NUTRITIONAL AND METABOLIC STUDIES IN TERM AND PRETERM INFANTS

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DECLARATION AND ACKNOWLEDGEMENTS

Although all of the studies described in this Thesis were conceived and initiated by myself, I am greatly indebted to a number of individuals whose invaluable practical and theoretical support enabled me to complete these studies.

During the development of the computerised indirect calorimetry system I was extremely grateful for the technical assistance I received from Mr David Avery, Technical Director of Airspec (UK), (now Chest Scientific Instruments Ltd) from whom I purchased the mass spectrometer and who, under my guidance, wrote the appropriate software. I am grateful to Mrs Anna Crichton, Research Nurse, who provided me with practical support during the technical and clinical evaluation of the calorimetry system.

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The study of the effect of early solid feeding on infant health was undertaken in parallel with other studies of Infant Feeding and Health, in which I collaborated with

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NUTRITIONAL AND METABOLIC STUDIES IN TERM AND PRETERM INFANTS.

SUMMARY

The nutritional management of the newborn infant continues to be plagued by fundamental issues which are the subject of much confusion and contradiction. There is not only an urgent need to clarify these issues, but also a need to widen our knowledge and understanding of many aspects of infant nutrition especially that relating to the preterm infant.

This Thesis considers nutritional issues in both term and preterm infants. The issues addressed in the term infant are first, the aetiology of the condition breast milk jaundice, and second, whether the practice of early solid feeding is harmful to the infant. The studies in the preterm infant are aimed at providing nutritional data on the sick ventilated infant.

MEASUREMENT OF ENERGY EXPENDITURE AND NUTRIENT UTILISATION IN VENTILATOR-DEPENDENT VERY LOW BIRTHWEIGHT INFANTS.

With neurodevelopmental outcome of very low birth weight infants being adversly affected by inadequate nutrition during the first few weeks of life, there is an urgent

need for more specific nutritional data on the sick VLBW ventilator-dependent infant. To date there is no calorimetry data on these infants because of technical difficulties relating to the practice of using continuous flow ventilators for neonatal ventilation. With differences in gas concentrations between inspiratory and expiratory circuits being so small, a system of considerable accuracy and precision is required.

STUDY 1 Development of a computerised indirect calorimetry system for measurement of energy expenditure in ventilator-dependent low birthweight infants.

The development of a new mass spectrometric gas analysis indirect calorimetry system which is non-invasive and can operate over several hours or days is described. The system, which is computer driven, consists of a respiratory mass spectrometer, a mesh pneumotachometer with differential pressure transducer, a 3-way elecromagnetic valve, and minor modifications to normal ventilator circuitry. The data from the mass spectrometer and the pneumotachometer are received by the microcomputer and oxygen consumption (VO_2), carbon dioxide production (VCO_2), respiratory quotient (RQ) and energy expenditure (EE) are calculated using the Weir equations.

Technical evaluation of each of the components of the

system indicates a total random error of <5%. Systematic error was determined using gas infusions which simulated carbon dioxide production and oxygen consumption. The relative error in the measurement of carbon dioxide production was <1.5% (CV 6.0%), and oxygen consumption <4.3% (CV 2.3%).

Clinical studies on 10 infants demonstrated a mean energy expenditure of 40.2 kcal/kg/day and a respiratory quotient in excess of 1.0.

CONCLUSION

A system for measuring energy expenditure in sick ventilated low birthweight infants has been developed and technical evaluation confirms that the system is precise and accurate. Clinical evaluation shows that continuous measurements can be made without interfering with the normal care of the infant. The energy expenditure of ventilated low birthweight infants may be less than previously indicated and the ratio of carbohydrate to fat and nitrogen content of parenteral nutrition regimen recommended for these infants may be inappropriate.

STUDY 2 Measurement of energy expenditure, oxygen consumption, carbon dioxide production and respiratory quotient in ventilated and non-ventilated preterm infants during the first 6 days of life.

Using the newly developed and evaluated calorimetry system, infants of birthweight less than 2000g were studied during the first week of life. Twelve of these infants were receiving artificial ventilation and 10 were breathing air spontaneously. All were receiving total parenteral nutrition.

On days 1-2, 3-4, and 5-6 there was no difference in energy expenditure between the two groups, but both groups demonstrated a trend of increasing energy expenditure during this time. The respiratory quotient was greater than 1.0 in both groups of infants.

CONCLUSION

The energy expenditure of ventilated infants during the first week of life is not greater than that of spontaneously breathing infants and may be much less if paralysing agents are prescribed. This has important implications for the nutritional management of these infants. With present regimens there is evidence of excess carbohydrate intake being converted to fat and this

increases carbon dioxide production. Modification of parenteral nutrition regimens may not only improve nutrient accretion rates but also reduce ventilator requirements of the infant.

INVESTIGATION OF THE AETIOLOGY OF BREAST MILK JAUNDICE

The aetiology of breast milk jaundice, an unconjugated hyperbilirubinaemia occurring in approximately 2-4% of breast fed infants, remains uncertain. It has been suggested that elevated free fatty acid concentrations within the breast milk inhibit hepatic glucuronyl transferase activity and their presence is the consequence of the activity of an abnormal lipase which has the chemical characteristics of bile salt-stimulated lipase but does not require prior stimulation by bile salts. For this conclusion to be drawn it was assumed that bile salts were not present in breast milk. There being no studies to support this assumption an investigation of breast milk for the presence of bile salts was undertaken.

STUDY 1. Study of breast milk for the presence of bile salts

Analysis of 28 random samples of breast milk demonstrated that small concentrations of cholate and chenodeoxycholate

were present in milk and with the ratios differing from that present in maternal blood, active transport mechanisms may be involved. A further study was performed to provide longitudinal data on bile salts in breast milk and their relationship to bile salt stimulated lipase (BSSL) in normal and jaundice milks.

STUDY 2. A study of the relationship between bile salts, bile salt-stimulated lipase and free fatty acids in breast milk of normal infants and those with breast milk jaundice.

Analysis of milk samples obtained at different times during lactation showed that there was a significant decline in both cholate and chenodeoxycholate levels with duration of lactation ($p<0.05$). There was also a significant fall in BSSL activity ($p<0.05$) but no correlation was found between BSSL activity and bile salt concentration. FFA concentrations were similar throughout lactation and were not related to either BSSL activity or bile salt concentration. There was a significant increase in the concentration of cholate and the cholate to chenodeoxycholate ratio in the milks of 12 infants with breast milk jaundice compared to normal milks, the BSSL activity was similar and contrary to previous reports, the FFA concentration was not increased in the milks of infants with breast milk jaundice.

CONCLUSION

This study does not support previous reports of increased lipase activity and increased free fatty acid concentration in breast milk of infants with breast milk jaundice. Further original data on the presence of bile salts in breast milk is reported and their relationship to the wide variation in free fatty acid composition of both normal and jaundiced milks requires further investigation.

A STUDY TO DETERMINE IF EARLY SOLID FEEDING IS HARMFUL TO THE INFANT.

Despite the professional advice that solid foods should not be introduced before 3 months of age the OPCS survey of 1980 showed that 56% of infants were introduced to solids before this time and the proportion increased to 62% when the survey was repeated in 1985. The reasons put forward for discouraging the premature introduction of solids include the possible risk of excessive weight gain, vulnerability of the gut to infection, and increased susceptibility to the development of allergic disease.

A prospective clinical study was undertaken to determine the influence of the early introduction of solid foods on weight gain, the risk of gastro-intestinal disease and the risk of allergic disorders during the first 2 years of life. Special consideration was given to design and

methodology, in particular, sample size, accuracy of feeding data, definition of outcomes, data collection and potential confounding variables.

In this study 9.7% of infants were commenced on solids before 8 weeks and 49.4% between 8 and 12 weeks of age. Solids were introduced earlier in infants who were male, who were from lower socio-economic groups and who were bottle fed. After adjustment for confounding variables the introduction of solids before 13 weeks of age was associated with increased weight gain in infants at 8, 13 26 and 52 weeks but not at 104 weeks.

Before and after adjustments for confounding factors, the incidence of gastro-intestinal infection was not influenced by the timing of the introduction of solids. Similarly, the early introduction of solids was not associated with an increased incidence of napkin dermatitis or wheeze. The incidence of eczema was increased at two years in infants who received solids between 8-12 weeks. There was increased incidence of respiratory illness at 14-26 weeks, and persistent cough at 14-26 and 27-39 weeks in early solid feeding infants.

CONCLUSION

This study concludes that many infants are receiving solids within the first three months of life but this

feeding practice is not associated with excessive weight gain or increased gastrointestinal illness. With the incidence of eczema being increased in the 8-12 week solid feeding group but not in infants receiving solids prior to this time, the association between early solid feeding and eczema remains inconclusive. Further follow-up may clarify this issue. The incidence of respiratory illness was increased during the first year of life in early solid feeding infants. Whether these symptoms are infective or allergic in origin cannot be answered by this study, but as the prevalence of asthma and other allergic disorders peak in later childhood, longer term follow-up of this cohort may clarify this issue. In the meantime, if parents wish to commence their infants on solid foods before three months of age they should be advised that there is an increased incidence of respiratory illness in early solid feeding infants.

CHAPTER ONE

GENERAL INTRODUCTION

As we approach the end of the twentieth century, it is remarkable that infant nutrition, one of the most vital ingredients for the continuation of human life, should continue to present so many unanswerable questions and require to be the subject of so much medical and scientific attention. Interest in infant nutrition has spanned many centuries with advocacy for the practice of breast feeding being recorded in several historical and religious texts including the Koran and the writings of Hippocrates. In his presentation to the Royal Society of Medicine in 1911[1], Dr David Forsyth, a London physician, provided a remarkable insight into infant nutrition during Elizabethan Times and discussed many of the infant feeding issues which continue to provoke debate today, notably the benefits of breast feeding, the safety of artificial feeds and the timing of weaning.

Why these issues remain unresolved today is not due to a lack of scientific interest which has been considerable, with many contributions being made by scientists, clinicians, psychologists, and sociologists. These publications have undoubtedly greatly extended our knowledge and understanding of infant nutrition but

unfortunately have also tended to raise further questions rather than provide unequivocal answers.

A reinforcement of the need for continuing scientific interest in infant nutrition has been provided by recent intriguing data from two separate centres. Lucas and co-workers in Cambridge, in a prospective clinical trial of neonatal diet on later brain development, demonstrated that a brief period of enhanced diet, had a significant impact on developmental quotient in infancy, and this advantage was still evident when the children were reassessed at 18 months and 4 years[2,3]. Barker and co-workers in Southampton have produced epidemiological data demonstrating that fetal and infant growth are strong predictors of later blood lipids, cardiovascular disease, hypertension, chronic bronchitis, and diabetes[4,5]. The data evolving from these centres strongly support the concept that early nutrition and growth are powerful determinants of later intellect and health.

Current research on infant nutrition can be divided into two principal areas of interest; studies which are attempting to resolve fundamental issues in the nutritional management of term infants, and studies which are aimed at increasing our knowledge and understanding of nutritional metabolism of the preterm infant. Both of these aspects of research of infant nutrition are

represented in this Thesis. The issues addressed in the term infant are first, the aetiology of the condition breast milk jaundice, and second, the question of whether the practice of early solid feeding is harmful to the infant. The studies in the preterm infant are aimed at providing much needed nutritional data on the sick ventilator-dependent infant.

CHAPTER TWO

MEASUREMENT OF ENERGY EXPENDITURE AND NUTRIENT UTILISATION IN VENTILATOR-DEPENDENT LOW BIRTHWEIGHT INFANTS.

2.1 BACKGROUND TO THE STUDY

Nearly 1% of all infants have a birth weight of less than 1500 g[6]. Although, in recent years, the mortality of this group has greatly diminished, there is still significant morbidity[7]. More than 60% of these very low birth weight (VLBW) babies suffer from respiratory distress syndrome and require artificial ventilation[8], the duration of which can be prolonged if the infant develops chronic lung disease. Attempts to reduce morbidity in this group of infants have concentrated on achieving improved cardiorespiratory function and haemodynamic stability during the immediate postnatal period[9]. However, recent human and animal studies have indicated that inadequate nutrition during this time may be a further potential contributing factor. A multicentre study has shown that preterm infants receiving a special low birthweight milk formula achieved significantly better developmental scores than controls at 18 months[2,3]. This study was undertaken on infants who were sufficiently well to tolerate enteral feeds. The special feed was only given for a median period of 28 days and the fact that

such a short period of enhanced nutritional support in a relatively low risk population can have such dramatic effects on neurodevelopment raises the question of whether more vigorous nutritional support during the period when these infants are most sick and requiring ventilation will result in a further improvement of neurodevelopmental scores. It is during this time that nutritional support is most difficult and growth failure most marked[10].

Recent animal studies have demonstrated that if nutrition is suboptimal, normal lung growth is impaired and recovery from inflammatory damage delayed[11]. Therefore, improved nutrition may not only reduce neurodevelopmental morbidity but also improve respiratory status thus allowing a shorter period of ventilation and earlier introduction of enteral feeds for these vulnerable infants.

To date, interest in the nutritional status and management of the VLBW baby has concentrated on healthy growing infants, their energy expenditure and nutritional balance being estimated while receiving different feeding regimes[12]. Indirect calorimetry has been the method of choice as it is practical and accurate for the spontaneously breathing infant. This method provides an estimate of oxygen consumption and carbon dioxide production from which the energy expenditure and respiratory quotient (RQ) can be calculated[13]. If

simultaneous measurement of urinary nitrogen is made, fat, carbohydrate, and protein utilisation can be calculated[14]. From these studies on healthy premature infants it has been suggested that the total energy intake for enterally fed infants should be in the region of 110-160 kcals/kg/day,[15] and 60-80 kcals/kg/day for infants receiving parenteral nutrition[16].

It cannot be assumed that this data is meaningful for the sick ventilator-dependent infant. The overall work of breathing in these infants will be influenced by the contribution of ventilator support, the frequency of spontaneous respirations and the compliance of the lungs. In adults, oxygen consumption is increased in trauma and sepsis[17], both of which are frequently present in sick infants. In healthy premature infants 15-20% of energy expenditure may be due to spontaneous activity[15] and this is likely to be reduced in the sick and the more premature infant. The overall effect of all of these factors on total energy expenditure in sick ventilated infants is at present unknown.

The lack of calorimetry data on sick ventilator-dependent infants is primarily related to the practice of using continuous flow ventilators for neonatal ventilation. These ventilators provide a continuous flow of oxygenated air across the proximal end of the endotracheal tube thus,

allowing the inhalation of fresh gas if spontaneous respirations are present. With the continuous gas flows being in the region of 6-8 l/min, and the volumes of oxygen consumed and carbon dioxide produced by the infant being only several mls/min, measurement of differences in gas concentrations between inspiratory and expiratory circuits requires a system of considerable accuracy and precision, particularly if the inspiratory oxygen concentration is high. An additional requirement of such a system is the ability to operate over several hours and to allow routine medical and nursing care of the infant to continue unhindered.

These criteria have been taken into consideration during the development of a indirect calorimetry system designed for use in ventilator-dependent infants.

