinetic data to achieve different plasma concentrations in each patient. A major goal of pharmacokinetic research is to identify those factors that may significantly alter a drug's pharmacokinetics (i.e. age, weight, disease states, other drugs) and to adjust appropriately the dosing scheme. A second goal is to quantitate the degree of inter- and intra-individual pharmacokinetic variability in the population. With this information one can predict the average concentration and the confidence bounds for a given drug administration scheme *(Maitre, et al. 1987)*.

# CHAPTER 3

## Present Study

### 3.0 AIMS OF THE STUDY

1. To evaluate the role of parenteral phenobarbitone in neonatal hyperbilirubinaemia in:
   a. decreasing serum bilirubin more rapidly than phototherapy alone
   b. decreasing duration of phototherapy and hospital stay.

2. To derive pharmacokinetic data for phenobarbitone in South African Black neonates.

### 3.1 PATIENTS AND METHODS

This was a prospective, randomised controlled study conducted at the neonatal unit of King Edward VIII Hospital, Durban.

All full-term jaundiced but otherwise healthy newborns selected for phototherapy were eligible for inclusion in this study.

The indications for eligibility into the study were bilirubin levels as follows :

| | | |
|---|---|---|
| Day 1 | : | 170-204 umols/l |
| Day 2 | : | 205-255 umols/l |
| Day 3 | : | 256-289 umols/l |
| Day 4 - 7 | : | 290-340 umols/l |

This guideline is derived from the protocol used at the King Edward VIII Hospital. For levels exceeding these an exchange transfusion was performed. The gestational age was $>$ 37 weeks as assessed according to the scoring system of *Dubowitz, et al. 1970.* The age ranged from day 0 to day 7. A mass of $\geq$ 2.5kg was a requirement for inclusion into the study. All patients were randomized to receive either phototherapy alone

(control group) or phototherapy plus phenobarbitone (study group). The dose of phenobarbitone (Sodium Gardenal® 200mg/ml) administered was 12mg/kg/body weight intramuscularly as a single dose. Informed and written consent were obtained from all parents prior to entry to the study. All neonates (both in the control and study groups) were placed under phototherapy units, eyes protected by a band to prevent retinal damage.

The **exclusion criteria** were as follows:

1. Patients who received any prior therapy for hyperbilirubinaemia.
2. Those patients with history or evidence of infections, cephalhaematomas, severe dehydration, birth asphyxia or any other illness.
3. Patients administered any medication known to interact with phenobarbitone or bilirubin (enzyme inducer or inhibitor).
4. Patients born to mothers who received enzyme inducing (e.g. phenobarbitone) or inhibiting agents (e.g. cimetidine) in the antenatal period.
5. Patients on any other concomitant medication.

## 3.2 PROCEDURES

The time of phototherapy or phenobarbitone administration was regarded as hour 0. Phenobarbitone was administered with a graduated insulin syringe for accuracy. Venous blood samples were obtained every 4 hours for the first 24 hours and 24 hourly thereafter and assayed for total serum bilirubin (TSB) and phenobarbitone. Total serum bilirubin determinations were performed immediately by the Blood Bank of the Natal Blood Transfusion Services, King Edward VIII Hospital, according to the spectrophotometric method *(Levkoff, et al. 1970)*.

All patients in the study were monitored at two hour intervals for the first 24 hours and 6 hourly thereafter by medical and nursing staff. Respiratory, neurological, cardiovascular and hydration status were assessed. Blood exchange transfusion was performed if a bilirubin level exceeded the recommended exchange level during the study.

## 3.3 INVESTIGATIONS

The following investigations were performed on all patients on entry into the study :

- Full blood count with smear and reticulocytes.
- Blood groups of mother and patient.
- Rhesus status of mother and patient.
- Coombs test.
- Liver function test and lactate dehydrogenase (LDH).
- Glucose-6-phosphate dehydrogenase levels (G-6-PD) (males and females).
- Reducing substances in the urine.

## 3.4 PHENOBARBITONE SERUM CONCENTRATION DETERMINATION

The serum phenobarbitone levels was assayed using a fluorescence polarisation immunoassay method *(Abbott TDx Clinical Analyzer)*, at the Drug Studies Unit, Department of Pharmacology, University of Durban-Westville. The lowest concentration that can be detected with 95% confidence by the TDx is 0.34 ug/ml. Venous blood samples were collected into plain glass venoject tubes, allowed to clot, and then centrifuged. The serum was separated into PVC tubes and stored at -20 degrees celsius until ready for analysis. Frozen samples were allowed to thaw at room temperature prior to analysis.

The instrument was operated according to manufacturer's instructions. After calibration, 3 quality control samples were analysed with every batch of 9 samples (representing 1 patient). Measured values which appeared to deviate from the expected profile were reassayed. Samples were also reassayed if the controls were not within 10% of the labelled concentration.

## 3.5 NONMEM ANALYSIS - METHOD

The population pharmacokinetic analysis was performed on a 80486 IBM-compatible personal computer using Double precision NONMEM - Version III - Level 1.2 and the PREDPP package at the Department of Pharmacology, University of Durban-Westville

*(Boeckmann, Sheiner and Beal, 1991)*. Whereas NONMEM is a general nonlinear regression analysis tool, PREDPP is specialised to the kind of predictions which arise in pharmacokinetic data analysis. Data was captured into Quattro Pro Version 4.0, written to a standard text file and then processed by the NM-TRAN programme, a control language translator and pre-processor to the NONMEM programme. The NM-TRAN programme re-organises the data into a format suitable for use in the NONMEM programme.

The sub-routine ADVAN 1 and TRANS 2 were chosen from the PREDPP library of sub-routines. ADVAN 1 implements the one-compartment (monoexponential) model, without first order absorption while TRANS 2 translates the elimination rate constant Ke into the pharmacokinetic parameters of clearance (Cl) and volume of distribution (Vd). In this study, the first blood sample was drawn many hours after drug administration, hence the assumption that absorption was complete, and, for purposes of data analysis, immediate. (An examination of Figure 7 confirms this assumption as valid).

## 3.6 STATISTICAL ANALYSIS

The results were subjected to appropriate statistical analyses including Repeated Measures Analysis of Variance (ANOVA), Chi-Square, and student t-test using the SAS SYSTEM statistics software package.

