

Neonatal Hyperbilirubinemia and Bilirubin Encephalopathy

Investigations into the diagnosis,
epidemiology, pathogenesis, management
and treatment of the jaundiced newborn

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DECLARATION

The research papers listed and referenced (and attached to this document) are the result of studies that I have performed personally or in which I have actively participated.

Papers no: 1-15, 17, 19, 22, 26-30, 34-36: I designed the study, participated in the data gathering and wrote the manuscript.

Papers no: 6, 18, 23: I helped to design the study and participated in gathering the data and writing the manuscript

Papers no: 20, 21: I helped in the design of the study and the writing of the manuscript.

Papers no: 24, 25, 33, 38: The first author was either a fellow in my training program or a junior member of our faculty. Under my direction, the study was designed and carried out. I was involved throughout the study in patient recruitment, study design and the drafting of the manuscript.

Papers no: 31 and 32: I initiated the ideas for these two reviews and participated together with Thomas B. Newman, M.D., in the drafting, writing and editing of these papers.

Paper no 37: I participated in the drafting and reviewing of this entire document and I contributed the original information on the risks of exchange transfusion.

This work has not been submitted to any other university for any other degree.

M. Jeffrey Maisels

DEDICATION

To the memory of Lisa Jean Maisels, 1965-2004, a person of piercing intellect who committed herself to helping others. She was a kind, considerate, thoughtful, courageous, optimistic and loving daughter and sister.

“March on my soul with courage”

(Judges 5:21, The Song of Deborah)

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INTRODUCTION

Jaundice is probably the most common newborn infant problem dealt with on a daily basis by the family practitioner and paediatrician. Jaundice occurs when the liver cannot clear a sufficient amount of bilirubin from the plasma. When the problem is excessive bilirubin formation or limited uptake or conjugation, unconjugated (i.e., indirect reacting) bilirubin appears in the blood and indirect hyperbilirubinemia is the predominant form of jaundice found in the newborn infant. In the vast majority of newborns, hyperbilirubinemia is transient and benign but, in rare cases, the serum bilirubin rises to a level that is toxic to the central nervous system. Understanding the pathogenesis and epidemiology of neonatal hyperbilirubinemia; recognizing, the problems involved in appropriate surveillance and monitoring of the jaundiced infant and the factors contributing to bilirubin encephalopathy; and implementing treatment of the jaundiced neonate in a timely fashion, are issues that have engaged clinicians and researchers for some 6 decades. This work will summarize my contributions to the field of neonatal hyperbilirubinemia and it includes papers published between 1971 and 2007. The description of this work will not follow its chronological sequence, but will be divided into the categories of diagnosis, epidemiology, pathogenesis, management, treatment, and bilirubin encephalopathy.

DIAGNOSIS – STUDIES USING TRANSCUTANEOUS BILIRUBIN MEASUREMENTS

When the bilirubin concentration in the serum increases, bilirubin is deposited in the skin and subcutaneous tissues producing the (yellow) physical sign of jaundice or icterus. There is a well established relationship between the total serum bilirubin (TSB)

concentration and the intensity of jaundice, and the assessment of skin color in quantifying the level of bilirubin in the plasma has been utilized as a clinical sign since the early part of the 20th Century. As it is neither possible, nor desirable to measure the serum bilirubin daily in every infant for the first week after birth, clinicians have used the clinical sign of jaundice as the trigger for deciding when to measure the TSB concentration.

Although there is a clear and semi-quantitative relationship between the yellowness of the skin and the TSB, the variations in colour perception by the human eye, differences in neonatal skin pigmentation, and variations in both the intensity and colour of the available light affect our ability to estimate TSB by assessing the degree of jaundice. Several studies have shown that practitioners provide wide ranges of estimates of TSB concentrations based on their clinical observations.

Recognition of the inaccuracy of the clinical estimation of TSB has led to the development of non-invasive instruments that can provide an objective measurement of skin colour. The first such electronic device made available for commercial distribution, the Jaundice Meter, was produced by the Minolta Camera Company and consisted of a hand-held instrument that measured the yellow color intensity of the skin and subcutaneous tissues. When a photoprobe is pressed against the infant's skin to a pressure of approximately 200g, a xenon tube produces a strobe light that travels through a fiberoptic filament to the photoprobe. The bright light penetrates the blanched skin and transilluminates the subcutaneous tissue. The scattered light returns through a

second fiberoptic filament and is carried to the spectrophotometric module. Inside the module, the light is divided by a dichroic mirror into two spectra, one of which passes through a blue filter (maximum absorption, 460nm) and the other through a green filter (maximum absorption, 550nm). Absorption at these spectra corrects for hemoglobin and the intensity of the yellow colour is measured as the difference between the optical densities of blue and green processed electronically to provide a digital meter reading. This instrument provided a read out of a “transcutaneous bilirubin index.” Based on correlative data from the particular hospital laboratory and patient population, this index could then be converted to a serum bilirubin level.

Transcutaneous Bilirubin Measurements in Full-Term Infants

To document the utility and accuracy of this instrument, in 1982 I performed 292 transcutaneous bilirubin (TcB) measurements in 157 full term infants.¹ Having established that TcB measurements correlated well with TSB determinations ($r=.93$, $p<.001$), I evaluated the ability of the Jaundice Meter to identify infants whose TSB concentrations exceeded 12.9 mg/dL (221 $\mu\text{mol/L}$). Using a TcB index value of 24, the TcB measurement had a sensitivity of 100%, a specificity of 97%, and a negative predictive value of 100% for identifying a TSB level of 12.9 mg/dL (221 $\mu\text{mol/L}$). Thus a TcB index < 24 correctly predicted the absence of TSB ≥ 12.9 mg/dL (221 $\mu\text{mol/L}$) in all cases.