2.2 DEVELOPMENT OF A COMPUTERISED INDIRECT CALORIMETRY SYSTEM FOR MEASUREMENT OF ENERGY EXPENDITURE IN VENTILATOR-DEPENDENT LOW BIRTHWEIGHT INFANTS

2.2.i The Indirect Calorimetry System

The system, which is computer driven, consists of a respiratory mass spectrometer, a mesh pneumotachometer with differential pressure transducer, a 3-way electromagnetic valve, and minor modifications to normal ventilator circuitry (Fig 1). The principle is similar to previously reported open circuit techniques in that samples of gas are drawn from the inspiratory and expiratory circuits and the oxygen consumption and carbon dioxide production are calculated from the product of the volume of the gas passing through the ventilator circuit and the changes in oxygen and carbon dioxide occurring between the two circuits. The data from the mass spectrometer and the pneumotachometer are received by the microcomputer and oxygen consumption (VO_2), carbon dioxide production (VCO_2), respiratory quotient (RQ) and energy expenditure (EE) are calculated using the Weir equations.[13]

VO_2 ml/min = Ve ml/min (FiO_2 - FeO_2)

VCO_2 ml/min = Ve ml/min ($FeCO_2$ - $FiCO_2$)

Energy Expenditure = 3.9 VO_2 + 1.1 VCO_2

(kcal/kg/min)

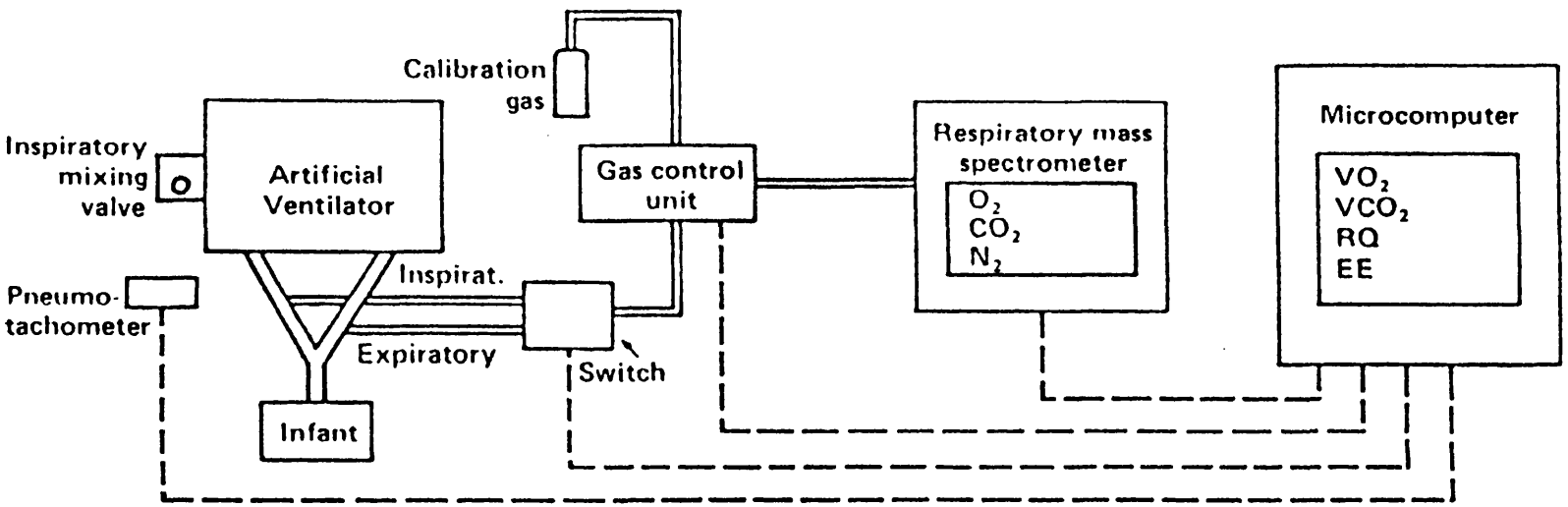


FIGURE 1

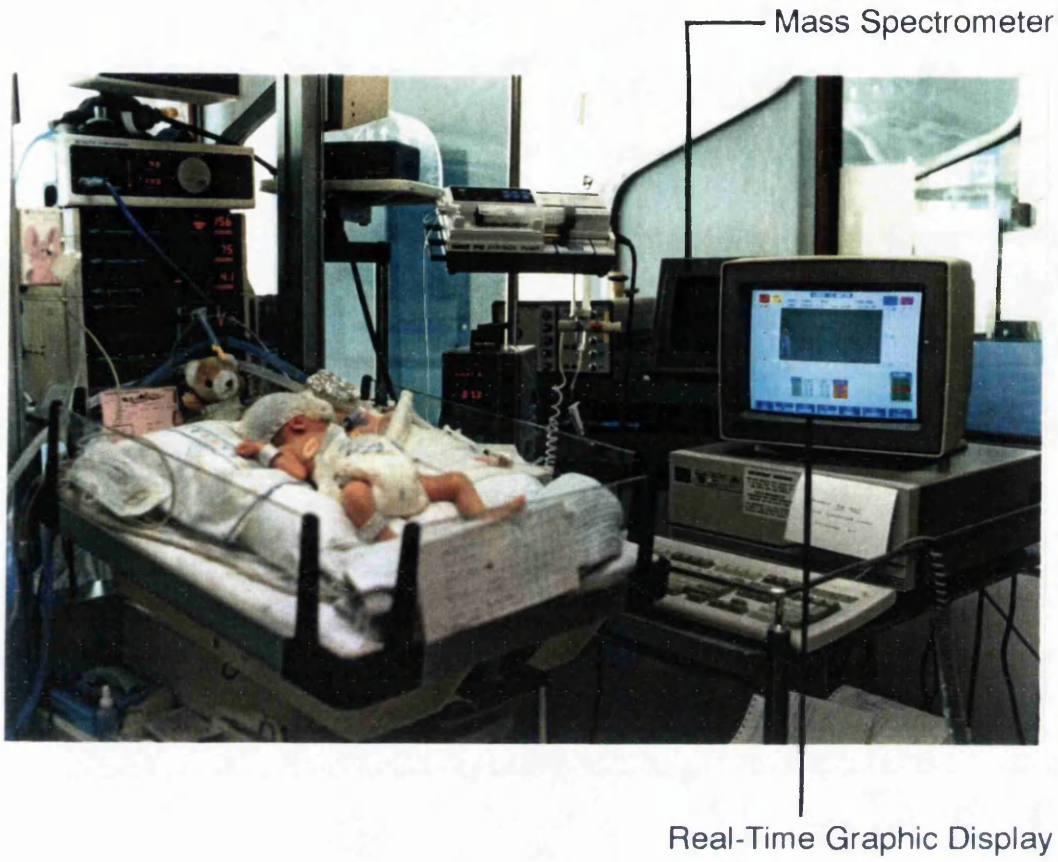
SCHEMATIC FLOW DIAGRAM OF INDIRECT CALORIMETRY FOR VENTILATOR-DEPENDENT INFANTS

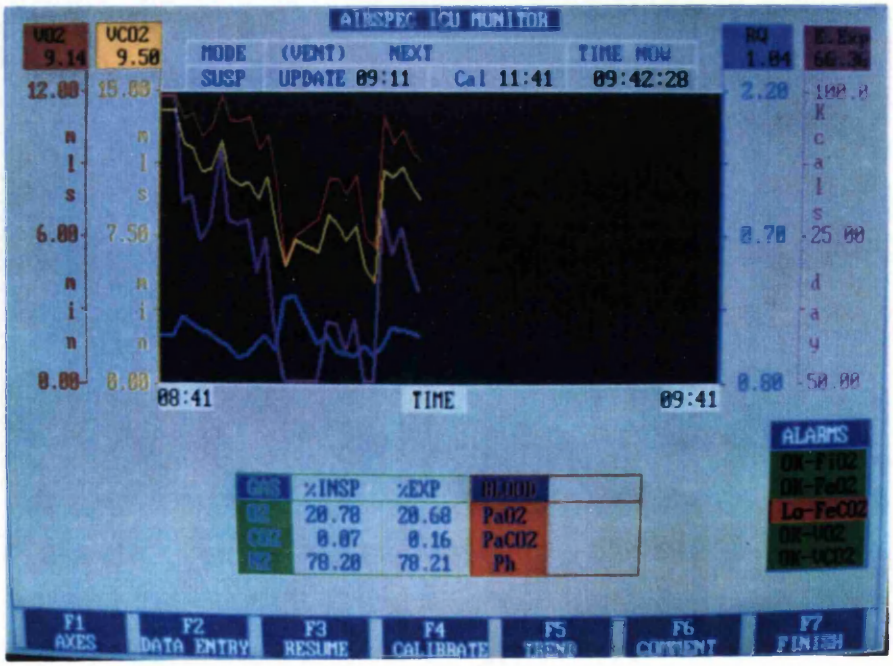
Each of the measured parameters is graphically displayed in real time on the computer screen (Photograph 1), stored in a data file and can be continuously printed (Fig 2). If urine is collected during the study, this enables the measurement of urinary nitrogen which will allow the determination of the non-protein RQ, fat, carbohydrate and protein utilisation[14], and additional nutritional parameters, which can be calculated by the dedicated software.

2.2.ii Technical Evaluation

The evaluation of the system was first approached by determining the errors of each of the components of the system. The calculations stated above include three measured values: the volume of gas flow, and the concentrations of oxygen and carbon dioxide in the inspiratory and expiratory circuits. The minimum expected error was therefore the sum of the errors sustained in obtaining these specific measurements. Other potential areas of error were the gas sampling process and the effects of varying pressure, humidity and temperature within the ventilator circuitry. It was also recognised that without a perfect "gold standard" against which the the system could be compared, a component of the systematic error could be due to error in measurement of

INDIRECT CALORIMETRY SYSTEM





GRAPHIC DISPLAY

Photograph 1

TIME	FiO2	FiCO2	FeO2	FeCO2	Ve	VO2	VCO2	RQ	Energy
9.22	22.71	0.03	22.61	0.12	6.74	6.13	5.33	0.87	42.85
9.24	22.68	0.03	22.57	0.13	6.77	7.08	6.53	0.92	50.12
9.26	22.67	0.03	22.58	0.10	6.78	5.41	4.39	0.81	37.34
9.27	22.67	0.03	22.58	0.11	6.77	5.73	5.15	0.90	40.37
9.29	22.67	0.03	22.59	0.12	6.80	5.16	5.32	1.03	37.39
9.31	22.67	0.03	22.57	0.11	6.78	6.56	4.69	0.71	44.26
9.32	22.67	0.03	22.59	0.09	6.78	4.91	3.68	0.75	33.41
9.34	22.68	0.03	22.59	0.09	6.80	5.72	3.92	0.69	38.30
9.36	22.68	0.03	22.58	0.11	6.81	6.14	5.05	0.82	42.48
9.37	22.68	0.03	22.59	0.11	6.79	5.42	5.22	0.96	38.69
9.39	22.68	0.03	22.59	0.11	6.79	5.86	4.81	0.82	40.54
9.41	22.69	0.03	22.61	0.10	6.81	5.36	4.33	0.81	36.99
9.42	22.70	0.03	22.61	0.10	6.81	5.55	4.50	0.81	38.29
9.44	22.71	0.03	22.63	0.11	6.82	5.41	5.04	0.93	38.39
9.46	22.72	0.03	22.63	0.10	6.79	5.21	4.13	0.79	35.80
9.47	22.72	0.03	22.64	0.10	6.79	5.09	4.05	0.80	34.99
9.49	22.72	0.03	22.63	0.11	6.76	5.66	5.11	0.90	39.85
9.51	22.73	0.03	22.62	0.13	6.77	6.95	5.91	0.85	48.40
9.52	22.74	0.03	22.64	0.12	6.87	6.40	5.45	0.85	44.58
9.54	22.74	0.03	22.65	0.10	6.86	5.81	4.62	0.80	39.92

or PgUp Pgdown:"T"ag or "U"ntag for lines to delete: ESC to Exit

FIGURE 2

PRINT-OUT OF CALORIMETRY DATA

the "gold standard" - in this case gas infusion studies - rather than the calorimetry system.

Infant ventilator

The infants were ventilated with a continuous flow, time-cycled infant ventilator (Sechrist Model IV-100B). Depending on the compressible volume of the circuit and the humidifier, a flow rate of 6-8 litres/minute is usually required to achieve adequate peak pressure and pressure rise time. Initial analyses of gases sampled from the inspiratory and expiratory circuits demonstrated that there was contamination of the gas samples taken from the expiratory circuit. This was found to be due to a low pressure flow of gas which, occurring during the exhalation phase, passed from the fluidic control system to the pressure alarm system via the pressure sense line and this line connected with the patient attachment at the proximal end of the expiratory circuit. This flow of gas was not essential for normal ventilator performance and after discussion with the distributing company (EME, Brighton) this gas flow was disconnected.

Flow measurements

The flow through the ventilator circuit was measured by a mesh-type pneumotachometer which was situated in the inspiratory circuit, proximal to the humidifier. The pneumotachometer was connected to a differential pressure transducer (Airspec,UK), which had an output of 5 volts

for 12 l/min and linearisation over a range of 0-12 l/min. Calibration of the pneumotachometer was undertaken using a calibrated syringe and a previously described computerised method which determines the conductance characteristics of the flowmeter[18]. The conductance values of the pneumotachometer correspond to pressure values which are determined by a weighted averaging technique, following multiple strokes of the precision calibrated syringe. The conductance values allow the measured differential pressures to be converted point by point into flows. The precision of repeated calibrations was +/- 0.3% of the syringe volume using a 50 stroke calibration process. The accuracy of measured volumes was shown to be within +/- 0.5% following a 100 stroke calibration process. Calibration was performed using room air and the effect of the difference in temperature, humidity and pressure from ventilation gas was considered to be minimal.

Mass spectrometer and ventilator connections.

Gas samples of approximately 100 mls/min were obtained through access points within the inspiratory and expiratory circuits and transmitted through capillaries to a standard miniature 3-way electro-magnetic valve (Airspec, UK), which was operated by computer control. The sampling times for inspiratory and expiratory gas can be individually programmed and in the reported clinical

studies the inspiratory time was 12 seconds and expiratory time 38 seconds. The valve directed the sample gas through a single capillary to the mass spectrometer. With both inspiratory and expiratory samples being fed in turn to the mass spectrometer through a common capillary the effects of pressure sensitivity on the analyses were removed.

Mass Spectrometer

Paramagnetic oxygen analysers and infra red carbon dioxide analysers require gas samples to pass through a drying chamber prior to analysis. This produces a significant delay in the response time and accurate synchronisation of the time of analysis with the collection time is necessary. This potential error can be avoided by measuring oxygen and carbon dioxide by mass spectrometry. With this method the gases are measured directly and the response time is such that the measurements can be considered immediate and simultaneous. In this system a quadrupole mass spectrometer (Airspec MGA 2000) was used. Factory evaluation of this instrument showed that reproducibility was better than 1% of value, drift was less than 1% in 24 hours, and linearity was better than 1% for uncorrected data. This instrument has a measurement resolution of 12 bits for 10 volts, and in the slow mode the analyser measures each gas every 20 milliseconds with

each data acquisition being the average of 256 measurements. The procedure of tuning and calibration of gases can be undertaken manually, or remotely by computer control. In this application the mass spectrometer was tuned to measure the concentration of oxygen, carbon dioxide, argon and nitrogen.

Measurement of Systematic Error

Initial validation studies of the complete system were undertaken using a dummy lung configuration. The dummy lung was a 0.5 litre rebreathing bag (M&IE Dentsply, Exeter, UK) and this was ventilated with settings similar to those required by an infant with moderately severe respiratory disease - rate 40/min, FiO_2 40%, and inspiratory and expiratory pressures 20/4 cm H_2O. The aims of this evaluation were to determine drifts in measurement of oxygen and carbon dioxide concentration over time, and to confirm zero gas exchange with this non-breathing system. Over the 24 hour study period drifts in oxygen concentration of +/- 0.5% were recorded and the carbon dioxide concentration remained within +/- 0.02%. To counteract the effect of possible drift on the calculations, the values taken for inspiratory gas concentrations were the mean of the measurements taken before and after the expiratory sample. With this modification, the mean VO_2 and VCO_2 measured using the

dummy lung configuration over 24 hours was 0.0 (+/- 0.1).

To assess the ability of the system to accurately measure gas exchange, gas infusion studies were designed to simulate levels of carbon dioxide production and oxygen consumption which are likely to occur in sick premature infants. The ventilator settings during the infusion studies were as above. In each case the relevant test gas was infused into the inspiratory circuit by a peristaltic infusion pump P-3 (Pharmacia, Sweden). The peristaltic pump was used in preference to a continuous flowmeter because the former emitted the gases in pulses and was therefore more accurately simulating the intermittent nature of respiration. The flow rate of the pump was measured on each occasion by infusing the gas into a previously calibrated U-shaped water manometer and from the volume of water displaced over a measured period of time, the flow rate calculated. The time periods were measured by a hand held stop-watch with a precision of 0.01 sec. The random error of the time measurement was assessed by recording repeated time measurements for the same displacement of water (7 ml). The mean(SD) of 20 measurements was 14.92(0.11) secs with the coefficient of variation (CV) of <0.75 %. The method of timing was therefore sufficiently precise. The use of the water manometer provided the additional advantage of allowing the measurement of the gas flow to be performed against a

pressure similar to that experienced when infusing into the ventilator circuit ie 4-20 cm H_2O. Saturation of the water with the infused gases was considered, but thought unlikely to occur, as the duration of infusion of gas through the manometer was always less than 60 secs. However, this was investigated by infusing oxygen, carbon dioxide and nitrogen over longer periods of time in order to identify changes in calculated flow rate. The flow rates were unchanged with time. The effect of any saturation was therefore considered to be negligible.