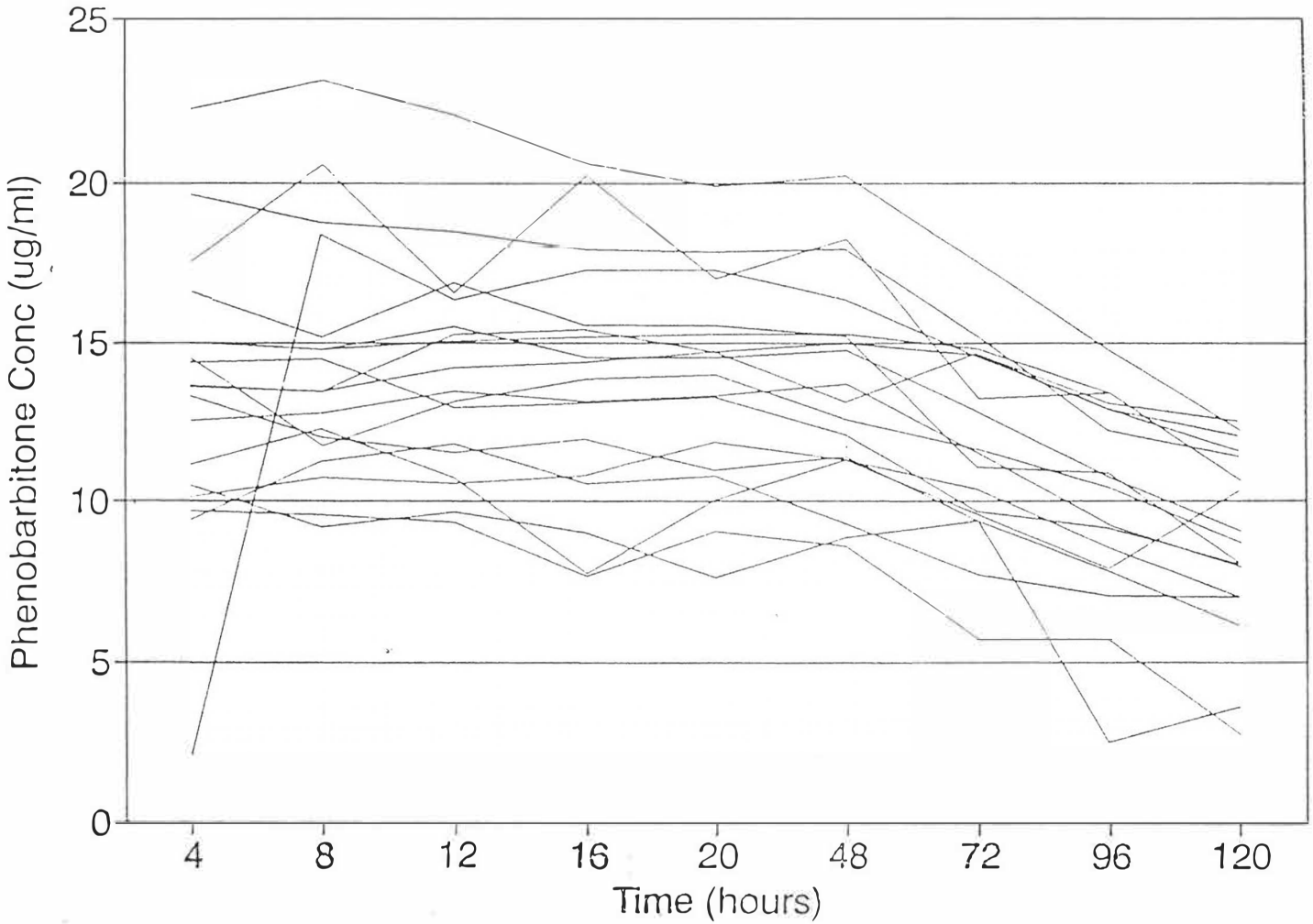



**FIG 7**

## INDIVIDUAL GRAPHS OF SERUM PHENOBARBITONE CONCENTRATION VS TIME

# CHAPTER 4

## Results

Forty four jaundiced Black neonates (27 males, 17 females) were included in the study (Table VI). All patients were otherwise, healthy newborn infants with a birth weight of $\geq$ 2.5kg. The gestational age of all patients was > 37 weeks. The study group comprised 22 newborns who received phenobarbitone in addition to phototherapy while the control group numbered 22 and received phototherapy only. ABO incompatibility (Table VII) was observed in both study and control groups (13 and 7 respectively). There were 6 Coombs positive patients in the study group and 4 in the control group. Five patients in the study group and 2 in the control group presented with both ABO and Coombs positivity. As seen from Table VIII most of the patients were in the post-natal age range Day 2 to Day 5.

### 4.1 PHENOBARBITONE ANALYSIS

A total of 198 serum samples from 22 subjects were analysed for serum phenobarbitone concentration (Appendix B). Phenobarbitone serum concentrations were below the level of detection by the instrument in 3 patients and unusually high in 1 patient. These patients were excluded from further data analysis.

Figure 7 represents individual graphs of serum phenobarbitone concentration versus time (n=18). Serum levels ranged between 5 to 25 ug/ml in the 120hr sampling period. With the exception of one, all other serum concentrations showed a steady decline with time.

Figure 8 is a NONMEM fit of the data (phenobarbitone concentration versus time) for all subjects represented in Figure 7. The curve labelled "typical patient" is the profile exhibited by the "average" patient in the population under study. The points depict the measured serum concentrations at each time point for each patient in the study.

TABLE VI

## DEMOGRAPHICS

| GROUP | NUMBER | SEX | | AVERAGE MASS |
|---|---|---|---|---|
| | (n) | male | female | (kg) |
| I | 22 / 44 | 12 | 10 | 2.97 |
| II | 22 / 44 | 15 | 07 | 3.10 |

TABLE VII

## HAEMATOLOGICAL STATUS

| GROUP | NUMBER | ABO INCOMP. | COOMBS (+) | ABO & COOMBS (+) | ABO COMP | Ma Rh (-) |
|---|---|---|---|---|---|---|
| 1 | 22 | 13 | 6 | 5 | — | 3 |
| 11 | 22 | 7 | 4 | 2 | 9 | 2 |

**TABLE VIII**

## NO. OF PATIENTS PER AGE IN DAYS

| GROUP | DAY 1 | DAY 2 | DAY 3 | DAY 4 | DAY 5 | DAY 6 | DAY 7 | TOTAL |
|---|---|---|---|---|---|---|---|---|
| I | 1 | 7 | 7 | 5 | — | 2 | — | 22 |
| II | 1 | 5 | 7 | 3 | 5 | 1 | — | 22 |

During the initial analysis using NONMEM, available demographic factors were plotted against the NONMEM predicted serum phenobarbitone concentrations in order to investigate the importance of these factors in explaining inter-individual variability. A strong relationship with mass, but no other demographic factor, was suggested. The influence of mass is important as it helps to determine whether neonatal phenobarbitone doses should be individualised according to body mass or whether a fixed dose could be used.

In an attempt to determine the influence of patient mass on phenobarbitone pharmacokinetics, various models were tested (Table IX). In deciding whether a particular model was useful or not, the primary criterion evaluated was the change in the minimum value of the objective function (MOBF). A change in the MOBF of 3.8 is statistically significant at the $p < 0.05$ level while a change of 7.8 is significant at $p < 0.005$ assuming a Chi-square distribution.

## 4.2 NONMEM HYPOTHESIS TESTING (TABLE IX)

### 1. Model 2 vs Model 1

The hypothesis that was tested was whether mass influenced Cl (and not Vd) in a linear relationship with a calculated intercept with the y-axis. Model 2 was rejected because the MOBF change was not significant (305.195 - 305.652 = -0.457).

### 2. Model 3 vs Model 1

The hypothesis that was tested was whether mass influenced Vd (and not Cl) in a linear relationship with a calculated intercept with the y-axis. Model 3 was accepted at the $p < 0.005$ level because a change in MOBF of more than 7.8 was noted (297.100 - 305.652 = -8.552).

### 3. Model 4 vs Model 3

The hypothesis that was tested was whether mass influenced both Vd and Cl in a linear relationship with a calculated intercept with the y-axis. Model 4 was rejected because the MOBF change was not significant (296.592 - 297.100 = -0.508).