I also compared the correlation between TcB and TSB when measured at the forehead or sternum and found that both of these measurements were equally accurate. I

concluded that, if used as a screening device, the TcB instrument would, with an acceptable degree of accuracy, identify those infants who required a serum bilirubin determination.

Interinstrument Variability

Because interinstrument variability of the Jaundice Meter had not been well studied, we evaluated the response of 4 TcB meters in a population of 72 newborn infants.²In this study, sequential readings were taken in random order from the forehead of each infant using 4 separate TcB meters. The order was randomized using a 4x4 Latin square design. Each instrument was calibrated by the manufacturer and the calibration was checked against the color standard provided. We used the average of the readings from the 4 instruments as the closest practical approximation to a theoretical “true mean value” and calculated the standard error of the estimate to provide the confidence limits about this mean. We found that 2 of the meters provided measurements that were consistently higher than the other 2 meters and that the differences were systematic and not random. Our calculations showed that, in 95% of cases, the measurement would provide a TcB index that was within ± 4.24 transcutaneous bilirubin units of the true value and this degree of error would represent approximately ± 2.8 mg/dL (47.9 $\mu\text{mol/L}$) of serum bilirubin. We therefore recommended that individual hospital laboratories should construct their own regression lines for each instrument using the serum bilirubin measurement as the independent variable and the transcutaneous bilirubin index as the dependent variable.

Cost Effectiveness

A question that must be asked about every screening test is: is this test cost effective? We therefore wanted to know whether or not the routine use of the Jaundice Meter as a screening tool in the newborn nursery would reduce both the need for serum bilirubin measurements and costs. In this study³ we first established the relationship between TSB levels and the TcB index in 356 newborns of ≥ 36 weeks gestation. We then introduced the routine clinical use of the jaundice meter and documented the impact of routine TcB measurements on the number of TSB measurements obtained over a 2-year period. In addition, we calculated the total direct costs for performing serum bilirubin measurements in the clinical laboratory and the TcB index in the nursery. We showed that the use of the jaundice meter produced a 36% reduction in the number of TSB measurements sent to the laboratory and generated cost savings of some \$1600/year.

Newer Jaundice Meters

The initial models of the Minolta jaundice meters had some significant disadvantages. They did not provide a read-out of the serum bilirubin concentration; a TcB index was displayed and TSB values were derived from the TcB index on the basis of data obtained in individual hospital laboratories. TcB readings were also significantly affected by gestation and skin pigmentation. As a result, these instruments achieved limited acceptance in hospitals in the United States, although they were widely used in Japan. Subsequently Minolta developed the JM-102 and, its latest model the JM-103 which has overcome some of the disadvantages of the earlier models. The JM-103 TcB

instrument uses two wavelengths and a dual optical path system. The principal of operation includes the formation of two beams, one of which reaches only the shallow areas of the subcutaneous tissue, while the other penetrates the deeper layers. The differences between the optical densities are detected by blue and green photocells. Measurement of bilirubin accumulated primarily in the deeper subcutaneous tissue should decrease the influence of other pigments in the skin, such as melanin and hemoglobin. These innovative technologies should reduce the effect of race, age, gestation and birth weight on TcB measurements. The JM-103 also contains an algorithm that converts the intensity of the yellow colour reading directly to a serum bilirubin concentration, (rather than an index that needs to be converted to a TSB value) and displays this as a digital readout.

In 2004 we published the first large study evaluating the new Minolta JM-103.⁴ In this study we compared TSB measurements with TcB measurements in 849 newborns \geq 35 weeks of gestation. This was a mixed population consisting of 59% White, 30% Black, 5% East Asian, 4% Middle Eastern and 3% Indian/Pakistani and Hispanic patients. We found a close correlation between TSB and TcB values in all of the population groups White (n=503, r=0.949); Black (n=253, r=.822); East Asian, Indian/Pakistani and Hispanic (n=93, r=.926). In the black population, the correlation was less close than in other groups but, because the tendency in blacks is for the TcB measurement to over estimate TSB levels, dangerous clinical errors are unlikely to occur. The measurement technique is rapid and simple and it is easy to perform repeated measurements over time, thus reducing the likelihood of error. TcB measurements with the JM-103 jaundice

meter should obviate the need for most serum bilirubin measurements in newborn infants ≥ 35 weeks of gestation. We now use this instrument in our newborn nursery as a routine screening device and the nurses obtain a daily TcB measurement on every infant during the midnight shift. Prior to its use, about 30% of infants in our nurseries had at least one TSB level measured. Using the JM-103 as a screening device, only 9% of infants currently have at least 1 TSB measured (unpublished data).

The Natural History of Bilirubinemia

There are currently no good published data on the natural history of bilirubinemia in the newborn. There is a nomogram that is widely used in nurseries throughout the United States and other countries to help identify infants who are likely or unlikely to subsequently develop hyperbilirubinemia. Unfortunately, because of significant biases in the population sampled, this nomogram does not represent the natural history of neonatal jaundice. To address this issue we performed 9,397 TcB measurements on 3,984 healthy newborn infants (gestational age ≥ 35 weeks) between 6-96 hours of age.⁵ All these measurements were performed with the JM-103 jaundice meter at 6-hour intervals. With this study we were able to achieve two objectives. The first was to establish a nomogram (the 5th, 25th, 50th, 75th and 95th percentiles) of the natural history of bilirubinemia in the first 96 hours of life, and the second was to establish the rate of rise of bilirubin levels over the first 96 hours. From these data we concluded that infants whose bilirubin levels were increasing more rapidly than 0.22 mg/dL (3.8 μ mol/L) per hour in the first 24 hours, 0.15mg/dL (2.6 μ mol/L) between 24-48 hours, and 0.06 mg/dL/hr (1 μ mol/L) after 24 hours need closer observation and evaluation as these

rates of rise suggest the presence of hemolysis. The data and the nomogram that we developed are useful in detecting aberrant trends and identifying infants who need additional evaluation as well as in planning for appropriate follow up for jaundiced newborns, particularly those in whom TcB measurements were obtained prior to discharge.