The results of the gas infusion studies are shown in Table 1. The carbon dioxide infusion (WCO_2) directly simulates CO_2 production (VCO_2). Nitrogen infusions simulate oxygen consumption by creating an O_2 difference which in gas exchange formulas is equivalent to O_2 consumption (VO_2). Using the measurements obtained during the infusion the volume of nitrogen infused (WN_2) and "oxygen consumption" can be calculated.

INFUSION	FLOW ml/min	MEASURED VCO_2 ml/min (SD)	CV %	RELATIVE ERROR %	MEASURED VO_2 ml/min	CV %	RELATIVE ERROR %	CALCULATED N_2 ml/min	%	CV RELATIVE ERROR %
CARBON DIOXIDE	3.86	3.80(0.23)	6.0	-1.5	-	-	-	-	-	-
	9.20	9.0(0.13)	1.4	-1.2	-	-	-	-	-	-
	13.98	13.83(0.53)	3.8	-1.1	-	-	-	-	-	-
OXYGEN	7.5	-	-	-	-7.8(0.31)	4.0	4.1	-	-	-
	9.36	-	-	-	-9.7(0.44)	2.8	4.3	-	-	-
	14.80	-	-	-	-15.10(0.42)	2.0	2.0	-	-	-
NITROGEN	10.10	-	-	-	1.90(0.9)	5.3	-	9.95 (0.41)	4.1	-1.5
	14.0	-	-	-	5.45(0.49)	8.9	-	14.20 (0.82)	5.7	1.4

Relative error = $\frac{\text{calculated value - true value}}{\text{true value}}$ x 100%

Table 1 Gas infusion studies

$$WN_2 = \frac{V(FiO_2 - FeO_2)}{FeO_2}$$

WN_2 is the volume of nitrogen infused

V is flow volume per minute

$$VO_2 = (V_1 \times FiO_2) - (V_2 \times FeO_2)$$

V_1 is inspiratory flow volume per minute

V_2 is the expiratory flow volume ie $V_1 + WN_2$

As a more direct method of measuring small oxygen differences between inspiratory and expiratory samples oxygen infusions were undertaken. In this test lung configuration the increased oxygen concentration was present in the expiratory sample producing a measurable "negative" oxygen consumption, this being calculated as above. Each gas infusion was a minimum of 60 mins duration (range 60-90 mins) and the means, standard deviations and coefficients of variation were calculated on a minimum of 40 data points (Table 1).

2.2.iii Clinical Evaluation

Clinical evaluation was undertaken on 10 preterm ventilated infants and the results are summarised in Table 2. The infants have been divided into those receiving total parenteral nutrition and those who were receiving dextrose infusion. The mean energy expenditure of all the infants studied was 40.2 kcal/kg/24hrs, with the lowest

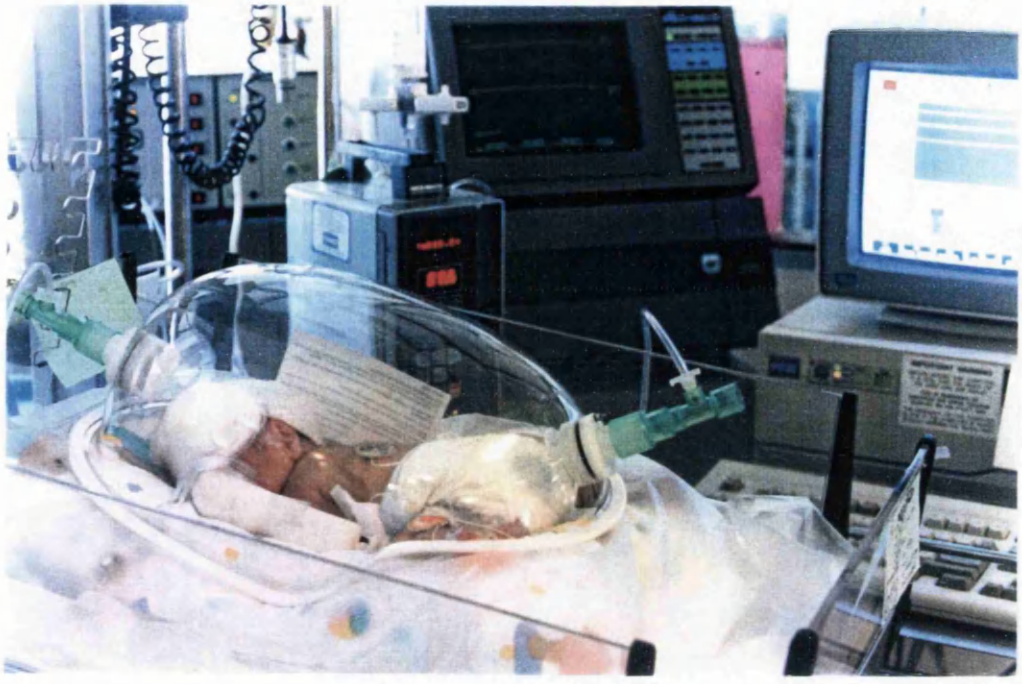
measurement of 28 kcal/kg/24hrs being in a 28 week gestation infant who was paralysed with a muscle relaxant. The mean RQ was greater than 1.0 in both groups.

	BIOLOGICAL DATA				VENTILATION		NUTRITIONAL INTAKE		CALORIMETRY			
	B.W. g	GEST WEEKS	AGE DAYS	WT g	RATE /MIN	F_1O_2 %	ENERGY INTAKE kcal/ kg/24 hrs	PROTEIN INTAKE g/kg/24 hrs	ENERGY EXPEND kcal/ kg/24 hrs	VO2 ml/kg /min	VCO2 ml/kg /min	RQ
TPN	1286	28.8	5	1126	30	32	75.0	2.1	42.3	5.8	6.17	1.07
n = 5	(125)	(2.0)	(0.7)	(159)	(20)	(6.5)	(23)	(0.6)	(8.5)	(1.1)	(1.5)	(0.16)
IV DEXTROSE	1862	30.2	2.4	1843	41.4	30.4	46.2	-	35.12	4.66	5.72	1.13
n = 5	(522)	(3.3)	(1.3)	(593)	(25)	(8.8)	(11)		(5.5)	(0.8)	(0.8)	(0.08)

Table 2 Clinical studies - ventilated infants

The possibility of respiratory gases by-passing the endotracheal tube was investigated at the start of each study by placing the capillary adjacent to the nose and the mouth of the infant. If CO_2 and/or O_2 were detected then a flow-through technique using a canopy which completely enclosed the infant was undertaken (Photograph 2). The only difference from the ventilator system was that the inspiratory capillary sampled air entering the canopy and the expiratory capillary sampled the air leaving the canopy. This was undertaken at the start and finish of the studies, and the mean of the results included in the overall calculations. The extra-tracheal VO_2 ranged from -0.1 to +0.2 ml/min and VCO_2 0.0 to +0.2 ml/min. In 5/10 infants there was no by-passing of the endotracheal tube.

INDIRECT CALORIMETRY SYSTEM



CANOPY SYSTEM FOR DETECTING
EXTRA-TRACHEAL GAS EXCHANGE

Photograph 2

2.2.iv DISCUSSION

An indirect calorimetry system for measuring energy expenditure and nutrient utilisation in sick ventilator dependent infants is described. Evaluation of the system shows there was no significant bias, and the instruments used were precise and accurate. Total system error during the worst test conditions was <5.0%. The inspiratory oxygen concentration during the studies was 40% and therefore the ratio of signal to noise very small during the studies with the low oxygen flows, but even in those conditions a relative error of 4% and coefficient of variation 4% would indicate that the system has adequate precision and accuracy to be used in the sickest infants.

The principal objectives of the system have been achieved in that it is non-invasive, compatible with continuous flow infant ventilators and operates without inconveniencing medical or nursing care. Studies of energy expenditure can continue for 24 hours or longer and can commence from the moment the infant is placed on the ventilator.

The rapid response time of the analyses and the real-time graphic display enables the acute and chronic effect of procedures and medications on energy expenditure and nutrient utilisation to be studied. The management of the

sick premature infant involves a number of interventions which are likely to influence the energy balance and nutritional status of the infant, in particular, the administration of surfactant, dexamethasone and transfusion of blood.

Failure to capture all of the expired respiratory gases is a potential source of error with this method. It is also possible that ventilator gas may leak from the distal end of the endotracheal tube without entering the infant's lower respiratory tract, this producing a falsely high measurement for oxygen consumption. Leakages are less likely in the more premature infants and those infants who have had an endotracheal tube in-situ for a short time. Our findings were similar to that of Lucas and colleagues[19] who found that by-passing of the endotracheal tube occurred in less than half the infants studied and was not more than 0.2 ml/min for carbon dioxide. In our studies we detected that the maximum leakage for both oxygen and carbon dioxide was 0.2 ml/min. The contribution of the skin to gas exchange has previously been reported[20], this being as much as 15% in the smallest infants. For these reasons in our canopy method the whole infant was enclosed and the results obtained are thought to reflect all extra-tracheal gaseous exchange. Ideally the canopy method should run simultaneously with sampling from the ventilator circuits and this may be possible with

the aid of a multiplex valve, but this has yet to be tested in this system. Modified face masks have been used to collect intra and extra-tracheal gases[21] but the placement of a mask on the face of an infant interferes with normal nursing care and because of its invasiveness may have an independent influence on the results obtained.

The clinical studies confirmed that the system did not affect the respiratory management of the infant and normal nursing and medical procedures were able to be undertaken. Preliminary results indicate that energy expenditure may be lower than previously suspected with the mean energy expenditure being 40.2 kcal/kg/day. From observation of these infants during the study periods, it is likely that this is partly due to the decrease in the work of breathing and also due to the reduced activity of the sick premature infant.

The other striking feature in the clinical studies was the finding that the respiratory quotient was generally greater than 1.0, this being most marked in some infants who were receiving intravenous dextrose alone. It has been demonstrated in adults that a high intravenous carbohydrate intake results in excess carbohydrate being converted to fat and this process results in a considerable increase in carbon dioxide production[22]. In order to eliminate this additional carbon dioxide, infants

who are receiving respiratory support are likely to require increased ventilatory requirements. One of the infants included in the clinical evaluation was being ventilated with air because of persistent carbon dioxide retention. He had been paralysed with a muscle relaxant, and calorimetry demonstrated that his resting energy expenditure was only 28 kcal/kg/day. His energy intake, which was solely carbohydrate for the first four days was almost double his expenditure. The respiratory quotient peaked at 1.4 on day 4 indicating a marked increase in carbon dioxide production as the excess carbohydrate was converted to fat. Persistently high blood sugars provided further evidence that the intake of carbohydrate was inappropriate. It is likely that a reduction in this infants carbohydrate intake would have resulted in a decrease in the rate of carbon dioxide production and reduction in carbon dioxide retention. This would have enabled the ventilator support (and the potential barotrauma) to be decreased and thus the risk of developing chronic lung disease reduced.

With the limited data from the clinical evaluation casting considerable doubt on the appropriateness of our present parenteral nutrition regimes for ventilator-dependent infants, more extensive data was required. Therefore, a further study of energy expenditure and carbon dioxide production in both ventilated and spontaneously breathing

infants was undertaken.

2.3 MEASUREMENT OF ENERGY EXPENDITURE, RESPIRATORY QUOTIENT, AND CARBON DIOXIDE PRODUCTION IN VENTILATED AND SPONTANEOUSLY BREATHING PRETERM INFANTS DURING THE FIRST 6 DAYS OF LIFE.

2.3.i INTRODUCTION

Data from the clinical evaluation indicated that, contrary to speculation, the energy expenditure of sick ventilator-dependent low birthweight infants may not be greatly in excess of that measured in spontaneously breathing infants. If this finding is confirmed, it is likely that energy intakes, especially carbohydrate, may be excessive for these infants. It was also noted that carbon dioxide production was usually greater than oxygen consumption resulting in the respiratory quotient being greater than 1.0, and this increased carbon dioxide production could have serious implications for infants in respiratory failure. More data is therefore required on energy expenditure and respiratory quotients in those infants. A study was therefore undertaken to compare these parameters in ventilated and spontaneously breathing low birthweight infants during the first week of life.

2.3.ii PATIENTS

Using the newly developed and evaluated calorimetry system, infants of birthweight less than 2000g were studied during the first week of life. Twelve infants, mean birth weight (SD) 1350 (274)g were receiving artificial ventilation and 10 infants, mean birth weight 1383 (372)g were breathing air spontaneously. All were receiving total parenteral nutrition. Each study took place between the hours of 10.00 and 13.00 and the minimum duration was 120 minutes.

2.3.iii RESULTS

On days 1-2, 3-4, and 5-6 there was no difference in energy expenditure between the two groups, but both groups demonstrated a similar trend of increasing energy expenditure during this time (Table 3). The respiratory quotient was greater than 1.0 in both groups of infants (Table 3).

	INFANTS	
	VENTILATED	SPONT. BREATHING
n	12	10
Weight g mean (SD)	1350 (274)	1383 (372)
EE (kcal/kg/day)		
Days 1-2	36.5 (6.3)	37.22 (6.9)
3-4	39.1 (9.9)	38.57 (4.8)
5-6	45.1 (7.0)	44.70 (7.2)
	ventilated v non-ventilated	p = 0.78
RQ		
Days 1-2	1.04 (0.12)	1.1 (0.11)
3-4	1.10 (0.11)	1.09 (0.1)
5.6	1.07 (0.10)	1.04 (0.1)
	ventilated v non-ventilated (Analysis of Variance)	p = 0.78

Table 3 Energy expenditure (EE) and respiratory quotient (RQ) of ventilated and spontaneous breathing very low birthweight infants during days 1-6.

ENERGY EXPENDITURE AND
NUTRIENT UTILISATION ?

28 week gestation 990g infant post surgery energy expenditure 184 kJ/kg/day respiratory quotient 1.1.

Photograph 3

2.3.iv DISCUSSION

Contrary to previous speculation the energy expenditure of ventilated infants during the first week of life is not greater than that of spontaneously breathing infants and it may be much less if paralysing agents are prescribed. This has important implications for the development of feeding regimes for these infants (Photograph 3). In both groups of infants the respiratory quotient was greater than 1.0 indicating that excess carbohydrate is converted to fat and carbon dioxide production is increased. Parenteral nutrition regimes traditionallly begin solely with dextrose infusion, then amino acids and fat emulsion are gradually introduced towards the end of the first week. It may be that a more acceptable formulation of energy intake for these infants during these first few days is a combination of fat and carbohydrate, as this may reduce the risk of hyperglycaemia and hypercarbia[22]. Although there have been concerns that the early introduction of fat emulsions may increase the risk of chronic lung disease [23,24,25], there is recent evidence indicating that fat emulsions may reduce lung damage[26]. Nutritionally, recent evidence from clinical tolerance studies indicates that fat emulsions are safe and likely to be beneficial[27]. To date there are no calorimetry data available on ventilated infants receiving fat emulsion during the first day of life and this requires

further investigation.

It is likely that inadequate protein intake contributes to the poor growth of these sick infants. High protein turnover rates have been reported and nitrogen retention is considerably less than that achieved by healthy preterm infants receiving enteral feeds[28]. Protein turnover data in ventilated infants is limited[29] and information on how protein turnover is affected by different rates of energy expenditure and different mixes of energy intake, is not available. This can be obtained by combining stable isotope labelled amino acid studies with the described indirect calorimetry system. Data from such studies will enable appropriate parenteral nutrition regimes to be developed which will not only improve nutrient accretion rates but also reduce the ventilator requirements of these infants.

2.4 CONCLUSIONS

A non-invasive, precise and accurate system for measurement of energy expenditure in sick ventilator-dependent low birthweight infants has been developed. Initial data indicates that energy expenditure in this group of infants may be less than speculated and the energy/protein mix in nutritional regimes for these infants may be inappropriate. The calorimetry system will be used in combination with stable isotope studies to provide more specific nutrient turnover data. The ultimate aim is the formulation of feeding regimes which will enable normal nutrient accretion and growth to be achieved, and will not exacerbate respiratory failure in these vulnerable infants.