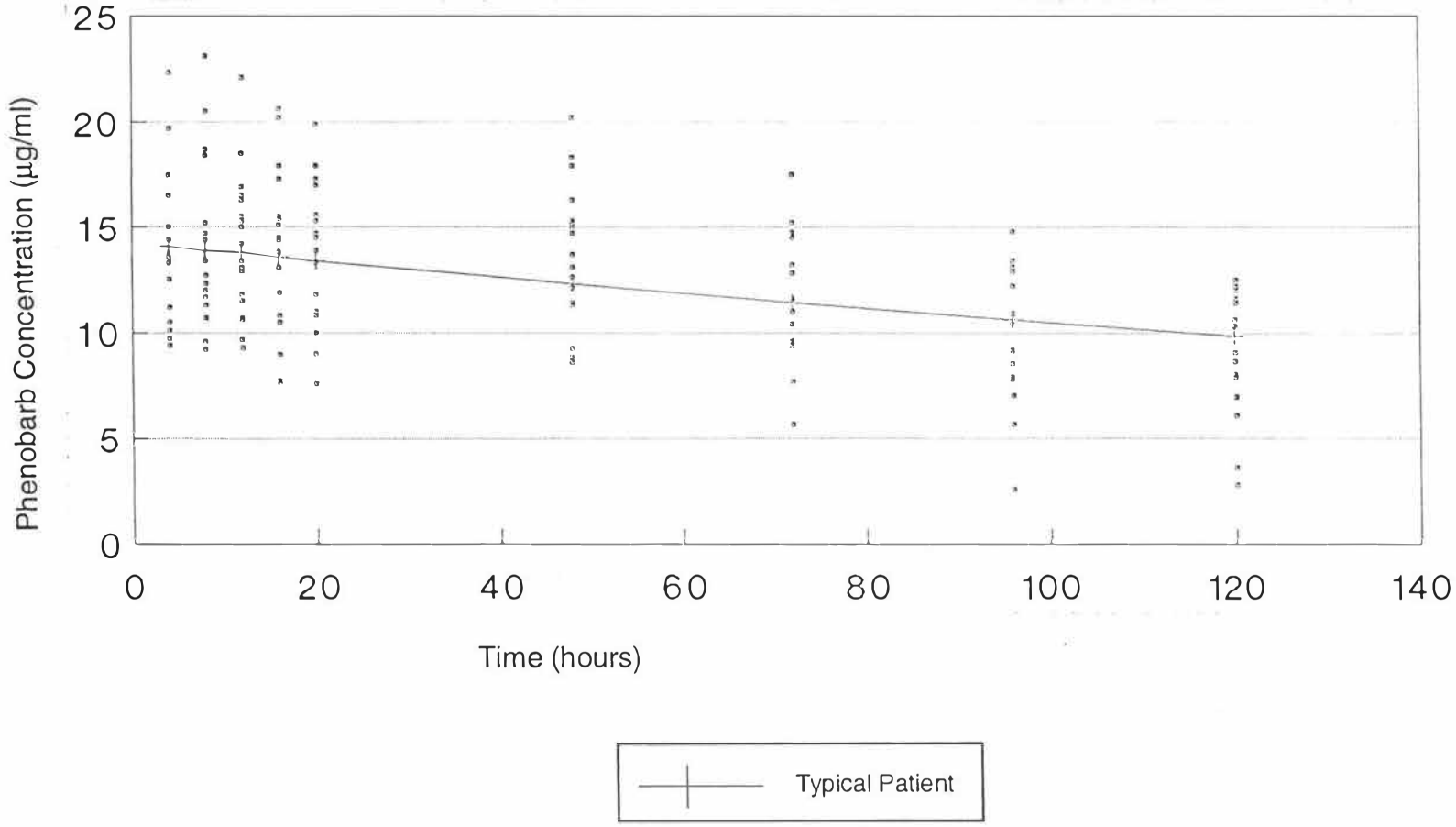



**FIG 8**

**PHENOBARBITONE CONCENTRATION (µg/ml) VERSUS TIME (hr) FOR ALL SUBJECTS - NONMEM FIT OF THE DATA**

**TABLE IX**

## NONMEM – HYPOTHESIS TESTING
### Influence of patient mass on phenobarbitone pharmacokinetics

| Model No. | Hypothesis | MOBF | Clearance (Cl) | Inter-individual variability in Cl (%) | (Vd) Vol. of Distribution | Inter-individual variability in Vd (%) | Intra-individual variability (%) |
|---|---|---|---|---|---|---|---|
| 1 | Cl and Vd not influenced by mass | 305.652 | 0.008 (l/hr) | 56 | 2.52 (*l*) | 25 | 9 |
| 2 | Cl influenced by mass (with intercept); Vd not influenced by mass. | 305.195 | Cl = 0.002 x mass + 0.002 (l/kg/hr) | 56 | 2.50 (*l*) | 24 | 8.9 |
| 3 | Cl not influenced by mass; Vd influenced by mass (with intercept) | 297.100 | 0.008 (l/hr) | 52 | Vd = 0.84 x mass + 0 (*l*/kg) | 25 | 8.8 |
| 4 | Cl and Vd influenced by mass (with intercept) | 296.592 | Cl = 0.002 x mass + 0.003 (l/kg/hr) | 53 | Vd = 0.84 x mass + 0 (*l*/kg) | 25 | 8.7 |
| 5 | Cl not influenced by mass; Vd influenced by mass (with intercept =0) | 297.100 | 0.008 (l/hr) | 52 | Vd = 0.84 x mass + 0 (*l*/kg) | 25 | 8.8 |

**4. Model 5 vs Model 3 - Refinement of Model 3**

The hypothesis that was tested was whether mass influenced Vd (and not Cl) in a linear relationship as conducted for Model 3. However in Model 5, the intercept was forced through zero. Model 5 was rejected because the MOBF did not change.
NONMEM analysis of neonatal serum concentration data in this study in neonates taking phenobarbitone resulted in clearance of 0.008 l/hr and a volume of distribution of 0.84 l/kg. The inter-individual variability in Cl and Vd was 52% and 25% respectively. Residual or intra-individual variability was 8.8% (Table IX).

### 5.4.2 SERUM BILIRUBIN ANALYSIS

The total serum bilirubin levels for 44 patients at each time point is presented in Appendix C. The mean serum bilirubin levels at various times are shown in Table X.

Figure 9 graphically represents the change in mean serum bilirubin concentrations with time during the study period. This graph comparing the study and the control groups showed a steady decline from baseline which became apparent from 8 hours in the study group and from 12 hours in the control group. At 72 hours the bilirubin level in the study group was below 200umol/l while in the control group it was above 220 umol/l. At 120 hours there was a coincidence of the two bilirubin levels.

Figure 10 is a linear fit of mean total serum bilirubin concentration for study versus control group against time. This graph depicts the linear fit of the rate of fall of bilirubin i.e. the elimination rate of bilirubin in both groups. At all time points after 4 hours, the rate of decline of bilirubin for the study group was greater than the control group. At 120 hours the levels for the study and control groups were approximately 190 and 210umol/l respectively. There was no significant differences between the two groups using the independent samples t-test (Appendix D).

**TABLE X**

## MEAN SERUM BILIRUBIN CONCENTRATION (umols/l) WITH SD

| GROUPS | TIME IN HOURS | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 4 | 8 | 12 | 16 | 20 | 24 | 48 | 72 | 96 |
| PHENOBARBITONE (I) | 282 ± 50 | 269 ± 42 | 258 ± 36 | 255 ± 40 | 248 ± 39 | 231 ± 47 | 213 ± 49 | 204 ± 60 | 206 ± 62 | 184 ± 36 |
| CONTROL (II) | 273 ± 43 | 253 ± 52 | 259 ± 59 | 265 ± 62 | 248 ± 51 | 235 ± 48 | 238 ± 44 | 223 ± 37 | 217 ± 31 | 202 ± 20 |
| (p. Value) | NOT SIGNIFICANT | | | | | | | | | |