Predicting Hyperbilirubinemia

Most recently we have evaluated the utility of using pre-discharge TcB measurements as a predictor of subsequent hyperbilirubinemia.⁶ In this study we were able to show that a pre-discharge TcB measurement above the 95th percentile indicated that the risk of that infant subsequently developing a TSB of 17 mg/dL (291 µmol/L) was 47.9 times greater than an infant whose pre-discharge TSB was below the 50th percentile. Of 4,854 infants with pre-discharge TcB levels below the 50th percentile, none developed a TSB > 20 mg/dL (342 µmol/L). Thus, a pre-discharge TcB is a valuable tool in helping to predict which infants are likely or unlikely to develop significant hyperbilirubinemia following discharge.

Outpatient Use of TcB Measurements

Almost all of the studies of TcB measurements to date have been performed in hospital settings and it was important to know whether this instrument would be valuable when used in an outpatient setting. To this end we have obtained TcB measurements in 4 private paediatric practices as well as in the paediatric outpatient clinics of the William Beaumont Hospital.⁷ In a population of 116 infants we found an excellent correlation

between TcB and TSB ($r=.846$, $p=0.0$). We were also able to show that the age of the infant (up to 12.6 days) had no effect on the difference between TSB and TcB. These data demonstrate that TcB measurements can be used with confidence in paediatric offices to identify those infants who do or do not require a TSB measurement.

Historical Perspectives

Finally I authored a paper reviewing the history and current use of transcutaneous measurements,⁸ in which I provide practical advice for the use of this measurement in paediatric practice.

EPIDEMIOLOGY – STUDIES OF BREASTFEEDING AND OTHER EPIDEMIOLOGIC FACTORS IN NEWBORN JAUNDICE

Breastfeeding, Weight Loss and Jaundice

In a series of publications⁹⁻¹⁴ I have identified, amongst other things, the major epidemiologic risk factors for the development of hyperbilirubinemia. In the first study we investigated the weight loss experienced by 40 breastfed infants and its association with jaundice and fever.⁹ There is surprisingly little published data on the normal weight loss to be expected for fully breastfed infants, particularly when these infants are divided into different weight group categories. In addition, it was unclear whether the fever that occurs occasionally in breastfed infants is related to under nutrition or dehydration. In this study we reviewed the charts of 100 infants from the well baby nursery, 25 infants in each of 4 weight groups (birth weight 2501-3000g, 3001-3500g, 3501-4000g, and >

4000g). These infants were selected only if they had been delivered vaginally and had been fully breastfed and had received no supplementation with water or formula. All infants were nursed by their mothers soon after birth and were breastfed subsequently on demand. In this study we found no relationship between serum bilirubin concentrations and weight loss and we also found that those infants who became febrile did not have excessive weight loss. The average weight loss for all infants was $5.8 \pm 3.2\%$ and we concluded that intervention was appropriate for infants whose weight loss exceeded 12%, not 5-7% as had been suggested.

The Role of Breastfeeding and Other Causes in Neonatal Jaundice

The association between breastfeeding and hyperbilirubinemia has been debated and extensively investigated over the last 30 years. We performed TSB determinations on 264 term infants who were consecutively delivered via the vaginal route.¹⁰ On the third hospital day, the mean (\pm SD) TSB level was 6.9 ± 3.6 mg/dL (118 ± 62 μ mol/L) in the breastfed infants and 6.5 ± 3.2 mg/dL (111 ± 55 μ mol/L) in formula fed infants. These TSB levels were determined in every case on the third hospital day. We concluded that breastfeeding did not affect the TSB level in the first 3 days although, because of the small sample size, there remained the possibility of a type II error. 41 infants had TSB concentrations > 12 mg/dL (246 μ mol/L) on the third day and additional investigations (ABO and Rh blood typing, direct antibody test, reticulocyte counts and red blood cell morphology) were performed on these infants in attempt to identify the cause for the hyperbilirubinemia. No cause could be found for the hyperbilirubinemia in 49% of these infants.

Normal Serum Bilirubin Levels and the Effect of Breastfeeding

Recognizing that the population that we had previously analyzed was quite small, we studied a substantially larger population to document normal serum bilirubin levels in the newborn and the effect of breastfeeding.¹¹ In this study we measured TSB concentrations in 2,416 consecutive infants admitted to the well baby nursery and found that the maximum TSB exceeded 12.9 mg/dL (221 μ mol/L) in 147 infants (6.1%). We compared these infants with 147 randomly selected control infants with maximum TSB levels \leq 12.9 mg/dL (221 μ mol/L). In 66 infants (44.9%) we identified an apparent cause for the jaundice but in 81 (55%) no cause was found. Of those infants for whom no cause for hyperbilirubinemia was found, 82.7% were breastfed versus 46.9% in the control group ($p < .0001$). Breastfeeding was therefore significantly associated with hyperbilirubinemia even in the first 3 days of life. The 95th percentile for formula fed infants was a TSB level of 11.4 mg/dL (195 μ mol/L) versus 14.5 mg/dL (248 μ mol/L) for the breastfed population. Of the formula-fed infants 2.24% had TSB levels $>$ 12.9 mg/dL (221 μ mol/L) versus 8.97% of breastfed infants ($p < .000001$). We therefore identified a strong association between breastfeeding and jaundice in the healthy newborn infant. We concluded that investigations for the cause of hyperbilirubinemia in healthy breastfed infants were not indicated unless a serum bilirubin level exceeded approximately 15 mg/dL (385 μ mol/L), whereas in the formula fed infants such investigations were indicated if the TSB exceeded approximately 12 mg/dL (205 μ mol/L).