CHAPTER 3

INVESTIGATION OF THE AETIOLOGY OF BREAST MILK JAUNDICE

3.1 BACKGROUND TO THE STUDIES

Breast milk jaundice which was first described nearly 30 years ago[30], is an unconjugated hyperbilirubinaemia which occurs in approximately 2-4% of otherwise healthy breast fed infants[31]. Despite the time interval since the original description, the aetiology remains uncertain. An aetiological mechanism which has been widely postulated is that the concentration of non-esterified fatty acids (FFA) in the milk of infants with breast milk jaundice is increased and elevated concentrations of free fatty acids have been shown *in-vitro* to inhibit the activity of the conjugating enzyme hepatic glucuronyl transferase [32]. Support for this mechanism comes from further *in-vitro* studies which have demonstrated that, after a period of storage, the breast milk of normal infants has a similar inhibitory effect on glucuronyl transferase activity. Further analysis of these milks indicated that lipolysis had occurred during the period of storage and the concentration of free fatty acids had increased[33].

An increased or abnormal lipase activity in the milk has been postulated as a possible explanation for the presence

of elevated free fatty acid concentrations in the milks of jaundiced infants[34]. Lipoprotein lipase (or serum stimulated lipase) was originally reported to be specifically increased in milk from mothers of infants with prolonged jaundice[35], but this was not supported by subsequent studies which demonstrated increased LPL activity in milk from mothers of normal infants[36]. This study also reported elevated FFA concentrations in milks of normal infants. Another group of investigators have been unable to demonstrate differences in LPL activity or free fatty acid concentrations in the breast milk of mothers of jaundiced and control infants[37].

The other human milk lipase, bile salt-stimulated lipase (BSSL), was studied by Poland and colleagues and they showed that BSSL activity did not differ between milk from mothers of infants with prolonged jaundice and control mothers[38]. But their data on free fatty acid concentrations in the milks did support previous data[32] by demonstrating increased concentrations of FFA in the milk of jaundiced infants. They also demonstrated increased lipase activity in those milks which they stated was due to an "unstimulated" lipase. The activity of this lipase was approximately twice as high in jaundiced milks when compared to the level in normal milks. The investigators subsequently postulated that this increased lipase activity was not due to LPL but an abnormal lipase which

has chemical characteristics similar to BSSL but does not require prior stimulation by bile salts[39]. For this conclusion to be drawn, it was assumed that bile salts necessary for stimulation of BSSL were not present in breast milk. There being no studies to confirm this assumption an initial study was undertaken to determine if bile salts are present in breast milk.

3.2 A STUDY OF BREAST MILK FOR THE PRESENCE OF BILE SALTS.

3.2.i Subjects and Materials

Twenty-eight samples of breast milk were obtained from 28 lactating mothers at various times during the first post-partum week. No attempt was made to standardise the collection at a particular time of day nor at a particular time after a feed. Thus, milk samples were effectively unselected. Random samples of blood were taken from 12 mothers for determination of serum bile acids in the first post-natal week.

Radioactive bile acids ([^{3}H]-cholic acid and [^{3}H]-chenodeoxycholic acid) were supplied by New England Nuclear Ltd., USA. Anti-bile acid sera were raised in our own laboratory and characterised as previously described[40]. Other chemicals and solvents were of analytical reagent grade.

3.2.ii Method

Radioimmunoassay (RIA) was carried out as described in an earlier report from this laboratory[40], but assay of unextracted milk failed to give reliable results. Consequently, milk (0.5 ml) was mixed well with 2 ml methanol and precipitated proteins were removed by centrifugation at 1000 x g. The supernatant was mixed

with a further 2 ml methanol and stored at 4°C overnight before a further centrifugation at 1000 x g. The supernatant was dried at 40°C under N_2 and the residue dissolved in 0.5 ml 70% w/v methanol-water. This was extracted twice with 2 ml n-heptane which was discarded. The methanol-water fraction was dried again under N_2, dissolved in 1 ml 0.01 M phosphate buffer, pH 7.4 (i.e. 1:2 dilution of original sample). An aliquot (0.1 ml) of this solution was used for assay of combined glycine and taurine conjugates of cholate and chenodeoxycholate. As a few samples indicated increasing concentrations when studied by progressive dilution, a heptane wash was incorporated into the extraction procedure. Following this step progressive dilution of samples gave the same values after assay and correction for dilution. We also confirmed that recovery of the two bile salts following this extraction procedure was similar (79.9% cholate, 87.5% chenodeoxycholate). Data were analysed using the Wilcoxon non-parametric rank sum test.

3.2.iii Results

Bile salts were found in all samples of milk analysed and also in colostrum (Table 4, Appendix 3.2 iii a). In both colostrum and milk there was a predominance of cholate, the ratio cholate conjugates to chenodeoxycholate conjugates being 2.6:1 and 3.4:1, respectively.

	Serum (umol/l)	Colostrum (umol/l)	Milk (umol/l)	Serum-milk ratio	P Value
Cholate					
Mean	0.93±0.13	0.24±0.05	0.89±0.13 (SEM)	1:1	NS
Range	0.32-2.17	0.15-0.43	0.29-2.86		
Chenodeoxycholate					
Mean	0.89±0.15	0.09±0.05	0.26±0.057 (SEM)	4:1	<0.01
Range	0.39-1.81	0.02-0.27	0.02-1.50		

Table 4. Concentration of bile salts in serum, colostrum and breast milk

3.2.iv Discussion

This study demonstrates for the first time that bile salts are present in colostrum and breast milk. There was a wide range of concentration for both cholate and chenodeoxycholate (Table 4), and it is likely that this reflects the random sampling in the study.

Many agents present in maternal serum are detected in breast milk[41]. In most instances their presence is secondary to passive diffusion and the concentration in breast milk is related to maternal serum concentration. In our study, maternal serum levels measured in the first post-natal week showed a cholate to chenodeoxycholate ratio of 1:1 (Table 4, Appendix 3.2 iv a). If bile salts enter the alveolar cell by passive diffusion then the primary bile salt ratio in the milk should be similar to that in serum or possibly show a predominance of the less polar dihydroxy bile salt, chenodeoxycholate. We found that there was an increased proportion of the more polar bile salt, cholate, the cholate to chenodeoxycholate ratio being 3.4:1. There was little difference between the serum and milk concentrations of cholate but there was a significant difference between the serum and milk concentrations of chenodeoxycholate ($P<0.01$). This cannot be explained by a difference in recovery as we found the recoveries of the two bile salts were similar.

Passive diffusion is often more marked during the colostral phase when breast circulation and permeability are increased[41]. We found that the concentration of cholate and chenodeoxycholate were lower in colostrum when compared with milk. Whether bile salts are actively transported by mammary tissue, as occurs in the distal small bowel[42], remains to be determined.

The concentrations of the bile salts in the milk are several orders in magnitude lower than the intraduodenal concentrations[43] required to stimulate bile salt stimulated enzyme activity in the gut, and BSSL activity is therefore unlikely to occur within the milk[44]. But the bile salt concentration within the mammary tissue remains unknown, and there may be specific compartmentalisation of bile salt and BSSL within the breast which might allow interaction between these agents. Such an interaction could explain the difference in free fatty acid concentration between normal breast milk and the milk of infants with breast milk jaundice. A more extensive study was therefore undertaken to determine the relationship of bile salts, bile salt stimulated lipase, and free fatty acid concentration in normal milks and the milks of infants with breast milk jaundice.

3.3 A STUDY OF THE RELATIONSHIP BETWEEN BILE SALTS, BILE SALT STIMULATED LIPASE AND FREE FATTY ACIDS IN BREAST MILK OF NORMAL INFANTS AND INFANTS WITH BREAST MILK JAUNDICE

3.3.i INTRODUCTION

The aims of this study were to provide further longitudinal data on the presence of bile salts in breast milk, and to investigate the relationship between the concentrations of bile salts, the free fatty acid concentration and the BSSL activity in the milk of normal infants and the milk of infants with breast milk jaundice.

3.3.ii PATIENTS

Determination of the concentration of bile salts in breast milk at different stages of lactation

Mothers of healthy newborn infants were recruited in the early neonatal period and 42 provided breast milk samples at 2 weeks, 30 at 6 weeks, 16 at 10 weeks, and 12 at 14 weeks. Each sample was taken from the same breast at the same time of day (mid-morning) using an Egnell breast pump. The sample was either analysed immediately or stored at -80^{o} before being analysed for bile salts, BSSL activity and free fatty acids.

Determination of the bile salt concentration, free fatty acid concentration and BSSL activity in milk from mothers

of infants with breast milk jaundice

During the period of study, 12 infants with breast milk jaundice took part in the study. The diagnosis was based on the presence of a prolonged unconjugated hyperbilirubinaemia (> 200 umol/l at 10 days of age) which was associated with negative laboratory investigations including tests of liver function, thyroid function, infection and blood group analysis. When breast feeding was discontinued for 24-48 hours, a decrease in the level of jaundice was demonstrated directly by measuring the serum bilirubin or indirectly using a bilirubin meter (Minolta / Airshields Meter 101). Milk samples using the above procedure were analysed for bile salt concentration, free fatty acid concentration and BSSL activity.

3.3.iii METHODS

Bile salt assay

Bile salts were extracted from the breast milk using a modification of our previously reported procedure[45]. Two mls human milk, 2 ml 0.05M NaOH and 0.5 g XAD7 resin were mixed for one hour on a rotary mixer before centrifugation at 1000 xg for ten minutes. The supernatant was extracted with more resin and both resin batches retained,

washed briefly with 1.0 ml of 0.05 M NaOH and the bile acids eluted with 2 x 2ml methanol. These eluates were combined, dried under N_2 and redissolved in 0.05 M NaOH (1 ml) which was extracted with 2 ml diethyl ether which was discarded. The alkaline solution was acidified with 0.25 ml 6 M HCl and the bile acids extracted three times into ethyl acetate (2 ml). These extracts were combined, evaporated to dryness and dissolved in 2 ml of methanol. Water (1 ml) was added and the solution passed through a Bond-elut column (Jones Chromatography Ltd, Llanbradach, Mid Glam.) primed with methanol. This procedure removed contaminating fatty acids (retained on the Bond-elut) while bile acids elute in the methanol/water. This was dried down and the bile acids, cholate and chenodeoxycholate were assayed by radio-immunoassay as previously described[46].

The chenodeoxycholate antiserum is specific for conjugated chenodeoxycholic acid and does not differentiate between glycine and taurine conjugates. The limit of quantitation is 0.01umol/l, corresponding to 1.0pmol per assay tube with a coefficient of variation of 17%. This figure improves to 5% when the concentration is 0.02umol/l. The cholate antiserum also does not differentiate between glycine and taurine conjugates, and we demonstrated that there was an 11.7% cross reaction with chenodeoxycholate conjugates. As the cholate levels

exceeded chenodeoxycholate levels by approximately 4 fold this will not result in a significant over-estimation of cholate levels.

BSSL activity

The emulsion substrate for lipase activity was prepared according to Mehta[47]. Briefly this involved emulsification of 10uCi of (^{14}C)-Triolein and 25mg of non-radioactive carrier Triolein in 1.25ml 1M Tris-HCL buffer pH 9.0 and 1.0ml of freshly prepared gum arabic (10%w/v). A Soniprep 150 was used for two periods of 60s at a power setting of 50 amplitude microns.
The incubation was carried out and terminated as described by Mehta[39], using methanol:chloroform:heptane (1.41:1.25:1.0). The incubation medium included 0.033 ml of the emulsion substrate, 0.15 sodium taurocholate (12 mM in 0.06 M tris, pH 8.6), 0.3 ml albumin solution (2.5% w/w in 0.06 M tris, pH 8.6) and 0.02 ml milk diluted in veronal buffer 5 mM, pH 7.4. Free fatty acids were partitioned by addition of 1.05ml of 0.05M potassium carbonate buffer pH 10.0, as described by Belfrage and Vaughan[48]. Extraction losses were corrected by use of (^{14}C)-Oleic acid tracer added to blanks prepared using emulsion which did not contain (^{14}C)-Triolein.

Free fatty acid assay

The milk (0.1ml) was mixed with 0.8 ml water and tricosanoic acid (10 ug) and acidified before extraction three times with 2 ml n-heptane containing 0.05% w/v bis-hydroxy-toluene. The extract was methylated with diazomethane and the fatty acid methyl esters assayed by GLC using a 30m Megabore column coated with DB225.

The results are expressed as medians with 95% confidence intervals and the data was analysed using rank correlation test, Wilcoxon one sample test, and regression analyses to determine the relationship between the different variables.

3.3.iv RESULTS

Bile salt concentration, BSSL activity and free fatty acids in normal milk

The median cholate and chenodeoxycholate concentrations in breast milk at 2, 6, 10 and 14 weeks are shown in Table 5. There was a marked decline in the concentration of both bile salts as lactation progressed ($p<0.05$). There was a marked dominance of cholate over chenodeoxycholate with the ratio peaking at 10 weeks (Table 5, Appendix 3.3 iv a). The concentrations of free fatty acid and BSSL activity at 2, 6, 10 and 14 weeks are also shown in Table 5. There was considerable intra - and inter - individual variation in levels of FFA but there was no significant trend in the concentrations at the different times of lactation. There was no significant association between bile salt and FFA concentration at any stage of lactation.

There was a marked decline in the BSSL activity with duration of lactation ($p<0.01$), but this was not significantly association with either the concentration of bile salts or FFA. Similar results to the cross-sectional data were obtained when the data from 12 mothers who provided samples at each of the sample times were analysed to determine individual trends. A less marked decline in the concentration of chenodeoxycholate was the

only noticeable difference (Table 6).

Concentration of bile salts, free fatty acid and BSSL activity in the milk from mothers of infants with breast milk jaundice

The median cholate and chenodeoxycholate concentrations are shown in Table 7. The only significant difference between the jaundice milks and the normal milks was the concentration of cholate which had a significant effect on the cholate to chenodeoxycholate ratio ($p<0.01$). The BSSL activity and free fatty acid concentrations are also shown in Table 7 (Appendix 3.3 iv b) and are not significantly different from the levels obtained in normal milks. There was no correlation between the bile salt concentration, BSSL activity and free fatty acid concentration.

WEEKS	2	6	10	14	
No. of samples	42	30	16	12	
CHOLATE umol/l	0.515 (0.46-0.63)	0.57 (0.47-0.64)	0.52 (0.43-0.64)	0.39 (0.35-0.45)	r=0.23 p<0.05
CHENO umol/l	0.21 (0.19-0.26)	0.20 (0.17-0.24)	0.15 (0.10-0.18)	0.15 (0.12-0.18)	r=0.34 p<0.01
CHOLATE/CHENO RATIO	2.52 (2.26-2.81)	2.80 (2.24-3.92)	4.88 (2.72-7.83)	2.53 (2.22-3.49)	r=0.13 NS
BSSL ACTIVITY*	64.2 (47.5-82.2)	45.0 (36.7-61.5)	40.9 (29.5-52.2)	21.2 (14.0-38.2)	r=0.34 p<0.01
FFA mmol/l	1.57 (1.23-2.11)	3.55 (1.99-5.35)	2.70 (1.58-4.99)	3.37 (1.08-6.37)	r=0.13 NS

* umol FFA produced/ml milk/min (95% CI)

Table 5. Median cholate and chenodeoxycholate concentrations, BSSL activity, and free fatty acid concentration in breast milk at 2, 6, 10, and 14 weeks.

Cross-sectional data from 44 mothers.

WEEKS	2	6	10	14	
No. of Samples	12	12	12	12	
CHOLATE umol/l	0.57 (0.42-0.71)	0.63 (0.52-0.79)	0.53 (0.42-0.67)	0.40 (0.36-0.47)	p<0.05
CHENO umol/l	0.22 (0.17-0.29)	0.17 (0.13-0.22)	0.12 (0.09-0.17)	0.17 (0.14-0.20)	NS
CHOLATE/CHENO RATIO	2.59 (2.14-2.89)	4.60 (2.63-7.12)	5.66 (2.79-10.56)	2.46 (2.12-3.40)	NS
BSSL ACTIVITY*	71.4 (45.0-99.5)	51.7 (34.2-90.5)	40.5 (28.2-52.2)	33.0 (15.2-46.2)	p<0.01
FFA mmol/l	2.37 (0.67-9.2)	4.17 (1.01-8.13)	2.25 (1.19-3.41)	3.07 (1.08-5.09)	NS

* umol FFA produced/ml milk/min (95%CI)

Table 6. Median cholate and chenodeoxylate concentration, BSSL activity,and free fatty acid concentration in breast milk taken from 12 mothers at 2, 6, 10, and 14 weeks.

