Central University of Technology, Free State

THE PREVALENCE OF CONTAMINATION AND NUTRITIONAL INADEQUACIES IN FEEDS INTRODUCED TO WEANING INFANTS IN THE BLOEMFONTEIN REGION

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DECLARATION OF INDEPENDENT WORK

I, LEZELLE JOOSTE, do hereby declare that this research project submitted to the Technikon Free State for the degree MAGISTER TECHNOLOGIAE: BIOMEDICAL TECHNOLOGY, is my own independent work that has not been submitted before to any institution by myself or any other person in fulfillment of the requirements for the attainment of any qualification.

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OPSOMMING

OPSOMMING:

Sedert die laat 1950's en gedurende die 1960's, het die groter beskikbaarheid, gemak van voeding, hoë mate van veiligheid, en relatiewe lae koste van suigelingformules in die Verenigde State en ander ontwikkelende lande daartoe gelei dat suigelinge kunsmatig gevoed is vanaf geboorte. Vandag, veral tydens die laaste 5 jaar, is aanvanklike borsvoeding meer algemeen. Die gebruik van suigelingformules speel egter nog steeds 'n groot bydraende rol in suigelingvoeding.

In Sub-Sahara Afrika, het die Verenigde Nasies (VN) agentskappe bepaal dat ongeveer 40% van kinders onder die ouderdom van 5 jaar onvoldoende groei getoon het; ongeveer 30% was ondergewig en 7% het 'n onvoldoende gewigstoename getoon. Diarree is een van die algemeenste oorsake van kindermorbiditeit en -mortaliteit in ontwikkelende lande. Daar word beraam dat oor die hele wêreld, uitsluitende China, 1400 miljoen episodes van diarree jaarliks voorkom by kinders onder die ouderdom van 5 jaar. Ongeveer 70% van diarree-episodes kan toegeskryf word aan patogene oorgedra deur voedsel.

Die Republiek van Suid-Afrika beskik oor 'n bevolking met 'n groot verskeidenheid van verskillende kulturele agtergronde. Elk van hierdie kulture het 'n ander siening rakende die spening van suigelinge. Die totale aantal sterfgevalle in die Vrystaat, gedurende 1999, beloop ongeveer 6870. The mortaliteitstempo van suigelinge in die ouderdomsgroep 0-5 jaar in die Vrystaat beslaan 15.6% van hierdie totaal. Sterftes in hierdie ouderdomsgroep kan beskou word as 'n akkurate indikator wat betref die beskikbaarheid van essensiële kinder- en omgewingsgesondheidsdienste. Infeksies is

die naasalgemeenste oorsaak van dood by kinders onder die ouderdom van 5 jaar, met diarree en gastroenteritis as die hoofoorsake. Die bydrae van speningswantoepassing tot hierdie syfers is onbekend.

Die hoofdoelwit van hierdie studie was om die voedings- en mikrobiologiese kwaliteit van die bottel-inhoud, verskaf aan spenende suigelinge in die Mangaung area te Bloemfoentein, vas te stel.

Die studie het bestaan uit 'n lukrake beskrywende ontwerp. Beide kwalitatiewe en kwantitatiewe data is versamel.

Die studiepopulasie het ingesluit 'n lukrake geselekteerde populasie vanuit 4 van die woonbuurte in die Mangaung streek, proporsioneel tot die populasiegrootte van die spesifieke woonbuurt. Mangaung is een van die tradisionele swart woonbuurte in Bloemfontein, die Vrystaatse hoofstad.

Monsters is lukraak versamel op grond van geografiese inligting verkry vanaf die Bloemfonteinse munisipaliteit. Oorspronklik is twee honderd moeders/dagmoeders van spenende suigelinge genader om monsters van suigeling-bottelvoedings te verskaf. 'n Groot verskeidenheid, waaronder ontbytgraan, suigeling-formulevoedings, "suurpap", gepasteuriseerde en ongepasteuriseerde melk, tee, koffie en vrugetesap is versamel. Chemiese analises is uitgevoer op die bottel-voedings insluitende vitamines A, C en E, laktose, vet, proteïene, vaste stowwe, kalsium, magnesium, sink en yster. Die inhoud is bepaal deur gebruik te maak van gestandardiseerde aanvaarbare tegnieke. Die mikrobiologiese analises is uitgevoer in ooreenstemming met die prosedures beskryf in Anneks A van Regulasie 1555 van 1997 van die Voedingsmiddels, Skoonheidsmiddels en Ontsmettingsmiddels Wet 54 van 1972. Minimale tellings is verkry omdat geen voorsiening gemaak is vir kieskeurige bakterieë nie.