Quantifying the Risk Factors for Jaundice

The data from this study population of 2,416 infants permitted us to do additional analysis.¹² Using stepwise logistic regression we were able to provide a quantifiable risk score for the risk of developing a TSB > 12.9 mg/dL (221 μ mol/L) depending on the presence or absence of certain risk factors. Thus a 38 week gestation, male infant of a diabetic mother whose labour was induced with oxytocin and who was breastfed and had a 10% weight loss, had an 84% chance of developing a TSB > 12.9 mg/dL (221 μ mol/L). On the other hand an infant born at 39 weeks gestation with none of the above listed risk factors and only a 5% weight loss had only a 1.4% risk of developing a TSB level of 12.9 mg/dL (221 μ mol/L).

We were therefore able to show that infants that have a number of risk factors can be expected to develop TSB levels that are substantially higher than those that do not have risk factors, and that recognizing the contribution of these risk factors to the TSB can avoid unnecessary laboratory investigations in an attempt to identify the cause of the jaundice. We pointed out that the definitions of normal bilirubin levels found in current texts of the time created, in essence, a class of healthy, jaundiced babies with “non-disease.” It was clear from these data that, in many jaundiced infants with known risk factors, as long as significant pathology such as hemolytic disease was ruled out, a policy of watchful expectancy was justified rather than a battery of laboratory tests. On the other hand an apparently normal (physiologic) TSB level in a low risk infant might arouse suspicion and require further investigation. This study showed that an awareness of these risk factors and their potential contribution to TSB levels permitted a

more rational approach to the action levels used for the investigation of jaundice in the newborn and what was needed was a new definition of what is and is not “physiologic jaundice.”

Length of Stay, Jaundice, and Hospital Readmission

In a subsequent large study we reviewed the number of infants who were readmitted to the hospital within 14 days of discharge with a diagnosis of hyperbilirubinemia.¹³ Over a period of 6 years (1988-1994), 247 (0.8%) of 29,934 infants discharged from the nursery were readmitted by the age of 14 days. 127 (51%) were admitted because of hyperbilirubinemia and 74 (30%) with the diagnosis of “rule out sepsis.” The factors that were associated with the readmission for jaundice were gestation \leq 38 weeks, the identification of jaundice during the nursery stay, length of stay in the nursery of $<$ 72 hours, male sex and breastfeeding. At this time, there was increasing pressure to discharge infants earlier from the hospital following birth and we were able to demonstrate that discharge at $<$ 72 hours significantly increased the risk for readmission with hyperbilirubinemia. The American Academy of Pediatrics (AAP) had previously recommended that infants discharged at $<$ 48 hours should be seen by a health care professional within 2-3 days of discharge. Our observations suggested that this recommendation should be extended to those discharged at $<$ 72 hours after birth and the AAP has, subsequently, amended its recommendation consistent with these observations.

Hyperbilirubinemia and Sepsis

Because bacterial infection is a potential cause of hyperbilirubinemia, some authors have suggested that newborns with significant unexplained indirect hyperbilirubinemia should be evaluated for sepsis. To look at this question we reviewed the charts of 306 newborns admitted to the pediatric ward within 21 days of birth with a diagnosis of indirect hyperbilirubinemia (peak serum bilirubin level 18.5 ± 2.8 mg/dL (316 ± 48 $\mu\text{mol/L}$).¹⁴ Sepsis was not identified in any of these 306 infants (upper 95% confidence limit for the risk of sepsis = 1%). 90% of these infants were fully or partially breastfed. We concluded that the overwhelming majority of newborns who require admission to hospital for indirect hyperbilirubinemia are healthy, breastfed newborns and unless they have clinical signs suggesting sepsis, they do not need to be investigated for sepsis.

PATHOGENESIS – STUDIES OF ENDOGENOUS CARBON MONOXIDE PRODUCTION

Why some newborns develop hyperbilirubinemia is a question that is still being investigated. Although it is clear that newborn infants produce more bilirubin than adults, have a limited ability to conjugate and excrete bilirubin and have an increased absorption of bilirubin via the enterohepatic circulation, which of these mechanisms predominates in individual infants is often not clear. Currently there is intensive investigation into the polymorphisms of the genes controlling conjugation and bilirubin uptake and there is evidence that they may play a far more important role in neonatal hyperbilirubinemia than has previously been appreciated. My interest in this area dates back to the late 1960's when I became aware of the work of Ronald Coburn at the

University of Pennsylvania. In a series of seminal studies, Coburn confirmed previous observations that carbon monoxide (CO) is endogenously produced in normal man and that approximately one mole of CO is produced per mole of heme catabolized. As one mole of bilirubin is also produced per mole of heme catabolized, the measurement of CO production is a direct measurement of heme catabolism and therefore bilirubin production.

Endogenous Production of Carbon Monoxide in Normal and Erythroblastotic Newborn Infants

In order to measure CO production, I developed a rebreathing system for the newborn infant.¹⁵ In this system, the infant's face is sealed into an airtight rebreathing mask for up to 3 hours, CO₂ is absorbed and oxygen supplied. Blood is sampled every 30 minutes and analyzed for carboxyhemoglobin. The rate of rise in carboxyhemoglobin corrected for a dilution factor (the increase of blood carboxyhemoglobin produced by the addition of a known amount of CO to the system) allows the calculation of total CO production. These data were then used to calculate mean red cell life span and the rate of bilirubin production. In 9 term infants (40-41 weeks gestation) studied at an average age of 45 hours, mean red cell life span was 88.1 ± 15.1 days and the calculated bilirubin production was 8.5 ± 2.3 mg/kg/24 hrs. Using the plasma bilirubin concentrations in these infants we were able to calculate the retention of intravascular bilirubin from the measured rise in serum bilirubin concentration per 24 hours and the plasma volume. These calculations indicated that, on average, these infants were excreting 5.6 ± 2.3 mg/kg/24 hr of bilirubin. We also studied 4 infants with

erythroblastosis fetalis, 3 with Rh disease and 1 with AO incompatibility. In these infants the CO production rates were 3 to 11 times greater than those in the normal infants and their rates of bilirubin production ranged from 23 to 96 mg/kg/24 hr.