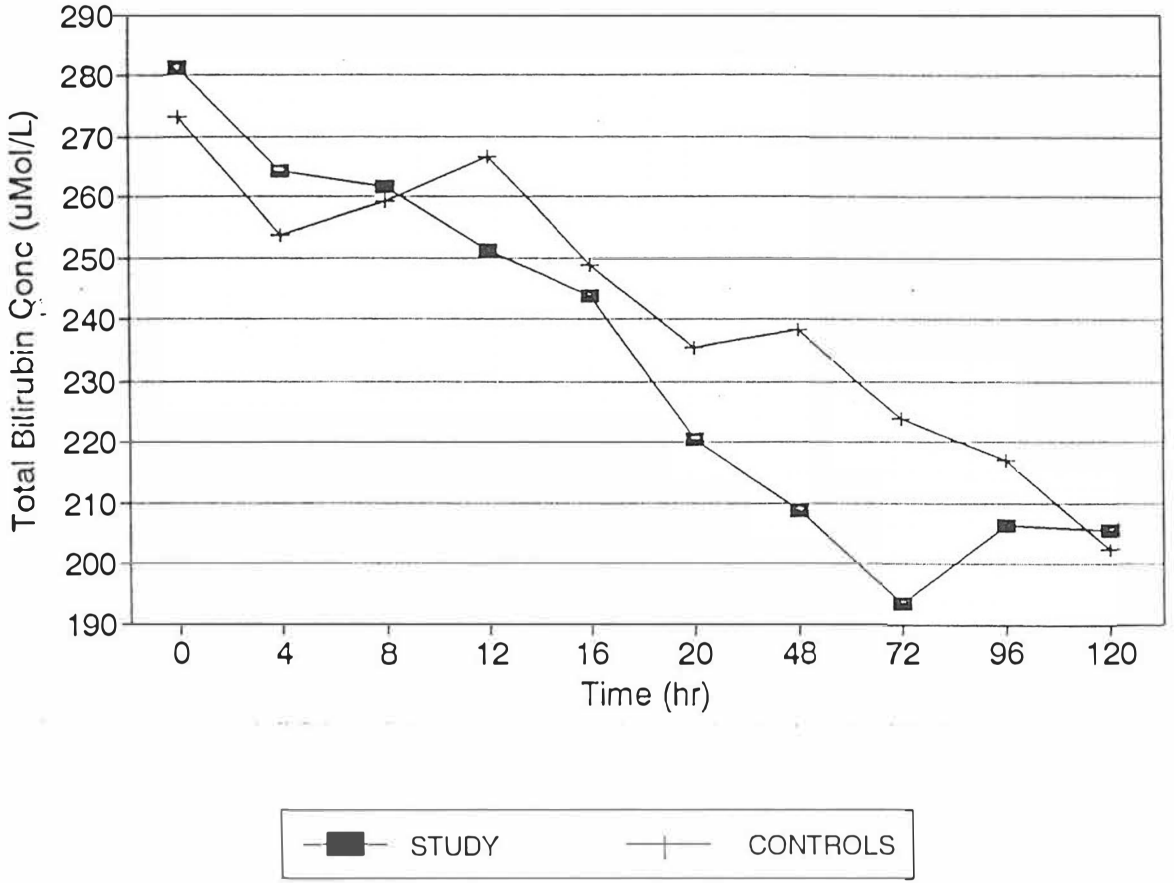



FIG 9

## MEAN SERUM BILIRUBIN CONCENTRATION FOR STUDY VS CONTROL AGAINST TIME

The area under the curve using the trapezoidal rule was determined for both groups (Appendix E). Both area under the curve (AUC) and the normalised area under the curve (NAUC) were calculated and subjected to independent samples t-test. There were no significant differences between the study and the control groups in the means of both AUC and NAUC. Repeated measure analysis of variance (ANOVA) was used to compare the serum bilirubin levels in the two groups (Appendix F) at each time point, to look at the effect over time and to determine if there was a significant group and time interaction (i.e. to determine if groups behaved differently over time). For this analysis (ANOVA), data up to 20 hours were analyzed since some observations were missing after 20 hours due to discharge from hospital. There were no significant differences between the study group and the control group in terms of bilirubin levels. There was no significant change over time. All the times differed significantly from baseline but the groups did not behave differently over time.

The number of patients with levels below 200umols/l of bilirubin at 48 and 96 hours were compared using the Chi-square test. At 48 hours, the study group numbered 12/18 (44.4%) while the control group was 6/22 (27%), a statistically non-significant result. At 96 hours, 15/18 (83.33%) in the study group and 14/22 (63.6%) in the control group were below 200umols/l ($p$ = ns). The Chi-squared test was also used to compare the two groups with regard to the average time it took to reach the 'discharge level' i.e. 200umols/l from hospital. The average time to reach 200umol/l in the was 67.37 hours in the study group (SD 45.26) and 73.68 hours (SD 40.86) in the control group. This difference was not statistically significant.

## 4.3 FURTHER ANALYSIS

A sub group analysis of ABO incompatibility versus ABO compatibility showed a significant difference at baseline ($p = 0.0160$) but no significant difference over time. Further sub-group analysis of the influence of Coombs positive and Coombs negative in the study from Day 4 to Day 6 was analysed using Unpaired samples t-test. No significant differences were found. Repeated measures analyses of variance were not performed due to small group sizes.

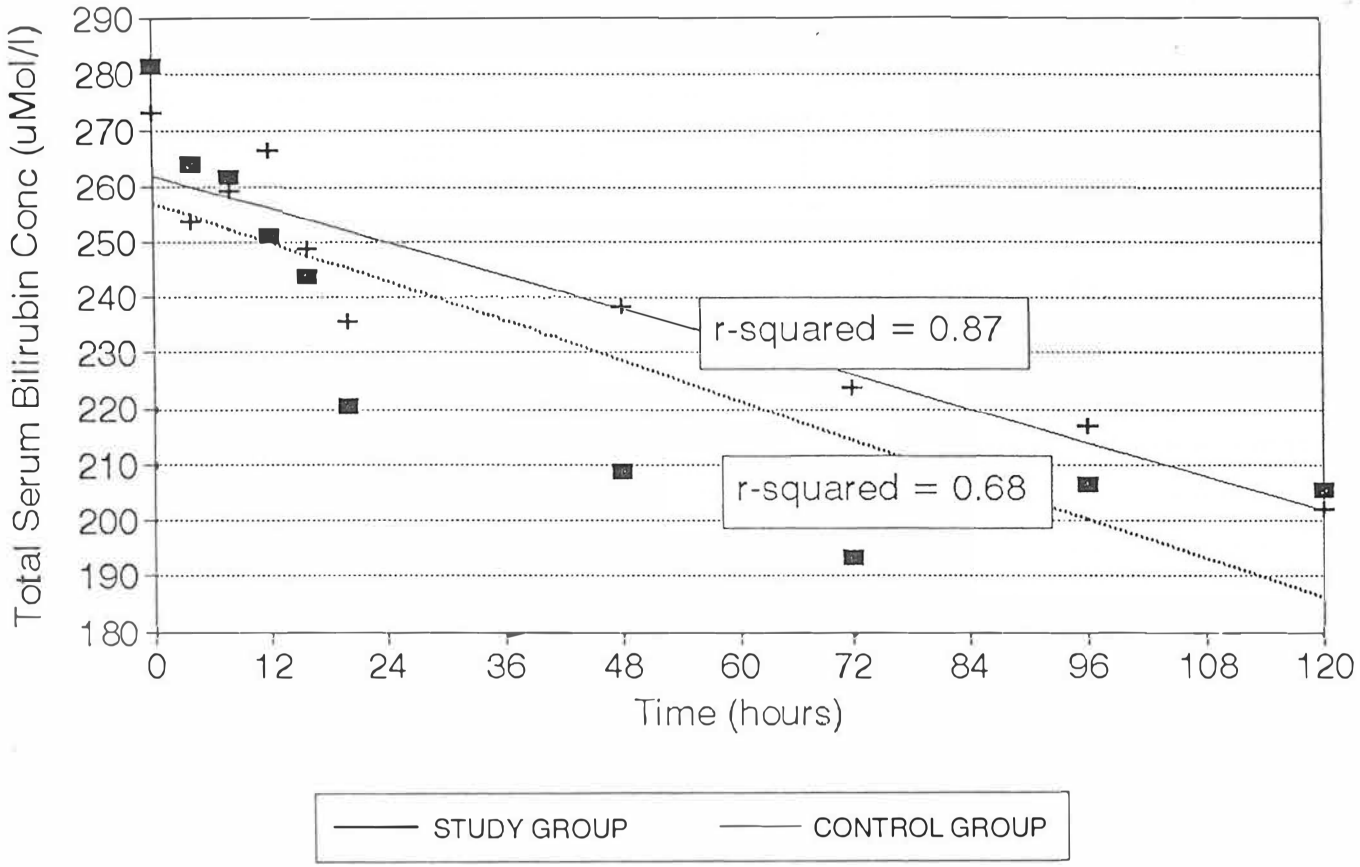



**FIG 10**

## LINEAR FIT OF MEAN TOTAL SERUM CONCENTRATION VS TIME

In addition, 2 of the male neonates had glucose-6-phosphate dehydrogenase deficiency. One neonate tested strongly positive for reducing sugars in the urine. However, on retesting two weeks later, the urine was found to be negative.