Data word beskryf deur frekwensies en persentasies vir gekategoriseerde data en gemiddeldes en standaardafwykings of mediane en persentieles vir kontinue data vir die

hele groep sowel as vir verskillende voedingsvloeistowwe. Die gemete inname van elke respondent is gekategoriseer en weergegee as persentasies van die RDA en die CODEX vir elke gemete nutrient.

Die CODEX standaarde verskaf voorgeskrewe standaarde waaraan elke kommersiële maatskappy moet voldoen rakende die voedingskwaliteit van sy produk, voor bemarking. Evaluasie van die gemiddelde waardes van die voedingsveranderlikes in vergelyking met die CODEX standaarde kon gebruik word om vas te stel of die bottel-voedings voldoende was in voedingswaarde. Vir die groep-data as 'n geheel was vitamiene A, E, vet, proteïene en laktose bokant die aanbevelings gestel deur CODEX. Die voedings-veranderlikes wat nie voldoen het aan die voorgeskrewe CODEX standaarde nie sluit in vitamien C, kalsium, magnesium, sink en yster. Evaluasie van die kontaminasievlak van suigelingvoedings wek kommer. Die kontaminasie is vasgestel deur die standaard-plaattellings en totale colivormige tellings te evalueer. Van die 188 voedings geanaliseer, het 145 'n standaard-plaattelling groter as 50 000 organismes/ml (Voedingsmiddels, Skoonheidsmiddels en Ontsmettingsmiddels Wet 54, 1972, standaarde vir menslike gebruik) gehad. Afhangende van die immuniteitstatus van die suigelinge sal voedings met sulke hoë tellings ongetwyfeld aanleiding gee tot diarree en siekte. By die evaluering van die colivormige tellings bepaal die Voedingsmiddels, Skoonheidsmiddels en Ontsmettingsmiddels Wet 54, 1972, dat tellings groter as 10 colivormige organismes/ml ongeskik is vir menslike gebruik. 'n Persentasie van 12.2 het positief getoets vir *E. coli*, bevestig deur die Indool toets. Die teenwoordigheid van *E. coli* in die bottelvoedings tesame met die hoë standaard-plaat- en colivormige tellings veroorsaak dikwels ernstge diarree.

Dit is duidelik dat daar 'n groot mate van kommer bestaan rakende die kwaliteit van bottelvoedings verskaf aan spenende suigelinge.

Na aanleiding van die standaarde daargestel deur die Voedingsmiddels, Skoonheidsmiddels en Ontsmettingsmiddels Wet 54, 1972, het 84.6% van die tellings van die bottel-voedings hierdie standaard oorskry. Dit is aanduidend dat hierdie

suigelinge vatbaar is vir diarree. Dit beklemtoon die bestaan van definitiewe faktore wat suigelinge beskerm teen diarree. Verdere ondersoeke rakende hiedie beskermde faktore word aanbeveel.

SLEUTELWOORDE: Suigelingvoeding, speningsmetodes, diarree, voedsel-kontaminasie, suigelingformule

SUMMARY

SUMMARY:

From the late 1950s through the 1960s, the greater availability, ease of feeding, high safety, and relatively low cost of infant formulae in the United States and other developed countries led to most infants being fed artificially from birth. Now, particularly during the past 5 years, more mothers are initially breast-feeding. However, infant formulae have continued to play a major role in infant nutrition.

In Sub-Saharan Africa, United Nations (UN) agencies estimated that approximately 40% of children under 5 years of age were stunted; about 30% underweight; and about 7% wasted. One of the most common causes of child morbidity and mortality in developing countries is diarrhoea. It is estimated that across the world, but excluding China, 1400 million episodes of diarrhoea occur annually in children under the age of 5 years. An estimated 70% of the diarrhoeal episodes could be due to pathogens transmitted through food.

The Republic of South Africa has a population with a large variety of cultural backgrounds. Each of these cultures has different views regarding the weaning of infants. The total number of deaths in the Free State for 1999 was approximately 6870. The mortality rate for infants in the age group 0-5 years in the Free State comprises 15.6% of this total. Deaths in this age group are considered to be an accurate indicator of access to essential child health and environmental health services. Infections were the next most common cause of death in children under five years old, with diarrhoea and gastro-enteritis as the most common causes. The contribution of weaning malpractice to this figure is unknown.