Recognizing that normal newborns produce bilirubin at more than twice the adult rate (per kg/24 hr) suggested that the inability to conjugate bilirubin (as a result of inadequate glucuronosyl transferase) may be less important than previously thought. Some 35 years later we were able to confirm this observation using a different method for measuring CO production (see below).

We subsequently reported data from an additional 17 infants in whom carbon monoxide production was measured using the rebreathing system.¹⁶ When we evaluated all 26 infants, we were unable to show a relationship between the measurements of CO production and either the peak TSB levels or reticulocyte counts. We concluded that a single measurement of CO production was unlikely to be useful in predicting which infants would subsequently develop hyperbilirubinemia and we confirmed these observations many years later (see below).

Exchange Transfusions and CO Production

We utilized our technique for measuring endogenous CO production to elucidate the mechanism of the “late” post exchange transfusion bilirubin rebound.¹⁷ During an exchange transfusion, extravascular bilirubin equilibrates with plasma bilirubin and immediately after the exchange further equilibration takes place, which is complete

within 30 minutes. Thus there is always some degree of bilirubin rebound following an exchange transfusion. In a significant percentage of infants with hemolytic disease, however, a further increase in bilirubin concentration occurs after the initial rebound necessitating one or more further exchange transfusions. In the days when Rh disease was common and before the advent of phototherapy, it was not unusual to have to perform as many as 3 or 4 exchange transfusions on an affected infant. Using our rebreathing system, we measured the endogenous production of CO in 4 infants before and after each exchange transfusion was performed. As anticipated, before the first exchange transfusion, every infant had a significantly increased rate of CO production, but markedly increased CO production rates were also found after 2, or in one case 3, exchange transfusions had been completed. Once CO production rates had fallen into the normal range, no further exchange transfusions were needed. These data were the first to confirm that a continued increase in heme turnover and bilirubin production was primarily responsible for the late bilirubin rebound that occurs after exchange transfusions.

End-Tidal Carbon Monoxide Measurements

The development of a technique for measuring end-tidal carbon monoxide, corrected for ambient CO (ETCOc), by David Stevenson and Henk Vreman at Stanford, greatly simplified the ability to use measurements of CO production as an index of heme catabolism. Measurement of ETCOc is completely non-invasive and requires the sampling of expired gas from the infant's nostril. The gas is drawn into an analyzer that measures the CO concentration with an accuracy of ± 0.3 ppm. The measurement

requires about 5-10 minutes. In a multinational study, ETCOc was measured in 1,370 neonates in 4 countries and 9 clinical sites.¹⁸ The hypothesis was that measurement of ETCOc at approximately 30 hours of life would identify infants who were producing more bilirubin (i.e., hemolysing) and were therefore at a greater risk for the subsequent development of hyperbilirubinemia. TSB levels were measured in all infants at age 96 hours. Although this study showed that an increase in ETCOc had some predictive value in indicating which infants might subsequently develop an elevated TSB level, the measurement of ETCOc was not as good as the measurement of a predischarge TSB alone in predicting the likelihood of hyperbilirubinemia.

Does Hemolysis Contribute to Early Jaundice in the Newborn?

As an extension of my original work on the measurement of CO production, I utilized ETCOc measurements to examine a fundamental question in the pathogenesis of neonatal jaundice: what is the contribution of increased heme turnover (hemolysis) to the development of hyperbilirubinemia in newborns in the first 4 days after birth. We measured ETCOc in 108 jaundiced newborns and 164 control newborns in our well infant nursery.¹⁹ All infants were ≥ 36 weeks of gestation and well. The jaundiced infants were identified with a standard nursery protocol that relied on TcB measurements to identify infants who required a TSB measurement. Those infants whose TSB concentrations exceeded the 75th percentile for age on the Bhutani nomogram were classified as cases. The control group was a convenience sample of infants of similar age in the well infant nursery. Infants qualified as controls if they were

not jaundiced at the time of the ETCOc measurement or, if they were jaundiced, their TSB level did not exceed the 75th percentile.

The mean ETCOc values decreased in the control group each day over the first 4 days but increased in the hyperbilirubinemic group, and the differences between the jaundiced and nonjaundiced infants were highly statistically significant on all days ($p=0.000$). Under normal circumstances, one would expect that approximately 50% of ETCOc measurements would be above the mean but in our jaundiced population, 82% of their ETCOc values were above the mean and 26% were more than 2 standard deviations above the mean value for the control infants. Thus, before hospital discharge, most infants at ≥ 36 weeks of gestation, with TSB levels above the 75th percentile are producing significantly more bilirubin than those with lower TSB levels. This suggests that increased heme catabolism, or hemolysis, is the primary mechanism responsible for hyperbilirubinemia in the first 4 days after birth.

MANAGEMENT

Management of Hyperbilirubinemia in the Breastfed Newborn

The association between breastfeeding and hyperbilirubinemia in the newborn is now well established but questions remain regarding the best approach to the management of jaundice in the breastfed infant. Recommended approaches include using phototherapy, supplementing the breastfeeding with formula, discontinuing breastfeeding and substituting formula, etc. We conducted a randomized, controlled trial at the Hospital Materno-Infantil “R. Sarda” in Buenos Aires.²⁰ In this study 125 full

term breastfed infants were evaluated each day for the presence of jaundice. Based on clinical judgment, a TSB was obtained and then repeated daily until the infants were discharged. Infants jaundiced at discharge were followed daily as outpatients until the bilirubin had reached its peak and started to decline. If the TSB concentration reached or exceeded 17 mg/dL (291 μ mol/L) the infants were enrolled in the study. Study infants were randomly assigned to one of 4 groups: 1) continue breastfeeding 2) discontinue breastfeeding, substitute formula 3) discontinue breastfeeding, substitute formula, administer phototherapy 4) continue breastfeeding, administer phototherapy. The largest decline in TSB levels occurred in both of the phototherapy groups (3 and 4) and the differences between groups (3 and 4) were not significant. The other outcome variable was how many infants developed a TSB > 20 mg/dL (342 μ mol/L). This occurred in only one (3%) of infants in group 3. On the other hand, it only occurred in 6/25 (24%) of infants in group 1, implying that if no intervention other than continuing breastfeeding is employed in an infant whose bilirubin level is 17 mg/dL, (291 μ mol/L) the TSB will not reach 20 mg/dL (341 μ mol/L) in 75% of these infants. Thus, watchful expectancy in this situation is quite justified.