	NORMAL	BMJ	
WEEKS	2	2	
No. of Samples	42	12	
CHOLATE umol/l	0.52 (0.46-0.62)	0.63 (0.55-0.71)	<0.05
CHENO umol/l	0.21 (0.16-0.48)	0.17 (0.11-0.23)	NS
CHOLATE/CHENO RATIO	2.52 (2.26-2.81)	3.91 (2.69-8.22)	<0.01
BSSL ACTIVITY*	64.2 (47.5-82.2)	59.2 (35.5-90.7)	NS
FFA mmol/l	1.57 (1.23-2.11)	1.73 (1.37-2.10)	NS

* umol FFA produced/ml milk/min (95% CI)

Table 7. Median cholate and chenodeoxycholate concentrations, BSSL activity, and free fatty acid concentrations in breast milk of normal infants and infants with breast milk jaundice.

3.3.v DISCUSSION

This study provides further data on the presence of bile salts in breast milk. We have extended our initial study by determining the levels of bile salts in breast milk at different stages of lactation. We have again demonstrated that cholate is the dominant bile salt in contrast to maternal serum where chenodeoxycholate is present in higher concentrations[45]. The levels of both cholate and chenodeoxycholate are inversely related to the duration of lactation.

We hypothesised that bile salts in breast tissue or less likely, in the milk, may be of sufficient concentration to interact with bile salt stimulated lipase and initiate the hydrolysis of triglyceride to free fatty acid. We measured bile salt concentration and free fatty acid concentration in breast milk of normal mothers and in the milk of mothers whose infants were diagnosed as having breast milk jaundice. There was no association between the bile salt concentration and that of FFA in either the normal controls or the breast milk jaundice group. In addition, we did not detect a significant difference in the free fatty acid concentration between the breast milk jaundice group and the normal controls. This finding is at variance with that reported by Poland[38] but in agreement with the findings of Constantopoulos[37]

This study provides additional data on changes in BSSL activity during lactation. Four previous studies have reported conflicting results[47,49,50,51]. Two indicated that there was no change with duration of lactation but their results were based on cross-sectional data. Another study reported a decline in BSSL with length of lactation and the other stated that BSSL was static but there was a decrease in bile-salt stimulated esterase activity. Both our cross-sectional data and the data from the mothers who provided samples at each of the sample times demonstrate a significant fall in BSSL activity with duration of lactation. We did not find any significant difference in BSSL activity between the normal controls and the breast milk jaundice group.

Some of the confusion regarding the relationship between human milk lipolytic activity, free fatty acid concentration and breast milk jaundice is that various investigators have measured different forms of lipase activity. Constantopoulos[37] measured lipoprotein lipase activity which he found not to be increased, Poland[38] measured bile salt stimulated lipase activity and in agreement with our findings found no significant difference in BSSL activity between the two groups. He also identified an "unstimulated" lipase, the activity of which corresponded with increased free fatty acid concentrations in the milk. Only nine mothers of infants

with breast milk jaundice were studied and the method used for storing the milk has since been shown not to prevent spontaneous hydrolysis of triglyceride to FFA[53,54]. In addition, the substrate for lipolytic activity was tributyrin, a triglyceride containing short chain fatty acids which can be hydrolysed to a certain extent by both lipoprotein lipase and BSSL without their specific cofactors [52]. A more recent study supports our findings by reporting the absence of higher BSSL, lipoprotein lipase or "unstimulated" lipase activity in milk of infants with breast milk jaundice[55].

Although we do not support the view that breast milk jaundice is associated with increased FFA concentrations within the milk, we do confirm that FFA concentrations vary widely between individuals and also within the one individual at different times. The mechanism of this is not known and whether bile salts have any role to play is still uncertain because of the lack of information on possible intra-mammary activity. Following the publication of this data, the possibility that bile salt concentrations may be higher in the mammary gland or that specific intracellular compartmentalisation of bile salts and lipase might affect their interaction within the cell has again been raised[56,57]. It is of interest that the concentrations of cholate were significantly higher than chenodeoxycholate and that it has been shown that cholate

is more effective in stimulating bile salt stimulated lipase[58]. Previous reports on serum bile salt concentrations in infants with breast milk jaundice show an increase in the cholate to chenodeoxycholate ratios[59], and it has been suggested that in breast milk jaundice there is increased enterohepatic circulation of bile acid as well as bilirubin[60].

More recently a further analysis of the fat composition of normal and jaundice milks has confirmed similar total concentrations of fatty acids within the two milks, but increases in specific fatty acids such as 18:3w6, 20:5w3 and total 6 desaturation products were found in the jaundice causing milks[52]. These fatty acids are only minor components of milk fat but because of their specific distribution in the milk fat globule membrane it has been suggested that these changes in concentration might affect the stability of the membrane and allow access of lipase to the triglyceride within the core. This interaction could be enabled by the elevated cholic acid levels found in our studies. This hypothesis requires further examination.

3.4 CONCLUSIONS

These studies report for the first time that bile salts are present in breast milk. The significance of this finding requires further investigation particularly regarding possible intramammary activity. The findings refute previous claims on the association of increased lipase activity and elevated free fatty acid concentrations with the aetiology of Breast Milk Jaundice. Marked intra- and inter-individual variation in free fatty acid concentration is noted in normal and jaundice milks. The mechanism of this is uncertain but an intra-mammary interaction between bile salts and the enzyme bile-salt stimulated lipase cannot be excluded and requires further investigation.

CHAPTER 4

IS EARLY SOLID FEEDING HARMFUL TO THE INFANT?

4.1 BACKGROUND TO THE STUDY

Successive reports on the Present Day Practice of Infant Feeding[61,62,63] have stated that solid food should not be introduced before three months and preferably not before four months of age. This view has received the support of both European and American Committees[64,65]. Despite this overwhelming professional advice, many infants receive solids before this time; the OPCS survey of 1980 showed that 56% of infants were introduced to solids before three months of age and the proportion increased to 62% when the survey was repeated in 1985[66]. The reasons for discouraging the premature introduction of solids include the possible risk of excessive weight gain[67,68], vulnerability of the gut to infection[69], and increased susceptibility to the development of allergic disease[70].

Although initial studies showed that infants who received early solids were significantly heavier than infants who were introduced to solids at the recommended time[67,68], more recent reports have been unable to confirm this association[71,72,73]. To determine the independent effect of early solids on weight gain, numerous other factors

which may influence infant weight gain such as maternal height, sex of infant, birth weight, and type of milk feed must be considered. To date, there are no studies demonstrating the effect of early solids on weight gain, after extensive adjustments have been made for each of these and other relevant factors.

Although the early introduction of solids in Third World countries has been shown to place the infant at increased risk of gastrointestinal infection[69], there is at present no data either to support or refute this claim for infants growing up in a developed society.

Studies on infant feeding and allergic disease have concentrated on the effect of breast milk and artificial formula[74], and there are few data on the role of solid feeding. Recent reports from one centre in New Zealand have shown an increase in the incidence of eczema at 2 and 10 years of age in children who were commenced on solid food before 4 months of age[75,76]. The incidence of asthma was not influenced by the early introduction of solids[77], and other allergic disorders were not considered in these reports.

A study was therefore undertaken to determine the influence of the early introduction of solid foods on weight gain, the risk of gastro-intestinal disease and the

risk of allergic disorders occurring during the first 2 years of life.

4.2 Study to determine if early solid feeding is harmful to the infant.

4.2.i Subjects

The infants, who were born between 38-42 weeks' gestation, were recruited to the study following a detailed verbal explanation to the parents. All mothers were delivered in the single obstetric unit, they were resident in the city of Dundee and living in a stable relationship.

4.2.ii Methods

Data collection

Infant feeding

Planned home visits were made by the mother's health visitor at 0.5, 1, 2, 3, 4, 6, 9, 12, 15, 18, 21 and 24 months. At each visit, using a standardised questionnaire (Appendix 4.2 ii a), details of infant feeding in the 24 hours prior to the visit were recorded, namely number of breast feeds, number and types of formula feeds, number of juice and water feeds and number of solid feeds per day (Appendix 4.2 ii b). As appropriate the dates were recorded of the first formula feed, first cow's

milk feed, first solid feed and last breast feed. Supplementary feeding was defined as the introduction of formula feeds, cow's milk or solid feeds, specifically omitting the use of juice or water. Mothers were supplied with feeding and infant illness record cards to facilitate accuracy of recall.

Milk Feeding Groups

On the basis of the infant feeding record mothers were allocated into one of four milk feeding groups as follows:

1. Exclusive breast feeding - mother breast feeding for 13 weeks or more who did not introduce supplements before that time.
2. Partial breast feeding - mothers breast feeding for 13 weeks or more who introduced supplements before that time.
3. Early weaners - mothers starting breast feeding but discontinuing before 13 weeks.
4. Bottle feeders - mothers starting bottle feeding from birth. Bottle feeders were further divided according to the initial milk formula received and subsequent changes of formula.

Solid Feeding Groups

The timing of the first solid food was recorded and infants were allocated to the appropriate solid feeding group -

1. Solids introduced before aged 8 weeks.
2. Between 8-12 weeks
3. After 12 weeks.

Anthropometry

Infant weight was recorded at 4, 8, 13, 26, 52 and 104 weeks. Weights were recorded by scales with 20g divisions (Marsden Weighing Machines, London) which were routinely checked three monthly.

Infant health

A standardised data sheet was used to collect the information on all episodes of infant illness (Appendix 4.2 ii c). Definitions for each illness were derived from those of Chandra[78] and have been previously reported[79]. Gastrointestinal illness was defined as vomiting and/or diarrhoea occurring as a discrete illness and lasting for 48 hours or more. This was differentiated from persistent posseting or episodes of regurgitation which were coded separately. Diarrhoea was diagnosed on the basis of frequent unformed stool of 48 hours duration or more and these episodes were distinguished from chronic diarrhoeal disease such as

cow's milk intolerance and malabsorption which were coded separately. Respiratory illness lasting 48 hours or more was coded under the predominant symptom - coryza, cough or wheeze. The diagnosis of eczema was based on the presence of the typical itchy papulovesicular rash and napkin dermatitis was coded separately.
For each episode of illness the health visitor was asked to record whether or not the General Practitioner had been consulted, had confirmed the diagnosis of disease, and/or prescribed therapy. Hospital admissions were also recorded and the diagnosis confirmed by scrutiny of the hospital case records. At the completion of the two years of follow-up the General Practitioner's records of the child were scrutinised to supplement the health visitor's data on childhood illness with previously unrecorded episodes being added to the record.

Biological, socio-economic and environmental factors.

A number of biological, socio-economic and environmental factors thought possibly to affect the health and growth of infants were identified and recorded either at recruitment or during the study (Appendix 4.2 iii d).

Biological factors

Maternal height, birth weight, gestation, sex of infant, Maternal history of allergic disease.

Socio-economic and environmental factors

Maternal age, parity, marital status, age of leaving school, maternal and paternal smoking, paternal age and social class.

Data analysis

The data were transferred to a main frame computer for analysis and the statistical package for the social sciences (SPSS-X) was used to describe the incidence of illness and the distribution of risk factors. The relation of illness or weight to several explanatory variables was investigated using multiple logistic regression. Generalised linear interactive modelling was used to perform the calculations and provide tests of significance and standard errors of the estimated variables in the function. Chi-square tests were used to compare differences in risk factors between groups. Where a weight was not measured at the exact scheduled age, it was estimated by extrapolating from the two nearest dates of measurement provided one of the measurements was within a specified period. These limits were 2, 7, 13, 15, and 20 weeks for the ages of 8, 13, 26, 52 and 104 weeks. Extrapolation assumed the relation between weight and log(age+40 weeks) was linear.

4.2.iii RESULTS

In this study 9.7% of infants were started on solids before 8 weeks and 49.4% between 8 and 12 weeks of age. There were several nutritional, biological and sociological differences between the early and late solid feeders. Solids were introduced earlier in infants who were male, and who were from lower socio-economic groups (Table 8). Early solid feeding was more frequent in bottle fed infants but even in the partial breast feeding group, 65% of infants received solids before 13 weeks. (Table 9). The early introduction of solids was not related to the type of formula the infant received (Table 10). There was no relationship between birth weight and the time of introduction of solids in contrast to the change from whey dominant to casein dominant formula in which there was a trend from remaining with whey dominant formulas in the lower birth weight groups to changing over to casein dominant formulas in infants of birth weight greater than 4000 g (Table 11).

The effect of nutritional, socio-economic and biological variables on weight gain were studied using multiple logistic regression analysis. Factors which had a significant independent effect on weight gain throughout the first two years of life were maternal height ($p<0.01$), birth weight ($p<0.001$), male sex of infant ($p<0.001$) and

gestation ($p<0.01$) (Appendix 4.2 iii a). At 8 weeks of age breast fed infants were heavier than bottle fed infants but thereafter there was no significant difference. Factors which did not have an independent effect on weight were parity, parental smoking, social class, gastrointestinal and respiratory illness.

Both unadjusted data and data adjusted for the above independent variables showed that early solid feeders (both groups <8 weeks and 8-12 weeks) were heavier than infants who received solids after 12 weeks at 8 weeks ($p<0.05$), 13 weeks ($p<0.001$), 26 weeks ($p<0.005$) and 52 weeks ($p<0.05$). At 104 weeks there was no difference between the three groups ($p>0.05$) (Table 12, Appendix 4.2 iii b). At each of the study periods there was no significant difference in weight between infants who received solids before 8 weeks and those who received solids between 8-12 weeks.

The analysis of weights included 584 (100%) infants at 8 weeks, 576 (99.3%) at 13 weeks, 544 (93.2%) at 26 weeks, 548 (93.8%) at 52 weeks and 392 at 104 weeks. As the sample at 104 weeks was only 67% of the sample at 8 weeks, this group was studied in more detail to determine whether it was truly representative of the earlier samples studied. The proportions of the three solid feeding groups at 8 and 104 weeks were almost identical -

9.7% v 9.4%, 47.4% v 48.0% and 42.9% v 42.6% respectively and association between rows and columns was not significant (X^2 2.9, p=0.24 for 2 degrees of freedom). Multiple regression analysis of the 392 infants followed to 104 weeks demonstrated that factors which had an independent effect on weight were identical to the 8 week group - birth weight, gestation, male sex and maternal height throughout the 2 years and breast feeding at 8 weeks. The mean weights of the 392 infants, at each of the study periods, for each of the solid feeding groups, were similar to the weights achieved by all the infants studied at those times. Sub-group analysis confirmed a similar pattern to the larger sample except that the effect of early solid feeding on weight gain was not significant by 52 weeks (Table 13).

Multiple regression analysis identified maternal age ($p<0.01$), socio-economic group ($p<0.001$), and type of milk feed ($p<0.001$) as having a significant influence on the incidence of gastrointestinal illness during the first two years of life. The analysis of the effect of early solid feeding on the incidence of gastrointestinal illness was studied using unadjusted data and data which had been adjusted for the above factors and in both analyses the frequency of gastrointestinal illness was not influenced by the early introduction of solids. (Table 14).

The effect of early solid feeding on the development of allergic disease was studied using skin disorders (eczema and napkin dermatitis), and respiratory illness (wheeze and persistent cough) as outcomes. For each of the solid feeding groups, adjustments were made for birthweight, gestation, parity, maternal age and maternal history of allergic disease. The early introduction of solids was associated with an increased incidence of respiratory illness at 14-26 weeks, and persistent cough at 14-26 and 27-39 weeks. Napkin dermatitis, and wheeze were not related to the early introduction of solids. At two years of age there was a significant difference in the incidence of eczema between the three feeding groups and this was primarily due to the difference between the 8-12 week and the after 12 week solid feeders (Difference 8.4%, 95% CI 2-15%, X^2 (1df) 5.8, p=0.02) (Tables 15, 16, 17, 18, 19).

The analysis of these illnesses was based on 665 (100%) infants at 13 weeks, 657 (98.8%) at 26 weeks, 650 (97.7%) at 39 weeks, 634 (95.3%) at 52 weeks, and 455 at 104 weeks. As the sample studied at 104 weeks was only 68.4% of the cohort at 13 weeks this group was also examined in more detail to determine whether it was representative of the larger samples. The proportions of the three solid feeding groups were similar to that of the 13 week sample 9.8% v 8.8%, 49.8% v 49.0%, and 40.4% v 40.2% and the

association between rows and columns was not significant. Multiple regression analysis of the 450 infants who were followed up for 104 weeks, demonstrated a similar pattern of relationships between solid feeding and illnesses (Appendices 4.2.iii.c,d,e) but the levels of significance were less than that of the larger samples - for respiratory illness (14-26 weeks, $p<0.05$) and persistent cough (14-26 weeks, $p=0.2$; 27-39 weeks, $p=0.06$). The pattern for solid feeding and eczema was similar, and the negative associations to the other outcomes present were confirmed. The 104 week sample was therefore considered to be representative of the larger study groups.