The main objective of this study was to determine the nutritional and microbial quality of bottle contents given to weaning infants, by their caregivers, in the Mangaung area of Bloemfontein.

The study was that of a randomised descriptive design. Both qualitative and quantitative data were collected.

The study population included a randomly selected population, from four of the neighbourhoods in the Mangaung region, proportional to the population size of the particular neighbourhood. Mangaung is one of the traditionally black townships in Bloemfontein, the capital city of the Free State.

Samples were obtained randomly, according to geographical information gathered from the Bloemfontein municipality. A total of two hundred caregivers of weaning infants were each requested to provide a sample of the infants bottle feed. There were a great variety of feeds which included cereal mixtures, infant formula feeds, gruels, pasteurised and unpasteurised cow's milk, tea, coffee and fruit juices.

Chemical analysis performed on the bottle feeds included vitamins A, C and E, lactose, fat, protein, solids, calcium, magnesium, zinc and iron. The contents were measured using standardised accepted techniques. Microbiological analyses were performed according to the procedures as described in Annex A of Regulation 1555 of 1997 of the Foodstuffs, Cosmetics and Disinfectants Act 54 of 1972. Counts recorded were minimum counts as no provision was made for fastidious bacteria.

The data is described by frequencies and percentages for categorical data and means and standard deviations or medians and percentiles for continuous data for the whole group as well as for the different beverage groups. The measured intake of each

respondent is categorised and described as percentages of the RDA and CODEX for each nutrient.

The CODEX standards provide prescribed standards that every commercial company must meet regarding the nutritional quality of its product, before marketing. Evaluation of the mean values of the nutritional variables in comparison with the CODEX standards could be used to assess whether the bottle feeds were adequate in nutritional content. For the group data as a whole vitamin A, vitamin E, fat, protein and lactose were above the recommendations set by CODEX. The nutritional variables that did not meet the prescribed CODEX standards included vitamin C, calcium, magnesium, zinc, and iron. Evaluation of the contamination level of the infant feeds elicits serious concern. The contamination level was determined by evaluating the standard plate and total coliform counts. Of the 188 feeds analysed, 145 had standard plate counts above 50 000 organisms/ml (Foodstuffs, Cosmetics and Disinfectant Act 54, 1972 standards for human consumption). Depending on the immune status of the infant, feeds with counts as high as this will definitely lead to diarrhoea and disease. When evaluating the coliform counts, the Foodstuffs, Cosmetics and Disinfectant Act 54, 1972 states that counts higher than 10 coliforms/ml are not fit for human consumption. A percentage of 12.2 were tested positive for *E. coli* confirmed by the Indole test. The presence of *E. coli* in the bottle feeds accompanied by the high standard plate and coliform counts often results in severe diarrhoea.

It is evident that there is certainly a great deal of concern regarding the quality of bottle feeds introduced to weaning infants.

According to the standards set by the Foodstuffs, Cosmetics, and Disinfectants Act 54, 1972, 84.6% of the counts from the bottle feeds exceeded the standards. This indicates that these infants were susceptible to diarrhoea. Therefore, this emphasises the existence of definite factors protecting the infants from infantile diarrhoea. Further investigation regarding these protective factors is recommended.

KEY WORDS: Infant nutrition; weaning practices; diarrhoea; food contamination; infant formula

ABBREVIATIONS

α	alpha
β	beta
&	and
$>$	more than
$\leq$	equal to or less than
$\approx$	approximately
°C	Degrees of Celsius
%	percentage
n	number of samples analysed
$\bar{x}$	mean
σ_{n-1}	standard deviation
dL	decilitre
g	gram
g/L	gram per litre
g/m^2	gram per square metre
IU	International Units
kcal	kilo calories
kg	kilogram
m	metre

mg	milligram
ml	millilitre
μg	microgram
μg/dL	microgram per decilitre
μgRE	microgram retinol units
nm	nanometre
1 α TE	1 mg d-alpha tocopherol

AAO	ascorbate oxidase
ADP	adenosine diphosphate
AMP	adenosine monophosphate
ATP	adenosine triphosphate

B.C.	Before Christ
β-complex	beta-complex
BMI	Body Mass Index

Calcitriol	1,25-dihydroxyvitamin D_3
Ca	calcium
Ca/day	calcium per day
Cat No	catalogue number
CFUs	colony forming units
CMV	cytomegelovirus