## 4.4 SIDE EFFECTS

During the trial, no patient presented with any serious side effects. The main side effects of phenobarbitone administration were drowsiness and excessive sleep. These patients were easily aroused and experienced no problems with breast feeding.

# CHAPTER 5

## Discussion

The object of this trial was to evaluate the efficacy of phenobarbitone in reducing serum bilirubin levels in neonatal jaundice and hence determining if there was any clinical benefit in instituting this treatment. The second objective was to derive, pharmacokinetic parameters for phenobarbitone in Black African jaundiced neonates.

A good deal of recent clinical research has explored the problem of jaundice in the healthy full-term infant. Hyperbilirubinaemia is of concern because of the relative risk of bilirubin toxicity to the central nervous system especially kernicterus. A review of the relationship between serum bilirubin and "brain damage" in term infants without haemolysis, found no evidence of any effect on cognitive impairment (IQ or development), neurologic abnormalities or hearing loss *(Newman and Maisels, 1992).* Hence, Newman and Maisels concluded that the risk of bilirubin induced damage is low in term infants without haemolysis. However, the difficulty arises from the fact that as with all other preventive measures, many subjects have to be treated to prevent the condition in a few.

Interest in using phenobarbitone to decrease neonatal hyperbilirubinaemia was generated following the retrospective study of *Trolle (1968)*, who reported a diminished incidence of neonatal jaundice among the offspring of epileptic and pre-eclamptic women treated with phenobarbitone during their pregnancies. This original report led to the assessment of the administration of phenobarbitone post-natally.
The administration of phenobarbitone in neonatal jaundice has been suggested to induce the enzyme glucuronyl transferase, which is important in the conjugation of bilirubin in the liver. A study by *Catz and Yaffe (1968)* showed that the reduction of serum bilirubin in animals was the result of enhanced glucuronyltransferase activity, with increased bilirubin conjugation and bile flow.

*Levi, et al. (1970)* found that with phenobarbitone therapy, the hepatic organic anion

binding protein 'Y' (i.e. Y-acceptor protein) was increased. This protein is responsible, in part, for the hepatic uptake of bilirubin. Such enhancement of liver function i.e. increased glucuronyl transferase activity and Y-acceptor protein probably increases the rate of bilirubin excretion and forms the basis of reducing the serum bilirubin concentration. Several clinical investigators have confirmed the capacity of phenobarbitone to reduce serum bilirubin levels *(Yeung and Field, 1969; Cunningham, et al. 1969, Wong and Wood, 1973)*. However, the outcomes remain controversial.

The investigation of phenobarbitone in neonatal jaundice is primarily directed towards a Third World population group with a high risk of severe neonatal jaundice and scarcity of resources for its management, especially exchange transfusion and its recognised associated risks. The opportunities arising from the use of phenobarbitone includes its relative ease of administration, inexpensiveness and availability, without increasing immediate morbidity and long term sequelae.

Phototherapy is effective in decreasing serum bilirubin concentration and appears to be safe as judged by neurodevelopmental outcome *(Schedit, et al. 1993)*. However, the separation of mother and infant by the barrier of phototherapy, together with the fear and anxiety engendered in the parents and the effects on bonding and breastfeeding are likely to have a negative impact on the mother. Two potential hazards of phototherapy are dehydration secondary to loose stools and retinal damage due to the effects of high intensity light.

In this phototherapy era, it is difficult to demonstrate the effectiveness of phenobarbitone alone in the management of neonatal jaundice. As with exchange transfusions, phototherapy has to be included in the protocol of studies on drug treatment because of its proven benefits. This results in blunting of the differences in bilirubin values between the control and the study groups. The additional difference between the study and control groups becomes the parameter by which the efficacy of the drug treatment may be judged.

A study by *Yeung, Chan, et al. (1971)* showed that the effect of phenobarbitone

treatment lasts longer than the course of administration. In the infant treated with phenobarbitone, the bilirubin levels remained significantly lower than that of the control infants for a further two days after cessation of treatment.

In the present study, a single intramuscular dose of 12mg/kg/body weight of phenobarbitone was used on the basis that a single, high but safe, dose would provide maximal induction of the UDP-Glucuronyl transferase since there is no literature which refers to the dose and the onset of induction in neonates. No sedation or withdrawal effects were observed after antenatal phenobarbitone, giving cord blood levels of 2-10ug/ml, when used for the prevention of neonatal jaundice. However, a sedative effect was observed with postnatal phenobarbitone particularly when high doses (20mg/kg) were used *(Guys Paediatric formulary, 1990)*. Therefore the dose of 12mg/kg used in this study was considered a safe dose.

In the present study, all patients were monitored at 2 hourly intervals for the first 24 hours (as recommended by the Ethics Committee of the University of Natal) and then 6 hourly for the rest of the study period. The safety of the dose was confirmed by the lack of any serious side effects during this intensive monitoring period. No patient presented with any complications. The only side effects observed of were drowsiness and excessive sleep. However, these patients were easily aroused and experienced no problems with breast feeding. Barbiturates have been studied extensively during pregnancy and the perinatal period, in view of their potential for immediate and long term untoward effects. Few immediate and no long term undesirable effects have been described after many years of therapeutic use of phenobarbitone in the perinatal period. The use of phenobarbitone for the prevention of neonatal jaundice constitutes, in many ways, a different type of perinatal exposure than results from its use in epileptic mothers, or for neonatal seizures or for intra-partum sedation and anaesthesia *(Goodman and Gilman, 1985)*.

In the present study, there were 27 males and 17 females. The average birthweight was 2.97kg in the study group and 3.10kg in the control group. While all patients had very high bilirubin levels, these values were below the recommended exchange levels as

practised at King Edward VIII Hospital. Of the total of 44 patients in the study most of the patients (45.45%) had ABO blood group incompatibility and 22.72% of these were Coombs positive. None of the patients in the study required exchange transfusion. The influence of Coombs positive and Coombs negative was not statistically significant. This latter analysis was deliberately excluded by *Vos, (1980)*, since in the South African Black population in particular, the raised development of immune anti-A or anti-B is known to be influenced strongly by many kinds of exogenous determinants in the immediate environment *(Vos and Kirk, 1958)*. The influence of ABO incompatibility over time in the present study was not statistically significant.

The retrospective study by *Vos, Adhikari and Coovadia (1981)*, showed that ABO blood group incompatibility was not significantly associated with overall jaundice. However, it was a very important cause of severe jaundice and accounted for nearly half of the infants with very high bilirubin values which necessitated exchange transfusion.

While there is a trend towards a decrease in serum bilirubin levels after phenobarbitone treatment the results were not statistically significant. Therefore the use of phenobarbitone in the treatment of neonatal hyperbilirubinaemia cannot be supported. Furthermore, the duration of hospital stay was unaffected.

In aiming for 'a kinder, gentler approach' *Newman and Maisels (1992)* recommend a relaxation in treatment schedules as follows :
*Phototherapy when the serum bilirubin concentration is 225-330umols/l in neonates who are sick or have haemolysis and a serum bilirubin 300-375umols/l in well neonates without haemolysis. They recommend exchange transfusion at a serum bilirubin concentration of 300-400umols/l in sick neonates or those with haemolysis and at 425-500umols/l in well neonates without haemolysis.*

A recommendation that will surprise many paediatricians is that breastfeeding should be interrupted in well neonates without haemolysis when the serum bilirubin is 275-425umols/l. These new recommendations are not universally accepted, noting the problems of kernicterus, and other toxic potential effects of bilirubin , as well as the

effects of moderate hyperbilirubinaemia on IQ. The recommendations in the prevention of jaundice by good breastfeeding strategies, support for the nursing mother as well as avoidance of water supplementation are suggested by one view and another makes a plea for early use of phototherapy, stressing it is more effective and reduces prolonged exposure to raised serum bilirubin concentrations *(Dodd, 1993)*. Before the above recommendations by Newman and Maisels can be fully endorsed, a controlled evaluation of the outcome of these proposals is awaited.