Breastfeeding Frequency and Bilirubin Levels

Although observational studies suggested that more frequent breastfeeding in the first few days after birth was associated with lower TSB levels, no randomized trial evaluating this process had been performed. We therefore studied the effect of breastfeeding frequency on TSB levels in the first 3 days after birth.²¹ 275 infants were randomly assigned to a frequent or demand breastfeeding schedule. In the frequent

group, infants nursed on average 9 times/day and in the demand group 6.5 times/day. The TSB level between 48 and 80 hours (median 53 hours) was 7.4 mg/dL (127 μ mol/L) in the frequent group and 8.0 mg/dL (137 μ mol/L) in the demand group ($p=.103$). Although we could not demonstrate a significant effect on TSB levels as a result of more frequent nursing in this population, there was a trend toward lower levels in those fed more frequently. Because of the limitations of the length of stay in hospital, it was necessary to measure the TSB levels on average at age 53 hours. If we had been able to follow these infants for longer we might well have been able to demonstrate that frequent feeding was associated with lower TSB levels.

TREATMENT – STUDIES ON EXCHANGE TRANSFUSION AND PHOTOTHERAPY

Exchange Transfusion and Ionized Calcium

Almost all exchange transfusions today are carried out using citrate phosphate dextrose (CPD) blood although, prior to its development, acid citrate dextrose (ACD) blood was commonly used. Because of the binding effect of citrate on the calcium ion, it was recognized that the ionized calcium would decrease when an exchange transfusion was performed with either ACD or CPD blood and it had long been a recommended practice to infuse calcium gluconate at regular intervals during the exchange transfusion in order to counteract the known binding effect of citrate on the calcium ion. Although widely practiced, there was no experimental evidence to indicate that the use of calcium gluconate had any beneficial effect on calcium ion homeostasis during exchange transfusion. We took advantage of the development of a calcium iron-selective electrode to study the effect of exchange transfusion and of added calcium gluconate on

the serum ionized calcium concentration.²² We studied 18 newborn infants during 27 exchange transfusions. We measured the total and ionized calcium concentration immediately before the exchange and after every 100 ml exchanged, and 2 hours and 4 hours after the exchange. When heparinized blood was used there were negligible changes in the ionized calcium concentration, whereas with ACD blood there was a significant fall in the ionized calcium. Nevertheless, this fall was not prevented by the infusion of calcium gluconate. Although the infusion of calcium gluconate temporarily increased the ionized calcium level, it immediately decreased as the exchange transfusion continued. It had also been a practice to infuse additional calcium if infants were noted to be irritable or crying or jittery during the exchange transfusion. We recorded the infant's clinical state as quiet or sleeping or crying or irritable during the exchange and correlated these findings with the serum ionized calcium concentrations. There was no association between the levels of ionized calcium and the clinical signs and symptoms indicating that other factors must be responsible for these changes in infant's behavior during exchange transfusion.

PHOTOTHERAPY

Phototoxicity to the Retina

With the introduction of phototherapy, it was generally accepted that it was important to protect the infant's eyes from direct exposure to the phototherapy lights because of the potential for damage to the newborn's retina. Although the toxic effect of light on the retina had been demonstrated in rats, piglets and pigeons, no such studies had been done in the newborn subhuman primate. In addition, in almost all of the published

studies, the animals' eyes were kept open with sutures, and mydriatic drops were used to provide pupillary dilatation. In our study we used newborn stump-tail monkeys, weighing 450-500g and exposed them to cool white fluorescent light from a standard phototherapy lamp for periods of 12hr, 24hr, 3 days and 7 days.²³ These monkeys were kept in standard infant incubators and a restraining device was used inside the incubator to maintain the monkey in a supine position facing the light source. On the other hand, no mydriatic drops were placed in the eyes and the monkeys could close their eyes as desired. The left eye was used as the study eye and the right eye was the control. The right eye was sutured closed and covered with a patch of black velour material. The exposed eyes showed progressive damage to the retina even after only 12 hours of exposure. We, therefore, concluded that there was a sound basis for the recommended practice of patching the eyes of infants undergoing phototherapy.

Fiberoptic Phototherapy

Fiberoptic phototherapy systems are convenient in that they can provide phototherapy without the need for eye patching and they allow a mother to hold her newborn while the infant receives phototherapy. On the other hand, because of the physical properties of a light source that is remote from the blanket or pad through which the phototherapy is delivered, the size of the phototherapy pads has been kept small in order to maintain a higher irradiance. Making the pads larger would decrease the irradiance although it would increase the surface area of the baby exposed to phototherapy. We conducted a randomized controlled trial to compare fiberoptic phototherapy with conventional phototherapy in healthy jaundiced newborns with birth weights > 2500 g.²⁴ Both

methods of phototherapy were effective in lowering the TSB but the mean TSB was lower after 18 hours of therapy in the conventional phototherapy group. On the other hand, the conventional phototherapy lights produced an average irradiance of 9.2 $\mu\text{W}/\text{cm}^2/\text{nm}$ versus 8.2 $\mu\text{W}/\text{cm}^2/\text{nm}$ in the fiberoptic group. In addition, the area of skin exposed to standard phototherapy lights is substantially greater than the area that receives phototherapy when fiberoptic pads or blankets are used and the exposure of a larger surface area of the infant to phototherapy produces a more significant decline in the TSB.