	SEX		SOCIAL CLASS		
	M	F	I, II	III	IV, V
SOLIDS:					
Before 8 wks	38(11.9)	27 (7.7)	9 (4.7)	37(11.5)	17(11.9)
8-12 wks	171(53.4)	161(45.9)	75(39.2)	166(51.4)	83(57.2)
After 12 wks	111(34.7)	163(46.4)	107(56.1)	120(37.1)	45(31.1)
Total	320	351	191	323	145
	X^2 10.6	P < 0.005	X^2 27.75	P < 0.0001	

Table 8 Relationship of time of introduction of solids to sex of infant and social class

	FEEDING CATEGORY				
	I	II	III	IV	TOTAL
	Breast Fed				
	Full	Partial	Early Weaners	Bottle Fed	
SOLIDS:					
Before 8 wks	0	7(5.3)	19(10.6)	39(14.8)	65(9.7)
8-12 wks	0	78(60)	98(54.4)	156(59.1)	332(49.5)
After 12 wks	97(100)	45(34.7)	63(35)	69(26.1)	274(40.8)
Total	97(100)	130(100)	180(100)	264(100)	671(100)

*X^2 refers to groups II, III, IV and therefore 4 degrees of freedom. *X^2 10.96 p = 0.03

Table 9. Relationship of time of introduction of solids to type of milk feed

DOMINANT (WD)	CASEIN DOMINANT FORMULA (CD)					WHEY FORMULA
	TIME OF CHANGE FROM WD FORMULA (WKS)					
	<1	1-3	4-7	8-12	>12	
SOLIDS:						
Before 8 wks	7(28)	3(16.6)	6(14.3)	7(18.9)	5(11.4)	11(11.2)
8-12 wks	12(48)	11(61.1)	25(59.5)	25(67.6)	28(63.6)	55(56.1)
After 12 wks	6(24)	24(22.2)	11(26.2)	5(13.5)	11(25)	32(32.6)
Totals	25	38	42	37	44	98

X^2 11.3 (10 df) p = 0.3

Table 10 Relationship of type of infant formula to timing of introduction of solids.

	Birth Weight (g)							
	2,500-2,999		3,000-3,499		3,500-3,999		4,000+	
	n	(%)	n	(%)	n	(%)	n	(%)
SOLIDS:								
Before 8 wks	8	(8.2)	21	(7.8)	28	(11.7)	8	(12.1)
8-12 wks	49	(50)	130	(48.5)	114	(47.7)	39	(59.1)
After 12 wks	41	(41.8)	117	(43.7)	97	(40.6)	19	(28.8)
Total	98		268		239		66	
					X^2 7.0 p = 0.32 (6df)			
CD FORMULA:								
Before 8 wks	8	(19)	35	(31.9)	26	(21.7)	16	(53.3)
After 8 wks	10	(23.8)	37	(32.7)	28	(34.1)	6	(20.0)
Always WD formula	24	(57.2)	38	(35.4)	28	(34.1)	8	(26.7)
Total	42		110		82		30	
					X^2 14.6 p = 0.02 (6 df)			

Table 11 Relationship of introduction of solids and type of infant formula to birth weight

AGE OF INFANT (WEEKS)	AGE OF INTRODUCTION OF SOLIDS (WEEKS) <7	8-12	>12	<7 v 8-12	<7 v >12	8-12 v >12
8	5110 (4996 to 5223)	5087 (5036 to 5138)	4960 (4905 to 5015)	0.72	0.02	0.0009
13	6051 (6190 to 5911)	5972 (5988 to 6035)	5779 (5712 to 5845)	0.31	0.0006	<0.0001
26	7781 (7580 to 7983)	7692 (7602 to 7782)	7418 (7322 to 7514)	0.48	0.005	0.0003
52	10007 (9742 to 10271)	9900 (9777 to 10024)	9691 (9559 to 9822)	0.47	0.04	0.03
104	12550 (12130 to 12982)	12644 (12455 to 12833)	12520 (12320 to 12720)	0.71	>0.8	0.37

Table 12 Relationship of weight (g)* (95% CI) of infants during first 2 years of life to age of introduction of solids.

* Weights adjusted for maternal height, birth weight, gestation and type of milk feed, and other variables (see text).

AGE OF INFANT (WEEKS)	AGE OF INTRODUCTION OF SOLIDS (WEEKS) <7	8-12	>12	<7 v 8-12	<7 v >12	8-12 v >12
8	5199 (5346 to 5052)	5102 (5167 to 5037)	4971 (5039 to 4902)	0.24	0.007	0.007
13	6097 (6275 to 5919)	5975 (6055 to 5895)	5824 (5908 to 5739)	0.22	0.008	0.01
26	7866 (8126 to 7605)	7670 (7781 to 7591)	7480 (7597 to 7362)	0.17	0.008	0.02
52	10029 (10352 to 9706)	9877 (10024 to 9730)	9767 (9922 to 9612)	0.4	0.15	0.31
104	12556 (12981 to 12130)	12643 (12831 to 12454)	12519 (12719 to 12319)	0.71	>0.8	0.37

Table 13 Relationship of weight (g)* (95% CI) of 392 infants measured at each of the time periods during the first 2 years of life to age of introduction of solids.

AGE OF BABY (WEEKS)	AGE OF INTRODUCTION OF SOLIDS (WEEKS) <8	8-12	>12	X^2 for adjusted rates
0-13	8(12.3, 5.7)	53(8.5, 9.1)	30(9.7, 10.4)	2.33
14-26	15(23.4, 20.7)	54(16.5, 15.8)	30(11.3, 12.9)	2.19
27-39	10(16.1, 13.1)	60(18.5, 15.9)	50(19.2, 23.2)	5.05
40-52	9(15.0, 12.2)	56(17.7, 15.6)	40(15.6, 18.7)	1.66
53-104	17(42.5, 41.6)	116(52.0, 50.9)	79(41.1, 42.6)	2.7

Table 14 Numbers (percentages and adjusted percentages) of infants with gastrointestinal illness according to age of introduction of solids.

AGE OF BABY (WEEKS)	AGE OF INTRODUCTION OF SOLIDS (WEEKS) <7	8-12	>12	X^2 for adjusted percentages
0-13	27(41.5, 36.1)	101(30.5, 29.1)	83(30.9, 33.9)	1.9
14-26	35(54.7, 52.0)	153(46.8, 46.9)	96(36.1, 36.6)	6.3*
27-39	34(54.8, 54.1)	158(48.8, 47.8)	107(41.0, 42.4)	2.6
40-52	31(51.7, 50.0)	156(49.2, 48.6)	112(43.6, 44.7)	0.8
53-104	33(82.5, 82.0)	194(87.0, 86.0)	149(77.6, 78.9)	2.7

*	X^2 (1 df)	p	Difference %	95% CI
<7 v 8-12	0.3	0.57	4.7	-8.6-18.2
<7 v >12	4.4	0.04	15.2	1.9-28.5
8-12 v >12	6.18	0.01	10.5	2.5-18.4

Table 15 Numbers (percentages and adjusted percentages) of infants with respiratory illness according to age of introduction of solids.

	AGE OF INTRODUCTION OF SOLIDS (WEEKS)			
AGE OF BABY (WEEKS)	<7	8-12	>12	X^2 for adjusted percentages
0-13	8(12.3, 10.7)	28(8.5, 8.0)	26(9.7, 10.7)	1.3
14-26	16(25.0, 23.3)	57(17.4, 17.5)	30(11.3, 11.7)	5.06+
27-39	19(30.6, 29.2)	58(17.9, 16.8)	30(11.5, 13.2)	7.36*
40-52	9(15.0, 12.8)	66(20.8, 19.4)	37(14.4, 16.7)	1.87
53-104	18(45.0, 43.2)	102(45.7, 44.4)	68(35.4, 36.8)	2.1
+ 14-26	X^2 (1 df)	p	Difference %	95% CI
<7 v 8-12	0.91	0.34	6.0	-4-16
<7 v >12	4.97	0.03	11.7	2.2-21.2
8-12 v >12	3.36	0.06	5.7	-0.03-11.5
* 27-39				
<7 v 8-12	4.4	0.04	12.3	1.7-22.9
<7 v >12	8.2	0.004	15.9	5.7-26.1
8-12 v >12	1.2	0.28	3.6	-2.3-9.5

Table 16 Numbers (percentages and adjusted percentages) of infants with persistent cough according to age of introduction of solids.

AGE OF BABY (WEEKS)	AGE OF INTRODUCTION OF SOLIDS (WEEKS) <7	8-12	>12	X^2 for adjusted percentages
Wheeze				
0-13	1(1.5, 1.0)	6(1.8, 1.4)	4(1.5, 2.2)	0.72
14-26	3(4.7, 3.6)	16(4.9, 4.2)	10(3.8, 4.9)	0.23
27-39	3(4.8, 4.2)	21(6.5, 5.8)	9(3.4, 4.5)	0.54
40-52	2(3.3, 2.6)	16(5.0, 4.4)	9(3.5, 4.4)	0.56
53-104	3(7.5, 6.5)	32(14.3, 14.5)	18(9.4, 9.4)	2.1

Table 17 Numbers (percentages and adjusted percentages) of infants with wheeze according to age of introduction of solids.

AGE OF BABY (WEEKS)	AGE OF INTRODUCTION OF SOLIDS (WEEKS) <7	8-12	>12	X^2 for adjusted percentages
0-13	8(12.3, 10.7)	68(20.5, 19.5)	42(15.6, 17.2)	3.38
14-26	11(17.2, 15.6)	61(18.7, 18.2)	26(9.8, 10.7)	5.05
27-39	9(14.5, 13.8)	42(13.0, 11.9)	23(8.8, 10.3)	0.61
40-52	4(6.7, 5.9)	42(13.2, 12.3)	22(8.6, 9.9)	2.76
53-104	4(10.0, 10.6)	39(17.5, 17.5)	39(20.3, 20.2)	1.91

Table 18 Numbers (percentages and adjusted percentages) of infants with napkin dermatitis according to age of introduction of solids.

AGE OF BABY (WEEKS)	AGE OF INTRODUCTION OF SOLIDS (WEEKS) <7	8-12	>12	X^2 for adjusted percentages
Eczema				
0-13	1(1.5, 1.2)	12(3.6, 3.5)	8(3.0, 3.2)	0.72
14-26	3(4.7, 4.0)	19(5.8, 5.2)	9(3.4, 4.2)	0.37
27-39	1(1.6, 1.8)	18(5.6, 5.6)	15(5.7, 5.6)	1.8
40-52	1(1.7, 1.7)	19(6.0, 6.6)	10(3.9, 3.1)	4.3
53-104	2(5.0, 5.4)	36(16.1, 17.0)	18(9.4, 8.3)	7.5*

*8-12 v >12 X^2 (1 df) 5.8, p=0.02. Diff 8.4% 95% CI 2-15%

Table 19 Numbers (percentages and adjusted percentages) of infants with eczema according to age of introduction of solids.

4.2.iv DISCUSSION

This study confirms the findings of previous reports[66], and demonstrates that many parents are not taking advantage of professional advice on when to introduce solid foods to their infants. Parents may be perceiving that early solid feeding is beneficial to their infant and therefore for the professional message to be accepted further data on the effects of early solid feeding are required. The principal aim of this study was to determine if the early introduction to solid foods was harmful to the infant and in particular whether these infants are at increased risk of excessive weight gain, gastrointestinal illness and allergic disease.

Early studies of the relation between early solid feeding and weight gain indicated that weight gain was increased in the early solid feeding group [67,68], but this has not been confirmed in recent studies[71,72,73]. Weight gain is influenced by a number of different demographic and nutritional factors and failure to give adequate consideration to these may explain the conflicting results. In this study potential growth factors such as maternal height, socio-economic status, sex of infant, birth weight, and type of milk feed were recorded and their relationship to early solid feeding examined.

Several of the demographic and nutritional findings in this study were similar to previous reports[66]. Solids

were introduced earlier for boys and for infants from the lower socio-economic groups and infants who were bottle fed were more likely to receive solids at an earlier stage. We were unable to support the previous evidence[66] that solids are given earlier to infants who are receiving casein-dominant as opposed to whey-dominant formulas. Although infants of heavier birth weight were more frequently changed to a less modified casein-dominant formula, the early introduction of solids was not shown to be more common in this group.

These, and other variables which were thought to be potential factors influencing weight gain during the first two years of life, were included in the regression analysis. Factors which had an independent significant effect on weight gain were maternal height, sex of infant, birth weight, type of milk feed and early solid feeding. After adjustment for these confounding variables, early solid feeding was shown to have a significantly positive effect on weight gain at 8, 13, and 26 weeks. The effect had greatly reduced by 52 weeks and was not significant at 104 weeks. Thus, the early introduction of solids may have an initial impact on the weight gain of the infant but this is not sustained during the second year of life. It would therefore seem unlikely that early solid feeding places the child at increased risk of excessive weight gain in later childhood. In support of this, a recent study has

shown that energy intakes are not significantly altered by the introduction of solids as the infant appears to reduce milk volume in order to maintain a similar caloric intake[80]. It has even been suggested that the early introduction of solids may reduce the risk of obesity, a recent report demonstrating that adiposity at 6 years of age was associated with late introduction of solids and prolonged breast feeding[81].

The incidence of gastrointestinal illness was similar within the three solid feeding groups and this finding persisted after adjustment for the confounding variables maternal age, socio-economic group and type of milk feed. This suggests that although there may be theoretical reasons for infants not being able to tolerate solids in the first few months of life[82], in practice there is no significant gastrointestinal upset. The incidence of chronic diarrhoeal illness in our cohort was too small for proper analysis but there was no difference between the early and late solid feeding groups. In Third World countries several studies have demonstrated increased gastro-intestinal infection in infants receiving early solid feeding[69] and this is thought to be related to inadequate sanitation conditions, poor nutritional status and poor quality solid foods. These conditions do not apply to the vast majority of infants in a developed society. In our previous report on this cohort[79], breast feeding for at least 13 weeks was shown to result in a

significant reduction in episodes of gastrointestinal illness during the first year of life. This did not imply exclusive breast feeding as the low incidence of gastrointestinal disease was seen in both the exclusively breast fed and the partially breast fed groups. In the latter group, 65% of infants received solids before 13 weeks of age. This data supports the view that early solids do not increase the risk of gastrointestinal illness and that the continuation of breast feeding is the most vital protective factor against gastrointestinal infection.

The incidence of respiratory illness was increased at 14-26 weeks in early solid feeding infants. Respiratory illness included the symptoms coryza, wheeze and persistent cough. Analysis of the latter two more serious symptoms showed that persistent cough was increased at 14-26 and 27-39 weeks, but wheeze was not influenced by the early introduction of solids. Respiratory illness in early childhood has been shown to be associated with environmental factors such as parental smoking[85] and poor social conditions[86]; adjustments were made for these confounding variables, and the association between early solid feeding and respiratory symptoms persisted. Whether the respiratory symptoms are a consequence of an atopic reaction or are due to recurrent infection cannot be answered by this study. An atopic element may become more apparent with longer term follow-

up and this will be undertaken. Fergusson did not find a significant relationship between early solid feeding and the development of childhood asthma when he followed-up his cohort to the age of 4 years[77].

In our earlier study we found that breast feeding for 13 weeks reduced the incidence of respiratory illness during the first 13 weeks of life but not thereafter. Data from this study indicates that by delaying the introduction of solids until after 12 weeks a further reduction in the incidence of respiratory illness will be achieved during the periods of 14-26 and 27-39 weeks.

The findings from our study showed an inconsistent relationship between early solid feeding and the incidence of eczema. Surprisingly, we found that eczema was less common in the 0-7 week solid feeding group and most common in the 8-12 week solid feeding group. Theoretically this could be explained on the basis of animal experimental work which has shown that hypersensitivity reactions precipitated by large antigenic loads are less extensive than hypersensitivity reactions precipitated by small antigenic stimuli[84]. In the newborn infant there is a gradual decrease in intestinal permeability during the first weeks of life[82], and therefore the antigenic load capable of being transported across the intestinal wall will be less in infants starting solids at 8-12 weeks. The effect of

very early versus *early* solids cannot elicited from the data from Fergusson and colleagues[75,76] as they defined early solid feeding as before 4 months and details of the solid feeding pattern within this time period are not available.

Further follow-up of this cohort may allow clarification of this issue as the prevalence of eczema and other allergic disorders peak in later childhood[83]. Fergusson and colleagues'[77] have now followed-up their study group for 10 years and confirmed their earlier report which highlighted the association of early solid feeding and eczema[76] .