The serum phenobarbitone levels measured in this study ranged from 5 - 25ug/ml and are consistent with predicted values based on the average mass of the study group (Figure 7). The decline in serum phenobarbitone levels is expected because all samples were drawn in the post-absorptive phase. Complete absorption from intramuscular injection occurs within 2 hours as shown by *Graham (1978)*. Patient E (Appendix B) displayed an increase in serum phenobarbitone concentration from 2.14 (time = 4 hours) to 18.37 (time = 8 hours) unlike the other subjects who showed a consistent decline in levels. This may be due to a number of reasons such as delayed absorption, the absorption site acting as a drug depot, sampling error and unusual individual pharmacokinetic characteristics.

The appearance of the concentration-time profile is similar to that reported by *Graham, (1978)* who showed complete absorption of phenobarbitone within 2 hours, peaking at 2 hours and declining thereafter.

In view of the ethical constraints in this neonatal study, blood sampling had to be restricted to the collection of samples that were clinically indicated. This precluded the conduction of a standard pharmacokinetic study which would have entailed the collection of numerous samples to fully characterise the absorption, distribution and elimination phases.

A clearance of 0.008 l/hr and a volume of distribution of 0.84 l/kg with an intra-individual variability of 8.8% was obtained in this study. An exhaustive survey of the literature failed to provide any pharmacokinetic data in jaundiced Black African

neonates. *This study therefore appears to be the first to report pharmacokinetic data for this population group in South Africa.* The clearance derived from this study is 0.008 l/hr. Clearance values for young children are reported as 0.012 l/kg/hr (Guelen, et al. 1975) which is in the upper range of the value obtained in the present study. The latter may be a consequence of the plasma clearance of phenobarbitone progressively increasing postnatally to a maximum at 4 weeks. In adolescence this value declines to 0.004 l/hr.

The apparent volume of distribution of phenobarbitone in neonates is reported to be 0.97 $\pm$ 0.20 l/kg *(Pitlick, et al. 1978)* and 0.93 $\pm$ 0.15 l/kg *(Painter, et al. 1989).* The value of 0.84 l/kg obtained in the present study is consistent with the literature.

Further studies in South Africa would validate these values and create a data base for national population pharmacokinetic parameters in neonates.

Population pharmacokinetic parameters are required not only to predict altered pharmacokinetics in patients but also to adjust doses through Bayesian forecasting. Bayesian forecasting uses a modified non-linear regression analysis to enable one to incorporate previous experience into a model using the probability distributions of the pharmacokinetic parameters for the model. Data collected during routine clinical care is potentially useful for investigations of drug disposition in patient populations. NONMEM has the power to analyse such data *(Tanigawara and Hori, 1991).*

The results obtained by the NONMEM analysis may be regarded as an average profile of the Black African neonatal population attending King Edward VIII Hospital in Durban. These results may form the basis for various clinical applications which include Bayesian forecasting programmes; dose prediction from steady state blood data and individualised dosage regimens. In addition, the pharmacokinetic parameters (Vd and Cl) so derived can be used in the calculation of loading and maintenance dose of phenobarbitone e.g. in neonatal epilepsy and intraventricular haemorrhage prophylaxis.

The decline of total serum bilirubin over time may be exploited as a surrogate for clinical end points in assessing the efficacy of phenobarbitone in neonatal jaundice. However, the high degree of intra-individual and inter-individual variability in absolute TSB concentrations presents a challenge in the assessment of drug effects on this marker. *Sanathanan and Peck (1991)* proposed the area under the curve (AUC) and normalised area under the curve (NAUC) as pharmacodynamic measures for assessing changes over time for CD4 counts in HIV patients. This may be extrapolated to TSB in this study.

NAUC = normalised area under the curve, is derived by calculating AUC directly from observed TSB values using the trapezoidal rule, and then adjusting the AUC value for the initial TSB and T, the length of the time interval over which the AUC is calculated. ie. AUC divided by the product of T and the baseline TSB value.
NAUC can be interpreted as the average relative change in TSB values from baseline.

In a neonate without a compromised bilirubin metabolism, the NAUC would be expected to remain constant at approximately 1. Any change in this value would imply altered bilirubin metabolism with time. Both control and study groups would be expected to display decreases in the NAUC values (below 1). In order to determine whether a greater decrease occurred in the study group, the independent samples t-test was performed (Appendix 4).
AUC and NAUC are measures of exposure to phenobarbitone plus phototherapy in the study group and phototherapy alone in the control group. Therefore any difference in these parameters in the study group will be due to phenobarbitone therapy. This concept forms the basis of the pharmacodynamic modelling.

Analysis of the AUC and the NAUC using the student independent t test found that there were no statistically significant differences between the two groups.
This result confirms that phenobarbitone (12mg/kg) treatment does not significantly decrease total serum bilirubin levels in neonatal jaundice.

## Recommendations

1. It remains theoretically possible that a spread of doses of phenobarbitone, greater than 12mg/kg, may significantly decrease total serum bilirubin in neonatal jaundice. However, there remains the possibility that higher doses of phenobarbitone may compete with bilirubin for binding to serum albumin, and hence displace the bilirubin. This may be compensated for by a greater increase in glucuronidation of bilirubin and hence its elimination over the displacement. Further studies to determine the trigger dose for maximum enzyme induction are required.

2. Blood sampling should be done at least at 10 minute intervals in the first hour; 15 minute intervals for the next hour and thereafter half hourly for the next 4 hours in order to derive absorption data.
   However, this poses an ethical problem as frequent blood sampling in neonates is frowned upon.
   In order to ascertain elimination data, prolonged hospitalization of the babies will be required as elimination is slow.

3. This study should be extended to other hospitals as this would include a more heterogeneous population with a greater number of subjects. The influence of environment and ethnicity may be evaluated. Pharmacokinetic data thus generated would be more representative of the South African neonatal population.

4. A study investigating day 1 term jaundiced babies with much higher bilirubin levels than has been included in this study protocol is recommended as they represent a very high risk group frequently requiring blood exchange transfusion.

## Conclusions

This prospective controlled study demonstrates that a single dose of phenobarbitone of 12mg/kg does not significantly lower total serum bilirubin levels or the duration of hospital stay in jaundiced Black African neonates. Furthermore, the pharmacokinetic data obtained in this study on Black South African jaundiced neonates are consistent with that reported in the literature. This study using phenobarbitone for neonatal jaundice and the pharmacokinetic data generated are the first to be reported in South Africa.