Double Phototherapy

The availability of fiberoptic phototherapy makes it easy to administer conventional phototherapy from above while the infant lies on a fiberoptic phototherapy blanket. In this study, newborns with birth weights <2500 g were randomly assigned to receive either single or double phototherapy.²⁵ After 18 hours of therapy, the serum bilirubin concentration declined by $31 \pm 11\%$ in the double phototherapy group and $16 \pm 15\%$ in the single phototherapy group. The irradiance delivered in the two groups was similar but the newborns receiving double phototherapy had a larger surface area exposed, so that the total dose of phototherapy was increased. These data demonstrated conclusively that increasing the surface area of the infant exposed to phototherapy significantly improves its efficacy.

Light Emitting Diodes

The most recent addition to the armamentarium of phototherapy is the light-emitting diode (LED). LED lights are power-efficient, durable light sources that provide high intensity light in the blue portion of the visible spectrum. LED lights do not emit significant infrared or ultraviolet radiation and produce minimum radiant heat. The small size, high luminous intensity and narrow wavelength band light of LED phototherapy makes this a useful method for delivering intensive phototherapy to newborn infants.

In a randomized, controlled trial, we compared the efficacy of LED phototherapy with special blue fluorescent (BB) tube phototherapy in the treatment of neonatal hyperbilirubinemia.²⁶ In this study we randomly assigned 66 infants ≥ 35 weeks of gestation to receive phototherapy using a LED device or BB. In addition to phototherapy from above, all infants also received phototherapy from below using 4 BB tubes or a fiberoptic pad. After 15 ± 5 hours of phototherapy the rate of decline in the total serum bilirubin (TSB) was 0.35 ± 0.25 mg/dL/h (6.0 ± 4.3 μ mol/L/h) in the LED group versus 0.27 ± 0.25 mg/dL/h (4.6 ± 4.3 μ mol/L/h) in the BB group ($p=0.20$). We concluded that LED phototherapy is as effective as BB phototherapy in lowering serum bilirubin levels in term and late preterm newborns.

“Homeopathic” vs Intensive Phototherapy

In 1996 I published a commentary entitled “Why use homeopathic doses of phototherapy.”²⁷ Although this publication took the form of a brief review, it did contain original data regarding the effect of the light source and distance from the light source on the irradiance delivered to the infant. In addition to discussing the efficacy of phototherapy and how it is affected by the light spectrum, the irradiance, the surface

area, and the spectral power delivered to the newborn, I noted that, although in use in the United States for more than a quarter of a century, the way in which phototherapy was delivered was frequently not effective. Phototherapy was used initially to prevent the TSB levels from reaching exchange transfusion levels in preterm infants. The subsequent move toward early discharge of infants from the nursery led to more infants being readmitted with significant elevations of serum bilirubin, some as high as 25 mg/dL (428 $\mu\text{mol/L}$). In these infants it was necessary to reduce the serum bilirubin level as rapidly as possible and for this type of infant intensive phototherapy was necessary. In addition to the general discussion of phototherapy presented in this paper, we measured the effect of the type of light source used, as well as the distance of the light source from the infant on the spectral irradiance. We were able to show that conventional phototherapy lights (daylight or regular blue lights) when placed 30 cm from the infant delivered an irradiance of about 8-10 $\mu\text{W/cm}^2/\text{nm}$. At the same distance from the infant, special blue fluorescent tubes delivered 30 $\mu\text{W/cm}^2/\text{nm}$ and, when placed 10 cm from the infant, the special blue tubes delivered 60 $\mu\text{W/cm}^2/\text{nm}$ whereas the conventional phototherapy lights delivered about 12-15 $\mu\text{W/cm}^2/\text{nm}$. We were thus able to demonstrate the importance of understanding the effect on irradiance of the type of light used and the distance between the light source and the infant.

The Effect of Phototherapy on Exchange Transfusion

In this paper I analyzed the impact of the introduction of phototherapy on the number of exchange transfusions performed.²⁸ I was able to show that in the 1950's and early 60's, prior to the introduction of phototherapy, the rate of exchange transfusions for all

infants was about 43/10,000 live births. Phototherapy was introduced in the late 1960's and, by the 1980's and 90's, the rate of exchange transfusions had fallen to 1.6/10,000 live births. During this time period, of course, the use of anti-D gamma globulin had been introduced to prevent Rh hemolytic disease and this undoubtedly had an impact on the number of exchange transfusions needed. Nevertheless, the use of intensive phototherapy has had a dramatic effect on decreasing the need for exchange transfusions in both ABO and Rh hemolytic disease. When one looks at infants with birthweights less than 1500 g the impact has been even more dramatic. In the absence of phototherapy, 36% of infants with birth weights <1500 g received at least one exchange transfusion. Between January 1988 and October 2007, not a single exchange transfusion has been performed at the William Beaumont Hospital on 2425 infants who weighted < 1500 g at birth.

Bilirubin Rebound Following Phototherapy

The use of intensive phototherapy has raised additional questions. It was well documented that, following the discontinuation of conventional phototherapy, some rebound in the TSB level usually occurred, but this question had not been studied in infants receiving intensive phototherapy.