4.3 CONCLUSION

This study concludes that many infants are receiving solids within the first three months of life but this feeding practice is not associated with excessive weight gain during the second year of life or an increased incidence of gastrointestinal illness during the first two years of life. The incidence of respiratory illness was increased at 14-26 weeks and persistent cough at 14-26 and 27-39 weeks in early solid feeding infants. The incidence of wheeze and napkin dermatitis was not related to the time of introduction of solids and the data relating early solid feeding to eczema was inconclusive.

Longer term follow-up is required. Parents who wish to introduce solids before 13 weeks can be informed that early solid feeding is associated with an increased risk of respiratory illness during the first 9 months of life. If infants are breast fed for 13 weeks, and solids are delayed until after 12 weeks, there will be a significant reduction in gastrointestinal and respiratory illness during the first year of life.

CHAPTER FIVE

FINAL CONCLUSIONS

This Thesis has addressed issues which in the nutritional management of the newborn infant have stimulated debate and uncertainty over many years.

The aetiology of Breast Milk Jaundice was investigated by the novel approach of determining whether bile salts in breast milk could be interacting with the enzyme bile-salt stimulated lipase. The studies show for the first time that bile salts are present in breast milk. The significance of this finding requires further investigation particularly regarding possible intramammary activity. The findings from the studies refute previous claims of the role of increased lipase activity and elevated free fatty acid concentrations in the aetiology of Breast Milk Jaundice. Marked intra- and inter variation in free fatty acid concentration is noted in normal and jaundice milks. The mechanism of this is uncertain but an intra-mammary interaction between bile salts and the enzyme bile-salt stimulated lipase cannot be excluded and requires further investigation.

The controversial issue of the most appropriate time to introduce solid foods was addressed. In order to define the specific effect of early solid feeding on infant

health, particular attention was paid to the many confounding variables which could influence the identified outcomes. The study concludes that many infants are receiving solids within the first three months of life but there is no evidence to suggest that this causes excessive weight gain or gastro-intestinal illness. The incidence of respiratory illness was increased at 14-26 weeks and persistent cough at 14-26 and 27-39 weeks. The incidence of wheeze and napkin dermatitis was not related to the time of introduction of solids and the data relating solid feeding to eczema was inconclusive. Longer term follow-up is required. Parents who wish to introduce solids before 13 weeks can be informed that early solid feeding is associated with an increased risk of respiratory illness during the first year of life. Parents should also be informed that breast feeding for 13 weeks will not only further reduce the frequency of respiratory illness but also reduce the risk of gastrointestinal illness.

With an increasing volume of evidence emphasising the need for optimum nutrition in order to achieve normal growth and development in the preterm infant, there is an urgent requirement for more specific nutritional data on the sick ventilated infant. A non-invasive, precise and accurate system for measurement of energy expenditure in these infants has been developed. Initial data indicates that energy expenditure in this group of infants may be less

than speculated and the energy/protein mix in nutritional regimes for these infants may be inappropriate. The described indirect calorimetry system will be used in combination with stable isotope studies to provide more specific nutrient turnover data. The ultimate aim is to develop feeding regimes which will not exacerbate respiratory failure and will enable normal nutrient accretion and growth for these vulnerable infants.

As stated in the general introduction, nutritional research tends to raise more questions than provide unequivocal answers and undoubtedly this is reflected in this Thesis. However, an attempt has been made to provide original data from which further progress can be made in advancing our knowledge and understanding of nutritional metabolism in the term and preterm infant.

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LIST OF APPENDICES

iii c Numbers (percentages and adjusted percentages) of infants followed up for 104 weeks with respiratory illness according to age of introduction of solids.

iii d Numbers (percentages and adjusted percentages) of infants followed up for 104 weeks with persistent cough according to age of introduction of solids.

iii e Numbers (percentages and adjusted percentages) of infants followed up for 104 weeks with eczema according to age of introduction of solids.

APPENDIX 3.2(iii a) STUDY OF BREAST MILK FOR THE PRESENCE OF BILE SALTS

Data File 1. Bile salts in breast milk

	Cheno umol/l	Cholate umol/l
Sample 1	0.14	0.72
2	0.10	0.67
3	0.09	0.50
4	0.06	1.03
5	0.14	0.56
6	0.30	0.82
7	0.22	0.62
8	0.24	0.94
9	0.52	1.93
10	0.37	1.58
11	1.50	2.50
12	0.23	2.24
13	0.58	2.86
14	0.15	0.44
15	0.11	0.61
16	0.17	1.14
17	0.74	0.94
18	0.02	0.41
19	0.31	0.34
20	0.42	0.49
21	0.29	0.66
22	0.03	0.49
23	0.16	0.82
24	0.17	0.34
25	0.05	0.42
26	0.07	0.31
27	0.05	0.29
28	0.09	0.45

APPENDIX 3.2(iv a)

Date File 2. Bile salts in maternal serum

	Cheno umol/l	Cholate umol/l
Sample 1	0.94	0.65
2	1.36	0.70
3	1.81	1.92
4	0.42	0.61
5	1.68	2.17
6	0.39	0.51
7	1.32	1.37
8	0.41	0.32
9	0.25	0.95
10	0.34	0.64
11	0.66	0.83
12	0.69	0.44

APPENDIX 3.3(iv a) STUDY OF RELATIONSHIP BETWEEN BILE SALTS, BSSL AND FREE FATTY ACIDS IN BREAST MILK OF NORMAL INFANTS AND INFANTS WITH BREAST MILK JAUNDICE

Data File 1. Normal milks

(i) 2 weeks
(ii) 6 weeks
(iii) 10 weeks
(iv) 14 weeks

	Cheno umol/l	Cholate umol/l	Ratio	Lipase pH7.4	Lipase pH8.6	Fatty acid mmol/l
Sample						
1(i)	0.12	0.68	5.67			
1(ii)	0.23	0.87	3.78			1.78
1(iii)				136.00	22.50	
2(i)	0.15	1.64	10.93			
2(ii)	0.29	0.83	2.86	7.50		2.34
3(i)	0.22	0.46	2.09	120.00	42.00	
3(ii)	0.33		0.00			
3(iii)	0.19	0.36	1.89	14.00	4.50	1.02
4(i)	0.17	0.35	2.06	28.50	30.50	
4(ii)	0.25	0.70	2.80	23.50	18.00	7.51
5(i)	0.04	0.21	5.25	26.00	18.00	
6(i)	0.20	0.64	3.20	49.50	26.00	2.75

APPENDIX 3.3(iv a) CONTINUED

	Cheno umol/l	Cholate umol/l	Ratio	Lipase pH7.4	Lipase pH8.6	Fatty acid mmol/l
Sample						
7(i)	0.21	0.51	2.43	115.00	49.00	1.78
7(ii)	0.19	0.30	2.79	62.50	28.50	
7(iii)	0.15	0.44	2.93		19.50	
8(i)	0.42	0.57	1.36	11.50	8.00	2.73
8(ii)	0.15	0.56	3.73		4.50	2.02
9(i)	0.15	0.49	3.27	7.00	22.50	2.51
10(i)	0.19	0.40	2.11	0.00	0.50	1.26
10(ii)	0.10	0.45	4.50	0.00	0.00	2.45
11(i)	0.20	0.51	2.55	0.00	14.50	5.03
11(ii)	0.13	0.30	2.31	4.50	13.00	3.00
12(i)	0.22	0.56	2.55	0.00	46.50	1.47
12(ii)	0.13	0.38	2.92	0.00	0.50	1.69
13(i)	0.28	0.84	3.00	0.00	0.00	1.67
13(ii)	0.17	0.38	2.24	0.00	4.50	0.27
14(i)	0.16	0.56	3.50	7.00	18.00	1.47
15(i)	0.10	0.49	4.90	141.00	57.00	
16(i)	0.14	0.45	3.21	141.00	47.30	1.33

APPENDIX 3.3(iv a) CONTINUED

	Cheno umol/l	Cholate umol/l	Ratio	Lipase pH7.4	Lipase pH8.6	Fatty acid mmol/l
Sample						
17(i)	0.25	0.51	2.04	100.00	2.50	
17(ii)	0.17	0.38	2.24	158.00	38.00	1.01
17(iii)	0.19	0.34	1.79	40.50	30.50	
17(iv)	0.25	0.36	1.44	59.50	26.00	
18(i)	1.57	2.87	1.83	115.00	42.50	0.78
18(ii)	0.61	0.78	1.28	45.00	30.50	0.20
19(i)	0.23	0.47	2.04	64.00	45.00	0.17
19(ii)	0.25	0.47	1.88	136.00	30.50	
19(iii)	0.26	0.42	1.62	64.00	21.00	
19(iv)	0.22	0.58	2.64	64.00	6.50	6.58
20(i)	0.32	0.65	2.03	45.00	26.00	2.54
20(ii)	0.22	0.40	1.82	18.50	16.50	4.13
21(i)	0.23	0.52	2.26	100.00	33.00	1.34
21(ii)	0.25	0.44	1.76	66.50	59.50	2.18
22(i)	0.21	0.64	3.05	62.50	33.00	1.19
23(i)	0.14	0.44	3.14	26.00	7.00	
23(ii)	0.18	0.79	4.39	45.00	18.50	1.95
23(iii)	0.14	0.39	2.79	36.00	18.50	3.41
23(iv)	0.16	0.35	2.19	14.00	14.00	2.23

APPENDIX 3.3(iv a) CONTINUED

	Cheno umol/l	Cholate umol/l	Ratio	Lipase pH7.4	Lipase pH8.6	Fatty acid mmol/l
Sample						
24(i)	0.17	0.40	2.35	26.00	23.50	
24(ii)	0.21	0.71	3.38	30.50	16.50	
24(iii)	0.14	0.34	2.43	28.50	18.50	2.14
24(iv)	0.12	0.27	2.25	0.00	0.00	5.09
25(i)	0.19	0.34	1.79	42.50	11.50	1.32
25(ii)	0.26	0.44	1.69	45.00	28.50	
25(iv)	0.14	0.36	2.57	14.00	11.50	1.97
26(i)	3.13	3.75	1.20	170.00	71.50	
27(i)		0.12	0.60	28.50	18.50	1.81
27(ii)	0.39	0.68	1.74	36.00	30.50	8.26
28(i)				52.00	30.50	1.29
29(i)	0.42	0.84	2.00	7.00	18.50	1.53
30(i)	0.16	0.31	1.94	69.00	40.50	0.40
31(i)	0.13	0.39	3.00	69.00	30.50	
32(ii)	0.22	0.47	2.14	54.00	33.00	2.23
33(i)	0.22	0.38	1.73	62.50	33.00	0.08

APPENDIX 3.3(iv a) CONTINUED

	Cheno umol/l	Cholate umol/l	Ratio	Lipase pH7.4	Lipase pH8.6	Fatty acid mmol/l
Sample						
34(ii)	0.42	0.79	1.88	38.00	14.00	11.08
35(i)	0.09	0.35	3.89	78.50	33.00	14.90
35(ii)	0.28	0.36	1.29	62.50	26.00	13.30
35(iii)	0.13	0.94	7.23	30.50	23.50	
35(iv)	0.07	0.32	4.57	33.00	16.50	
36(i)	0.16	0.42	2.63	144.00	42.50	3.14
36(ii)	0.23	0.38	1.65	16.50	14.00	0.18
36(iii)	0.04	0.78	19.50	11.50	14.00	0.64
36(iv)	0.12	0.53	4.42	16.50	16.50	
37(i)	0.17	0.42	2.47	26.00	23.50	0.99
38(i)	0.19	0.49	2.58	16.50	18.50	0.98
38(ii)	0.32	0.43	1.34	66.50	33.00	1.49
39(i)	0.22	0.43	1.95	59.50	36.00	0.54
39(ii)	0.26	0.90	3.46	23.50	16.50	
39(iii)	0.16	0.64	4.00	8.00	11.50	1.12
40(i)	0.72	1.79	2.49	38.00	23.50	1.18
40(ii)	0.16	1.17	7.31	38.00	36.00	10.29
40(iii)	0.07	0.61	8.71	64.00	28.50	1.98
40(iv)	0.17	0.38	2.24	28.50	14.00	1.20

APPENDIX 3.3(iv a) CONTINUED

	Cheno umol/l	Cholate umol/l	Ratio	Lipase pH7.4	Lipase pH8.6	Fatty acid mmol/l
Sample						
41(i)	0.29	0.52	1.79	82.00	40.50	3.50
41(ii)	0.07	0.84	12.00	69.00	23.50	2.97
41(iii)	0.10	0.52	5.20	40.50	38.00	1.19
41(iv)	0.15	0.43	2.87	28.50	16.50	0.98
42(i)	0.30	0.91	3.03		49.50	0.70
42(ii)	0.16	0.55	3.44	64.00	26.00	0.76
42(iii)	0.06	0.53	8.83	64.00	26.00	2.70
42(iv)	0.15	0.39	2.60	8.00	8.00	1.05
43(i)	0.36	0.97	2.69	69.00	38.00	
43(ii)	0.16	0.42	2.63	71.50	42.50	
44(i)	0.20	0.40	2.00	71.50	30.50	
44(ii)	0.09	0.68	7.56	42.50	8.00	7.58
44(iii)	0.15	0.40	2.67	47.50	23.50	4.70
44(iv)	0.20	0.49	2.45	62.50	16.50	5.23
45(i)	0.23	0.45	1.97	28.50	11.50	3.62
45(ii)	0.09	0.71	7.89	14.00	14.00	
45(iii)	0.04	0.52	13.00	4.50	16.50	1.09
45(iv)	0.22	0.39	1.77	0.00	2.00	2.25

APPENDIX 3.3(iv a) CONTINUED

	Cheno umol/l	Cholate umol/l	Ratio	Lipase pH7.4	Lipase pH8.6	Fatty acid mmol/l
Sample						
46(i)	0.19	0.43	2.26	117.00	36.00	
46(ii)	0.15	0.86	5.73	45.00	26.00	2.23
46(iii)	0.17	0.81	4.76	52.00	26.00	2.87
46(iv)	0.17	0.42	2.47	14.00	23.50	1.08

APPENDIX 3.3(iv b) A STUDY OF RELATIONSHIP BETWEEN BILE SALTS, BSSL AND FREE FATTY ACIDS IN BREAST MILK OF NORMAL INFANTS AND INFANTS WITH BREAST MILK JAUNDICE

Date File 2. Breast milk jaundice milks

	Cheno umol/l	Cholate umol/l	Ratio	Lipase pH7.4	Lipase pH8.6	Fatty acid mmol/l
Sample						
1	0.22	0.65	2.95	49.50	21.00	1.82
2(i)	0.04	0.54	13.50	49.50	26.00	1.76
2(ii)				23.50	14.00	0.91
3	0.18	0.61	3.39	55.1	51.5	2.10
4	0.18	0.51	2.83	47.50	18.50	0.42
5	0.25	0.56	2.24	2.00	21.00	1.71
6	0.22	0.65	2.95	82.00	36.00	0.65
7	0.06	0.71	11.83	69.00	57.00	2.92
8(i)	insufficient milk					1.23
8(ii)	0.36	0.82	2.28	33.00	33.00	1.57
8(iii)	0.13	0.54	4.15	21.00	16.50	
8(iv)	0.04	0.60	15.00	11.50	14.00	2.80
9	0.30	0.83	2.77	38.00	16.50	0.84
10	0.13	0.71	5.46	69.00	30.50	2.53

APPENDIX 3.3(iv b) CONTINUED

	Cheno umol/l	Cholate umol/l	Ratio	Lipase pH7.4	Lipase pH8.6	Fatty acid mmol/l
Sample						
11(i)	0.10	0.65	6.50	69.00	30.50	1.96
11(ii)	0.15	0.47	3.13	172.00	47.50	1.38
11(iii)	0.17	0.36	2.12	64.00	26.00	
12(i)	0.21	0.51	2.43	132.00	38.00	1.23
12(ii)	0.10	0.58	5.80	52.00	18.50	
12(iii)	0.17	0.36	2.12	36.00	21.00	2.45

APPENDIX 4.2 ii a

INFANT FEEDING DATA

Question 1 (To breast feeding mothers only)
How many breast feeds did you give in the last 24 hours?

Question 2 (To all mothers)
How many feeds of formula milk did you give in the last 24 hours?

Question 3 (i) What brand of formula milk are you using?

(ii) Have you used any other brands of formula milk since my last visit?

Question 4 How many feeds of juice or water have you given the baby in the last 24 hours?