## THE CHILD APPEAL

*I am the child*
*You hold in your hands my destiny*
*You determine, largely, whether*
*I shall succeed or fail*
*Give me, I pray, those things that*
*make for happiness,*
*train me, I beg you, that I*
*may be a blessing to the world.*

**From**
**Every Child**

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APPENDIX A

# BLOOD BANK (KING EDWARD VIII HOSP) STATISTICS FOR NEONATAL JAUNDICE

| APRIL 1992 TO MARCH 1993 | TOTAL (YR) | AVERAGE MONTH |
|---|---|---|
| 1. NEONATAL NURSERY, WARDS, CLAIRWOOD | 2799 | 254 |
| 2. EXCHANGE TRANSFUSIONS | 195 | 15 |
| -ABO INCOMP. (COOMBS NEGATIVE) | 24 | 02 |
| -ABO INCOMP. (COOMBS POSITIVE) | 54 | 4.5 |
| -ABO COMPATIBILITY | 18 | 1.5 |

**1984 – 1992**

| | | |
|---|---|---|
| 3. NURSERY STATISTICS | | |
| 1984 | 1428 | 119 |
| 1985 | 1595 | 133 |
| 1986 | 1223 | 102 |
| 1987 | 1009 | 84 |
| 1988 | 1132 | 94 |
| 1989 | 1310 | 109 |
| 1990 | 1510 | 126 |
| 1991 | 1594 | 133 |
| 1992 | 1432 | 119 |
| AVERAGE | 1359 | 113 |

APPENDIX B

## SERUM PHENOBARBITONE CONCENTRATION (ug/ml) WITH TIME

| PATIENT | NUMBER OF HOURS POST DOSE | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | HOUR 4 | HOUR 8 | HOUR 12 | HOUR 16 | HOUR 20 | HOUR 48 | HOUR 72 | HOUR 96 | HOUR 120 |
| A | LQ | 0.36 | 0.52 | LQ | LQ | LQ | LQ | LQ | 0.37 |
| B | 9.71 | 9.56 | 9.30 | 7.65 | 9.02 | 8.59 | 5.67 | 5.68 | 2.80 |
| C | 10.47 | 9.20 | 9.65 | 9.00 | 7.61 | 8.86 | 9.39 | 2.57 | 3.60 |
| D | 11.16 | 12.26 | 10.73 | 7.74 | 10.02 | 11.30 | 9.55 | 7.89 | 10.31 |
| E | 2.14 | 18.37 | 16.32 | 17.25 | 17.25 | 16.31 | 14.54 | 12.89 | 11.55 |
| F | 19.67 | 18.74 | 18.46 | 17.90 | 17.85 | 17.92 | 15.20 | 12.22 | 11.36 |
| G | 9.42 | 11.25 | 11.80 | 10.52 | 10.75 | 9.28 | 7.70 | 7.04 | 6.97 |
| H | 17.52 | 20.55 | 16.53 | 20.22 | 16.98 | 18.25 | 13.22 | 13.40 | M |
| I | 12.49 | 12.74 | 13.43 | 13.13 | 13.29 | 13.66 | 11.49 | 9.22 | 7.91 |
| J | 22.26 | 23.10 | 22.06 | 20.58 | 19.88 | 20.19 | 17.50 | 14.75 | 12.24 |
| K | 14.97 | 14.72 | 15.03 | 15.15 | 15.25 | 15.26 | 14.78 | 13.39 | 10.65 |
| L | 14.99 | 14.74 | 15.47 | 14.52 | 14.51 | 14.73 | 12.76 | 10.73 | 9.06 |
| M | 14.45 | 11.69 | 13.12 | 13.80 | 13.93 | 12.56 | 11.60 | 10.42 | 8.65 |
| N | 13.28 | 11.98 | 11.52 | 11.92 | 10.98 | 11.36 | 9.38 | 7.80 | 6.08 |
| O | 13.59 | 13.44 | 15.27 | 15.39 | 14.70 | 13.10 | 14.66 | 13.05 | 12.50 |
| P | LQ | 0.63 | 0.68 | LQ | LQ | LQ | LQ | LQ | LQ |
| Q | LQ | 0.52 | LQ | LQ | 0.50 | LQ | LQ | LQ | LQ |
| R | 16.55 | 15.17 | 16.85 | 15.52 | 15.56 | 15.23 | 11.04 | 10.85 | 8.04 |
| S | 13.64 | 13.44 | 14.18 | 14.38 | 14.71 | 14.97 | 14.61 | 12.89 | 12.03 |
| T | 34.48 | 35.74 | 32.23 | 31.04 | 32.33 | 30.80 | 29.11 | 28.07 | M |
| U | 14.37 | 14.44 | 12.91 | 13.06 | 13.26 | 12.09 | 9.63 | 9.12 | 8.02 |
| V | 10.13 | 10.71 | 10.55 | 10.77 | 11.83 | 11.29 | 10.37 | 8.53 | 6.98 |

Results excluded from further analysis  M = Missing  LQ = Low Quantity

APPENDIX C

# SERUM BILIRUBIN LEVELS WITH TIME

| ID | GR. | AGE | HRO | HR4 | HR8 | HR12 | HR16 | HR20 | HR48 | HR72 | HR96 | HR120 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 3 | 276 | 280 | 249 | 258 | 285 | 268 | 247 | 284 | 234 | 174 |
| 2 | 1 | 2 | 266 | 350 | 258 | 326 | 279 | 286 | 282 | 265 | 131 | 115 |
| 3 | 1 | 2 | 203 | 190 | 192 | 190 | 195 | 195 | 218 | 244 | 259 | 182 |
| 4 | 1 | 3 | 279 | 238 | 315 | 180 | 183 | 195 | 201 | 171 | 99 | — |
| 5 | 1 | 2 | 320 | 263 | 257 | 248 | 310 | 269 | 320 | 270 | 220 | 212 |
| 6 | 1 | 4 | 333 | 319 | 285 | 284 | 297 | 155 | 148 | 128 | — | — |
| 7 | 1 | 1 | 170 | 231 | 233 | 265 | 231 | 249 | 241 | 178 | — | — |
| 8 | 1 | 2 | 236 | 248 | 245 | 283 | 251 | 251 | 257 | 195 | — | — |
| 9 | 1 | 3 | 253 | 275 | 219 | 272 | 253 | 213 | 182 | 144 | — | — |
| 10 | 1 | 2 | 259 | 203 | 207 | 186 | 177 | 138 | 140 | 105 | — | — |
| 11 | 1 | 2 | 235 | 228 | 281 | 288 | 281 | 304 | 291 | 298 | 294 | 232 |
| 12 | 1 | 2 | 263 | 272 | 265 | 251 | 243 | 232 | 254 | 295 | 245 | 193 |
| 13 | 1 | 2 | 234 | 219 | 207 | 198 | 171 | 164 | 195 | 181 | — | — |
| 14 | 1 | 4 | 332 | 262 | 285 | 245 | 207 | 194 | 183 | — | — | — |
| 15 | 1 | 6 | 385 | 337 | 343 | 340 | 273 | 286 | 250 | 226 | 182 | — |
| 16 | 1 | 4 | 298 | 285 | 268 | 235 | 226 | 175 | 107 | — | — | — |
| 17 | 1 | 3 | 321 | 285 | 282 | 256 | 289 | 208 | 200 | 139 | — | — |
| 18 | 1 | 3 | 289 | 291 | 220 | 252 | 261 | 265 | 197 | — | — | — |
| 19 | 1 | 4 | 311 | 300 | 255 | 265 | 253 | 260 | 210 | 177 | — | — |
| 20 | 1 | 3 | 297 | 270 | 231 | 280 | 265 | 210 | 195 | — | — | — |
| 21 | 1 | 3 | 256 | 214 | 254 | 222 | 245 | 204 | 190 | 169 | — | — |
| 22 | 1 | 6 | 372 | 310 | 315 | 298 | 309 | 306 | 200 | — | — | — |
| 1 | 2 | 2 | 298 | 302 | 311 | 320 | 293 | 267 | 311 | 259 | 223 | 226 |
| 2 | 2 | 4 | 316 | 285 | 335 | 339 | 283 | 294 | 264 | 282 | 252 | 187 |
| 3 | 2 | 3 | 211 | 200 | 205 | 203 | 214 | 205 | 203 | 198 | 180 | 175 |
| 4 | 2 | 3 | 262 | 242 | 270 | 256 | 296 | 281 | 292 | 266 | 249 | 229 |
| 5 | 2 | 5 | 307 | 294 | 282 | 299 | 300 | 294 | 282 | 183 | 155 | — |
| 6 | 2 | 2 | 197 | 171 | 228 | 294 | 204 | 119 | 123 | — | — | — |
| 7 | 2 | 2 | 289 | 252 | 265 | 265 | 227 | 217 | 239 | 183 | — | — |
| 8 | 2 | 3 | 256 | 281 | 290 | 294 | 314 | 271 | 262 | 250 | 217 | 182 |
| 9 | 2 | 1 | 186 | 190 | 192 | 196 | 189 | 193 | 195 | 190 | — | — |
| 10 | 2 | 2 | 207 | 117 | 105 | 124 | 132 | 138 | — | — | — | — |
| 11 | 2 | 5 | 303 | 345 | 281 | 261 | 262 | 249 | 244 | 235 | 205 | — |
| 12 | 2 | 4 | 321 | 290 | 367 | 375 | 283 | 271 | 264 | 275 | 255 | 211 |
| 13 | 2 | 5 | 312 | 329 | 340 | 396 | 295 | 300 | 272 | 250 | 240 | 200 |
| 14 | 2 | 4 | 294 | 267 | 271 | 283 | 289 | 254 | 280 | 264 | 211 | 209 |
| 15 | 2 | 5 | 309 | 290 | 222 | 214 | 200 | 198 | 185 | 179 | — | — |
| 16 | 2 | 5 | 295 | 233 | 246 | 211 | 238 | 224 | 233 | 193 | — | — |
| 17 | 2 | 3 | 264 | 239 | 253 | 251 | 300 | 263 | 253 | 240 | 200 | — |
| 18 | 2 | 6 | 342 | 298 | 324 | 300 | 291 | 233 | 210 | 198 | — | — |
| 19 | 2 | 3 | 261 | 229 | 211 | 260 | 253 | 245 | 233 | 206 | — | — |
| 20 | 2 | 3 | 285 | 243 | 281 | 261 | 228 | 229 | 195 | — | — | — |
| 21 | 2 | 2 | 232 | 247 | 200 | 222 | 155 | — | — | — | — | — |
| 22 | 2 | 3 | 262 | 236 | 223 | 210 | 228 | 200 | 227 | 178 | — | — |