We studied two groups of infants - those who received intensive phototherapy in our well baby nursery during their birth hospitalization and a second group who had been discharged from the nursery and were readmitted for phototherapy.²⁹ In the infants who received phototherapy during their birth hospitalization, 8.2% had a rebound sufficient to

require additional phototherapy whereas only 1 (0.7%) of 144 infants who first received phototherapy on readmission required additional phototherapy. We concluded that it was not necessary to keep infants in the hospital to check for rebound although it was clear that those who required phototherapy during their birth hospitalization were more likely to have a significant rebound than those admitted later. The incidence of ABO blood group incompatibility with a positive direct Coombs' test in the infants receiving phototherapy before discharge was 22% compared with only 3.5% in those infants who were readmitted for phototherapy. Thus, the infants receiving phototherapy before discharge from the hospital are much more likely to have some degree of hemolysis and are therefore more likely to have a rebound in serum bilirubin.

Does Intensive Phototherapy Produce Hemolysis?

The other question that has arisen regarding intensive phototherapy is - could it produce hemolysis? There is some in vitro evidence that phototherapy can increase the erythrocyte osmotic fragility and produce lipid peroxidation of the red cell membrane leading to hemolysis.

We measured end tidal CO concentration corrected for ambient CO (ETCOc) before, during and after phototherapy in infants receiving intensive phototherapy.³⁰ In a study of 27 infants \geq 35 weeks of gestation, there was a steady decrease in the mean ETCOc over the course of phototherapy and we were able to conclude that there was no evidence that intensive phototherapy produced hemolysis in these infants.

BILIRUBIN ENCEPHALOPATHY

Does Hyperbilirubinemia Damage the Brain of Healthy Full-Term Infants?

A question that has long been asked is whether or not hyperbilirubinemia is damaging to the brain of healthy full-term infants. While it is certain that if TSB levels are high enough, they can cause kernicterus (see below), it was a standard recommendation in textbooks that whenever the TSB level reached 20 mg/dL (342 μ mol/L) an exchange transfusion should be performed, irrespective of the cause of the hyperbilirubinemia. In this paper, Thomas B. Newman and I reviewed the world's literature on this subject in considerable detail looking at all of the published studies dealing with the effects of hyperbilirubinemia on cognition, neurologic abnormality and on hearing.³¹ This analysis included a discussion of the effects of confounding and effect modification, whether or not the correct "risk factor" was being measured, and finally the importance of clinical versus statistical significance. We were able to conclude that no evidence exists to support the view that bilirubin levels of 20 mg/dL (342 μ mol/L) are hazardous to healthy term infants and this conclusion has been confirmed in recent studies.

New Guidelines for Management

A follow up on this review was a more focused discussion of the management of hyperbilirubinemia in which we proposed new guidelines for the treatment of these infants.³²

An Animal Model of Bilirubin Encephalopathy

In most animal species, hyperbilirubinemia alone fails to produce kernicterus but when accompanied by additional insults to the nervous system, such as asphyxia, kernicterus can occur. To further elucidate this process, we examined the effect of hyperbilirubinemia on the brain stem auditory evoked responses (BAER) in the paralyzed, ventilated adult rat.³³ The infusion of bilirubin alone failed to produce changes from control values in the BAER. In asphyxiated animals, however, progressive deterioration in the BAER was seen in 58% of the surviving asphyxiated animals who received bilirubin. These changes were not seen in any of the asphyxiated animals who did not receive bilirubin. The secondary deterioration of the BAER involved all 4 major waves, suggesting that the toxicity affected either the cochlea/auditory nerve (represented by wave I) or the brainstem auditory pathway, reflected by subsequent waves. These data confirmed that monitoring the BAER could be useful as a non-invasive means of identifying bilirubin toxicity in the newborn.

Kernicterus in Healthy Term Newborns

If TSB levels are sufficiently elevated, kernicterus will occur and we documented this in a report of 6 infants born between 1979-1991 whose peak recorded TSB levels occurred 4-10 days after birth and ranged from 39.9-49.7 mg/dL (667-850 $\mu\text{mol/L}$).³⁴ None of these infants had laboratory findings consistent with hemolysis and no cause was found for the hyperbilirubinemia other than its association with breastfeeding. We concluded that although rare, classic kernicterus can occur in apparently healthy, full-

term breastfed newborns who do not have hemolytic disease or any other discernable cause for their jaundice.

Kernicterus is Still Occurring

I subsequently authored a commentary to bring the attention of the paediatric community to the issue of kernicterus which is still occurring today.³⁵ This commentary was followed by the Clinical Practice Guideline of the American Academy of Pediatrics on the management of hyperbilirubinemia in the newborn infant 35 or weeks of gestation.³⁶ I was the principal author of this guideline which is widely used throughout the world. As background to this guideline, it was necessary to produce an evidence-based review of the issues that were being considered by the academy's subcommittee. I participated in this technical report and contributed the information on the risks of exchange transfusion.³⁷

Bilirubin as an Antioxidant

Although bilirubin is known to be toxic to the central nervous system, it is also one of the most powerful antioxidants that we possess and, at the tissue level, exerts a more powerful antioxidant effect than does alfa-tocopherol (vitamin E). It is known that oxidative injury contributes to the development of retinopathy of prematurity (ROP) and, because of its antioxidant properties, we wondered whether or not bilirubin might have a role in preventing ROP in the extremely low birth weight infant. We therefore evaluated the relationship of ROP to bilirubin levels in 157 infants born at 23 to 26 weeks estimated gestational age.³⁸ For each infant we calculated the average of all bilirubin

levels obtained on each day for the first 15 days of post natal life. The mean bilirubin level over the first 15 days in infants with mild ROP was 5.0 ± 0.8 mg/dL (428 ± 14 μ mol/L) and 4.7 ± 0.7 mg/dL (80 ± 12 μ mol/L) in those with severe ROP (not significant). When the groups were further subdivided into the individual stages of ROP, no significant differences in mean TSB levels were found. However, when the groups were arbitrarily divided by average bilirubin levels, we did find that a significantly higher proportion of infants with severe ROP had average bilirubin levels of < 5 mg/dL (85 μ mol/L). This result could have been a function of the fact that those infants with severe ROP were smaller and less mature and would therefore have received earlier and more aggressive phototherapy.

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