Question 5 How are you sterilising the water you are giving to the baby?

Question 6 How many feeds of solids have you given in the last 24 hours?

Question 7 (For the baby after 6 months of age)
How many meals did your baby have in the last 24 hours?

Question 8 Who gave the feeds to the baby in the last 24 hours?

Question 9 Have you introduced formula milk for the first time since my last visit?

If "yes", when did you start this?

Question 10 Have you introduced cow's milk for the first time since my last visit?

If "yes", when did you start this?

Question 11 Have you introduced solid food since my last visit?

If "yes", when did you start this?

Question 12 (For breast feeding mothers only)
Have you stopped breast feeding <u>completely</u> since my last visit?

If "yes", when was the last breast feed given?

Question 13 (For breast feeding mothers who have stopped breast feeding)

Why did you stop breast feeding?

INFANT GROWTH

Question 14 Please record weight of infant, if recorded since last visit and state where the baby was weighed.

Question 15 Please record OFC, if measured since last visit and state where the measurement was made.

INFANT SUPERVISION

Question 16a (i) Has your baby been cared for in a day nursery?

(ii) If "yes"; Has the day nursery been full time or part time?

Question 16b Is baby being cared for by anyone apart from mother?

If "yes", record who is the carer.

Question 17 Have any of the other children in the house been ill since my last visit?

If "yes", please record:

(i) Age of child
(ii) Nature of illness
(iii) Dates of the start and finish of the illness.

Question 18 Have you been ill yourself since my last visit?

If "yes", please record:

(i) Nature of illness
(ii) Dates of the start and finish of the illness.

ILLNESS IN BABY

GASTROINTESTINAL

Question 19 (i) Has your baby been vomiting since my last visit?

If "no", record as negative.

If "yes", ask following supplementary questions:-

(ii) Is the vomiting more than a mouthful at at time?

(iii) Is this a change from his usual pattern?

If "yes" to both supplementary questions, record the dates the vomiting started and stopped.

THERAPEUTIC ENQUIRY

(i) Did you use any treatment by yourself?

(ii) Did you consult your General Practitioner?

(iii) Did your General Practitioner give any treatment?

(iv) Was your baby admitted to hospital?

If "yes", record admission dates.

Question 20 (i) Has your baby had diarrhoea since my last visit?

If "no", record as negative.

If "yes", ask following supplementary questions:-

(ii) How many dirty nappies did the baby have each day?
(iii) Was this a change from the usual pattern?
(iv) What consistency were the stools?
(v) What colour were the stools?
(vi) Were the stools offensive?

If the diarrhoea was a change from normal bowel habit and if the stools were watery, of abnormal colour or offensive, record the dates the diarrhoea started and stopped.

If diarrhoea present ask <u>THERAPEUTIC ENQUIRY</u> as per Question 19 above.

Question 21

(i) Has your baby had colic since my last visit?

If "no", record as negative.

If "yes", ask following supplementary questions:-

(ii) What does baby do when it has colic?

Use following probing questions:-

(iii) Does baby scream during an attack?
(iv) Does baby draw up his/her legs during an attack.

(v) At what time of day does the 'colic' occur?

(vi) Is the baby well between attacks?

If baby has intermittent attacks when he/she screams and draws up legs and is well between attacks, diagnose as colic and record the dates the colic started and stopped.

If colic present ask THERAPEUTIC ENQUIRY as per Question 19 above.

RESPIRATORY

Question 22 (a), (b) and (c)

(i) Has your baby had any colds since my last visit?

If "no", record as negative.

If "yes", ask following supplementary questions.

(ii) Did baby have a runny nose?

If "yes", record as coryza.

(iii) Did baby have a persistent cough?

(iv) Did baby have a wheeze?

If "yes", record dates respiratory symptoms started and finished.

If respiratory infection present, ask

THERAPEUTIC ENQUIRY as per Question 19 above.

OTITIS

Question 23 (a), (b) and (c)

(i) Has your baby had sore ear since my last visit?

If "no", record as negative.

If "yes", ask the following supplementary questions:-

(ii) Did your own doctor examine the ear?

(iii) Was the soreness on the outside or the inside of the ear?

If "outside", record as otitis externa.

If "inside", record as otitis interna if confirmed by medical examination.

(iv) Has your baby had a discharging ear since my last visit?

If ear infection present, ask THERAPEUTIC ENQUIRY as per Question 19 above.

SKIN PROBLEMS

Question 24 (a), (b)

(i) Has your baby had a sore bottom since my last visit?

If "no", record as negative.

If "yes", ask the following supplementary questions:-

(ii) Was the red area confined to the skin covered by the nappy?

If "yes", record as "nappy rash".

(iii) Has your baby had any other skin rash?

If "yes", ascertain from examination or from medical attendants if rash was typical of eczema. If eczema present, record as positive. If skin problem present, ask THERAPEUTIC ENQUIRY as per Question 19 above.

OTHER INFECTIONS

Question 25 (a), (b), (c) and (d)

(i) Has your baby had a mouth infection since my last visit?

(ii) Has your baby had a sticky eye since my last visit?

(iii) Has your baby had any other infections since my last visit?

If "no" to above questions, record as negative.

If "yes", to any above questions, record diagnosis made by doctor, if G.P. consulted.

If any of above infections present, ask THERAPEUTIC ENQUIRY as per Question 19 above.

OTHER ILLNESSES

Question 26

(i) Has your baby had any other illness since my last visit?

(ii) Have you consulted your G.P. for any other reason since my last visit?

(iii) Has your baby been in hospital or seen at the hospital for any reason since my last visit?

If "yes" to any of the above, please specify reasons.

FORM B | REF. NO. : | AGE OF BABY :

FEEDING DATA : During 24 hours prior to visit | DATE OF VISIT :

1. Breast feeds (number) [ ][ ]
2. Formula milk feeds (number) [ ][ ]
3. Brands of formula milk (i) .....
   (ii) .....
4. Juice/water feeds [ ][ ]
5. Method of sterilising water .....
6. Solids (number) [ ][ ]
7. Number of meals per day [ ][ ]
8. Feeds given by : (a) Mother (number) [ ][ ]
   (b) Father (number) [ ][ ]
   (c) Other relative (number) [ ][ ]
   (d) Friends/other (number) [ ][ ]

The following three questions only need to be answered ONCE (if they apply since last visit).

9. Date of FIRST formula milk feed .....
10. Date of FIRST cow's milk feed .....
11. Date of FIRST solid food .....
12. Date of LAST breast feed .....
13. Reason(s) for stopping breast feeding .....

INFANT GROWTH (Every three months)

14. Weight ..... Kg Date ..... Place of weighing .....
    OFC ..... cm Date ..... Place of measurement .....

INFANT CARE

16. Child in day nursery YES/NO ..... If yes : part-time/full time .....
    Child cared for by Minder/Relative/Other .....

ILLNESS IN CHILDREN IN HOUSE (Since last visit)

17 (a) Child's age ..... (b) Child's age .....
    Nature of illness ..... Nature of illness .....
    Started (date) ..... Started (date) .....
    Finished (date) ..... Finished (date) .....

ILLNESS IN MOTHER (Since last visit)

18. Nature of illness .....
    Started (date) .....
    Finished (date) .....

Appendix 4.2 ii b. Infant feeding data sheet

FORM B

ILLNESS IN BABY (Since last visit)

	Date Started	Date Finished	No B	Self Medic.	GP Consult.	GP gave Medicine	Hosp. Admiss.	Comments
GASTROINTESTINAL								
19. Vomiting								
20. Diarrhoea								
21. Colic								
RESPIRATORY								
22. (a) Coryza								
(b) Persistent Cough								
(c) Wheeze								
OTITIS								
23. (a) Otitis Externa								
(b) Otitis Interna								
(c) Discharging ear								
SKIN PROBLEMS								
24. (a) Nappy rash								
(b) Eczema								
OTHER INFECTIONS								
25. (a) Mouth Infection								
(b) Eye Infection								
(c) Other Infection (Please specify)								
OTHER ILLNESSES								
26. (a)								
(b)								
(c)								
OTHER COMMENTS								

Appendix 4.2 ii c. Infant illness data sheet

FORM C REF.NO. : GROUP: POST CODE: [D D]

ANTENATAL DATA

1. (a) Mother's age at booking ..... 1. (a) ☐☐
   (b) Parity (live births only) ..... (b) ☐
   (c) Marital state (1) Married (2) Single (3) Cohab. (4) Other (9) N/K (c) ☐
   (d) Occupation - Present or premarital ..... (d) ☐☐
   (e) Age left school ..... (e) ☐☐
   (f) Height (cms) ..... (f) ☐☐☐
   (g) Smoking (number/day) (00 = Nil; 99 = N/K) ..... (g) ☐☐

2. (a) Father's age ..... 2. (a) ☐☐
   (b) Occupation ..... (b) ☐☐
   (c) Employed (1) Yes (2) No (9) N/K (c) ☐
   (d) Smoking (number/day) (00 = Nil: 99 = N/K) ..... (d) ☐☐

Existing maternal (chronic)medical conditions :

3. (a) Infective (1) Yes (2) No 3. (a) ☐
   (b) Allergic (1) Yes (2) No (b) ☐
   (c) Psychiatric (1) Yes (2) No (c) ☐
   (d) Other (1) Yes (2) No (d) ☐

4. A & E attendance with S.I.I./O.D./S.I. (1) Yes (2) No 4. ☐
5. Pregnancy (1) Planned (2) Unplanned (9) N/K 5. ☐
6. Initial reaction to pregnancy : 6. ☐
   (1) Pleased (2) Displeased (3) Ambivalent (4) Upset (9) N/K
7. Attended Mothercraft : 7. ☐
   (1) Yes (2) No (9) N/K
8. Obstetric booking clinic visit (weeks gestation) ..... 8. ☐☐
9. Default of A/N Clinic (>1) : 9. ☐
   (1) Yes (2) No
10. Number of A/N admissions ..... 10. ☐

LABOUR AND DELIVERY

1. Onset (1) Spontaneous (2) Induced (3) Elective C/S 1. ☐
2. Mode (1) SVD (2) Forceps (3) LUSCS (4) Breech (5) Other 2. ☐

Appendix 4.2 ii d. Biological, socio-economic and environmental data sheet

FORM C

	MOTHER IN PUERPERIUM		
	COMPLICATIONS		
1.	(a) Infection	(1) YES (2) NO	1. (a) ☐
	(b) Psychological	(1) YES (2) NO	(b) ☐
	(c) Social problems	(1) YES (2) NO	(c) ☐
	FEEDING		
2.	Initial feeding method:	(1) Breast (2) Breast and bottle (3) Bottle	2. ☐
3.	Feeding on discharge :	(1) Breast (2) Breast and bottle (3) Bottle	3. ☐
4.	Bottle fed babies : type of formula used in hospital		4. (a) ☐ (b) ☐☐
	BABY		
1.	D.O.B.		1. ☐☐☐☐☐☐
2.	Sex	(1) Male (2) Female	2. ☐
3.	Birthweight (Kgms)		3. ☐.☐☐
4.	OFC (cms)		4. ☐☐.☐
5.	Gestation by assessment		5. ☐☐
6.	Apgars (1 min and 5 min)		6. ☐☐
7.	Admitted to S.C.B.U.	(1) NO (2) Less than 24 hours (3) More than 24 hours	7. ☐
	SPECIFIC PROBLEMS		
8.	(a) Jaundice with phototherapy	(1) YES (2) NO	8. (a) ☐
	(b) Infection : 00 = NO If YES - classify as Form B		(b)1. ☐☐ (b)2. ☐☐
	(c) Other : 00 = NO If YES - classify as Form B		(c) ☐☐

	CARD A DATA	CARD A
1.	Number of rooms in house (including kitchen)	1. ☐
2.	(1) = Inside toilet (2) = Outside only toilet	2. ☐
3.	Number of adults in house (over 16 years)	3. ☐
4.	Number of children in house (excluding new baby)	4. ☐
5.	Refrigerator in house (1) YES (2) NO	5. ☐

Appendix 4.2 ii d. Biological, socio-economic and environmental data sheet

APPENDIX 4.2(iii a) EFFECT OF POTENTIAL VARIABLES ON WEIGHT GAIN DURING FIRST 2 YEARS OF LIFE

Date File 1. Analysis of variance

	AGE (WEEKS)				
	8	13	26	52	104
Covariates					
Maternal age	0.791	0.848	0.710	0.739	0.496
Birth weight	<0.001	<0.0001	<0.001	<0.001	<0.001
Gestation	0.239	0.027	0.015	0.017	0.009
Paternal age	0.939	0.773	0.309	0.205	0.739
Maternal height	<0.001	0.001	0.002	<0.001	0.003
GI infection	-	0.081	0.555	0.476	0.771
Respiratory infection	-	0.391	0.747	0.639	0.904

Significance of F

APPENDIX 4.2(iii a) CONTINUED

Date File 2. Analysis of variance

	AGE (WEEKS)				
	8	13	26	52	104
Main effects					
Parity	0.779	0.329	0.086	0.089	0.907
Maternal smoking	0.889	0.901	0.442	0.464	0.235
Paternal smoking	0.829	0.860	0.824	0.882	0.262
Parental social class	0.229	0.533	0.595	0.115	0.674
Marital status	0.829	0.569	0.565	0.182	0.444
Sex of infant	<0.001	<0.001	<0.001	<0.001	0.018
Milk feed	<0.001	0.014	0.761	0.989	0.897
Solid feeding	<0.01	<0.001	0.001	0.078	0.753

Significance of F

APPENDIX 4.2(iii b) EFFECT ON WEIGHT (g) OF TIMING OF INTRODUCTION OF SOLIDS

	AGE (WEEKS)				
	8	13	26	52	104
Grand mean (g)	4035	5897	7585	9825	12582
Solid feeding groups					
1	+110(75)	+202(153)	+286(196)	+315(182)	+186(-26)
2	+47(52)	+76(75)	+131(107)	+147(77)	+76(61)
3	-77(-74)	-129(-118)	-214(-166)	-245(-132)	-127(-62)

Numbers in parenthesis are weight changes adjusted for maternal height, sex of infant, birth weight and type of milk feed

AGE OF BABY (WEEKS)	AGE OF INTRODUCTION OF SOLIDS (WEEKS) <7	8-12	>12	X^2 for adjusted percentages
0-13	19(46.3,40.5)	72(31.7,30.5)	56(29.6,32.4)	1.51
14-26	18(43.9,40.7)	116(51.1,51.2)	69(36.5,37.1)	6.4
27-39	23(56.1,56.8))	111(48.9,48.5)	78(41.3,41.6))	3.0
40-52	21(51.2,49.1)	114(50.2,48.7)	84(44.4,46.7)	0.1
53-104	33(82.5,82.0)	194(87.0,86.0)	149(77.6,78.9)	2.7

Appendix 4.2 iii c Numbers (percentages and adjusted percentages) of infants followed up for 104 weeks with respiratory illness according to age of introduction of solids

AGE OF BABY (WEEKS)	AGE OF INTRODUCTION OF SOLIDS (WEEKS) <7	8-12	>12	X^2 for adjusted percentages
0-13	6(14.6,12.4)	18(7.9,6.9)	19(10.1,11.8)	3.2
14-26	8(19.5,18.0)	45(19.8,18.9)	20(10.6,12.0)	2.89
27-39	12(29.3,29.3)	42(18.5,17.3)	21(11.1,12.6)	5.37
40-52	4(9.8,8.2)	50(22.0,20.6)	29(15.3,17.4)	4.2
53-104	18(45.0,43.2)	102(45.7,44.4)	68(35.4,36.8)	2.1

Appendix 4.2 iii d Numbers (percentages and adjusted percentages) of infants followed up for 104 weeks with persistent cough according to age of introduction of solids

AGE OF BABY (WEEKS)	AGE OF INTRODUCTION OF SOLIDS (WEEKS) <7	8-12	>12	X^2 for adjusted percentages
0-13	0(0,0)	11(4.8,4.4)	6(3.2,3.7)	4.4
14-26	1(2.4,1.9)	12(5.3,4.4)	7(3.7,4.9)	0.91
27-39	0(0,0)	13(5.5,5.7)	9(4.8,4.8)	3.9
40-52	0(0,0)	13(5.7,6.5)	8(4.2,3.4)	5.37
53-104	2(5.0,5.4)	36(16.1,17.0)	18(9.4,8.3)	7.5

Appendix 4.2 iii e Numbers (percentages and adjusted percentages) of those infants followed up for 104 weeks with eczema according to age of introduction of solids