APPENDIX D

# INDEPENDANT SAMPLES t-TEST ON MEAN TOTAL SERUM BILIRUBIN CONCENTRATION – STUDY VERSUS CONTROL

| | STUDY | CONTROL |
|---|---|---|
| | 281.2222 | 273.1364 |
| | 264.2222 | 253.6364 |
| | 261.6111 | 259.1818 |
| | 251.1111 | 266.5455 |
| | 243.7222 | 248.8182 |
| | 220.5000 | 235.4762 |
| | 208.8333 | 238.3500 |
| | 193.5000 | 223.8333 |
| | 206.3333 | 217.0000 |
| | 205.5000 | 202.3750 |
| MEAN | 233.6556 | 241.8353 |
| SD | 30.4145 | 22.6809 |
| INDEPENDENT SAMPLES t-TEST ON STUDY VERSUS CONTROL: p = 0.505 | | |

APPENDIX E

# TABLE OF AREA UNDER THE CURVE (AUC) AND NORMALISED AREA UNDER THE CURVE (NAUC)

| GROUP | AUC | NAUC | GROUP | AUC | NAUC |
|---|---|---|---|---|---|
| STUDY | 19048 | 6.170251 | CONTROL | 3450 | 3.71770 |
| STUDY | 31760 | 7.478125 | CONTROL | 15350 | 5.89313 |
| STUDY | 14806 | 5.352853 | CONTROL | 32138 | 8.55033 |
| STUDY | 18702 | 0.076470 | CONTROL | 32228 | 8.18196 |
| STUDY | 19958 | 7.830508 | CONTROL | 23440 | 8.5355 |
| STUDY | 10538 | 5.729249 | CONTROL | 31976 | 9.15076 |
| STUDY | 10718 | 4.760618 | CONTROL | 23602 | 7.05211 |
| STUDY | 34204 | 0.631910 | CONTROL | 7608 | 5.96954 |
| STUDY | 18506 | 6.828897 | CONTROL | 16496 | 5.88581 |
| STUDY | 3976 | 4.247863 | CONTROL | 29768 | 9.36718 |
| STUDY | 12522 | 4.644578 | CONTROL | 13878 | 7.22043 |
| STUDY | 26810 | 6.310390 | CONTROL | 2602 | 3.14251 |
| STUDY | 10234 | 4.848993 | CONTROL | 23630 | 7.03300 |
| STUDY | 16954 | 5.668224 | CONTROL | 32354 | 8.24299 |
| STUDY | 13208 | 5.385522 | CONTROL | 32096 | 8.58333 |
| STUDY | 14484 | 6.021484 | CONTROL | 30280 | 8.06292 |
| STUDY | 13612 | 5.139752 | CONTROL | 14448 | 5.02589 |
| STUDY | 17536 | 6.741636 | CONTROL | 13680 | 5.62372 |
| CONTROL | 16784 | 6.37739 | CONTROL | 23646 | 7.69318 |
| CONTROL | 11016 | 5.20000 | CONTROL | 17100 | 5.63157 |

**INDEPENDENT SAMPLES T-TEST ON AUC GROUPED BY GROUP**

| GROUP | N | MEAN | SD |
|---|---|---|---|
| 1.000 | 18 | 17087.556 | 7600.349 |
| 2.000 | 22 | 2O466.818 | 9590.964 |

| | | | |
|---|---|---|---|
| SEPARATE VARIANCES | T = -1.243 | DF = 38.0 | PROB = 0.221 |
| POOLED VARIANCES | T = -1.214 | DF = 38.0 | PROB = 0.232 |

**INDEPENDENT SAMPLES T-TEST ON NAUC GROUPED BY GROUP**

| GROUP | N | MEAN | SD |
|---|---|---|---|
| 1.000 | 18 | 6.326 | 1.758 |
| 2.000 | 22 | 6.825 | 1.717 |

| | | | |
|---|---|---|---|
| SEPARATE VARIANCES | T = -0.902 | DF = 36.1 | PROB = 0.373 |
| POOLED VARIANCES | T = -0.904 | DF = 38.0 | PROB = 0.372 |

APPENDIX F

# MEAN SERUM BILIRUBIN LEVELS WITH STANDARD DEVIATION (SD)

| GROUP 1 | | | | | |
|---|---|---|---|---|---|
| VARIABLE | N | MEAN | STD DEV | MINIMUM | MAXIMUM |
| HR 0 | 24 | 282.458 | 50.230 | 170.000 | 385.000 |
| HR 4 | 24 | 269.875 | 42.675 | 190.000 | 350.000 |
| HR 8 | 24 | 258.250 | 36.686 | 192.000 | 343.000 |
| HR 12 | 24 | 255.791 | 40.392 | 180.000 | 340.000 |
| HR 16 | 24 | 248.708 | 39.974 | 171.000 | 310.000 |
| HR 20 | 24 | 231.208 | 47.809 | 138.000 | 306.000 |
| HR 48 | 24 | 213.041 | 49.410 | 107.000 | 320.000 |
| HR 72 | 18 | 204.388 | 60.118 | 105.000 | 298.000 |
| HR 96 | 9 | 206.888 | 62.008 | 99.000 | 294.000 |
| HR 120 | 7 | 184.714 | 36.586 | 115.000 | 232.000 |
| **GROUP 2** | | | | | |
| HR 0 | 22 | 273.136 | 43.489 | 186.000 | 342.000 |
| HR 4 | 22 | 253.636 | 52.913 | 117.000 | 345.000 |
| HR 8 | 22 | 259.181 | 59.532 | 105.000 | 367.000 |
| HR 12 | 22 | 265.181 | 62.243 | 124.000 | 396.000 |
| HR 16 | 22 | 248.818 | 51.088 | 132.000 | 314.000 |
| HR 20 | 21 | 235.476 | 48.457 | 119.000 | 300.000 |
| HR 48 | 20 | 238.350 | 44.248 | 123.000 | 311.000 |
| HR 72 | 18 | 223.833 | 37.242 | 178.000 | 282.000 |
| HR 96 | 11 | 217.000 | 31.527 | 155.000 | 255.000 |
| HR 120 | 8 | 202.375 | 19.970 | 175.000 | 229.000 |