DNA	Deoxyribonucleic acid
E. coli	*Eschericiae coli*
EFAs	essential fatty acids
FAO	Food and Agricultural Organisation
FAs	fatty acids
Fe	iron
FS	Free State
HANES	Health and Nutrition Examination Survey
Hb	haemoglobin
HPLC	High performance liquid chromatography
LCP	long-chain polyunsaturated essential fatty acids
LDH	low-density lipoproteins
Mg	magnesium
MTT	3-(4, 5 dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide
N.S.	not specified

PCM	protein calorie malnutrition
PEM	protein energy malnutrition
PKU	phenylketonuria
PMS	5-methylphenazinium methosulphate
PTH	parathyroid hormone
PUFAs	polyunsaturated fatty acids
RDA	Recommended Daily Allowances
RE	retinol equivalents
RNA	Ribonucleic acid
ROP	retinopathy of prematurity
RSA	Republic of South Africa
SA	South Africa
SAVACG	South African Vitamin C Consultative Group
STD	standard
TNTC	too numerous to count
UN	United Nations
UNICEF	United Nations Children's Fund
UOFS	University of the Free State
USA	United States of America
UV	ultra violet

WHO	World Health Organisation
Vitamin A	retinol
Vitamin C	ascorbic acid
Vitamin E	alpha-tocopherol
Zn	zinc

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CHAPTER 1

1.1 INTRODUCTION

Growing "bodies have the most innate heat; therefore, they require the most food, for, otherwise, their bodies are wasted." Hippocrates, 460-370 B.C., Aphorisms [1].

The term "wean" has been defined in the past by a variety of authors, all with different perceptions. Whitehead, 1985 described weaning as the time when breast-feeding alone becomes inadequate in either protein or energy [2]. Barness defined weaning as complete discontinuation of breast suckling, bottle sucking, or addition of supplemental foods [3]. Kretchmer preferred to describe weaning as the time when milk is not important in the diet and when needs are better met by a variety of supplemental foods [4]. These are just a few versions of the definition by well-known pediatricians. However, it is evident that there is a great deal of controversy regarding the specific definition of infant weaning. The *Oxford English Dictionary* defines the term "wean" as: "To accustom a child or animal to the loss of its mother's milk: to cause to cease to be suckled" [from the term "wenian," "to accustom"] [5]. For future references within this study, the term "wean" will represent the time when breast-feeding is inadequate to serve the nutritional needs of the infant. However, this excludes some practices associated, for instance, with allergic babies, unwillingness of the mother to breast-feed or problems regarding lactation.

In the past, weaning was mostly a breast-feeding issue, and is regarded as the contemporary universal nutritional link par excellence for the entire human race – north, west, south and east, all 5700 million of us [1, 6]. Historically, it is also a vital nutritional link in the human family's unending chain; it helps to define our place in the parade of generations as much in terms of those who came before us as of those who will come after [6]. Later,

with the introduction of artificial feeding, it was described as the time when anything besides breast-milk, like formula – even water – was introduced [1]. Although after 60 million or so years of mammalian evolution – or what many attribute to the perfect hand of the Creator – a synthetic product usually based on the milk of other species could hardly be expected to measure up [6]. Throughout most of human history infant mortality rates have averaged 15-25% and have been as high as 90% in orphans who did not have ready access to a wet nurse. As recently as 100 years ago, one-quarter of all infants in the United States died before reaching their first birthday [7]. Many of these deaths were due to malnutrition and infectious diarrhoeal diseases associated with the poor sanitary conditions that were endemic in poor urban immigrants [7, 8]. Thus, until the 20th century, breast-feeding was almost a life-or-death proposition for newborns. In fact, attempts to simulate human milk by making various adjustments to cow's milk were the basis for early American paediatric theory and practice. The founders of American paediatrics, such as Holt, Rotch, and Jacobi, devoted much of their careers to defining the nutritional needs of infants and developing safe infant feedings by modifying cow's milk with water, sugar and cream [7].

From the late 1950s through the 1960s, the greater availability, ease of feeding, high safety, and relatively low cost of infant formulae in the United States and other developed countries led to most infants being fed artificially from birth [7, 9]. The trend reversed in the 1980s, and now, particularly during the past 5 years, more mothers are initially breast-feeding. However, with the increasing numbers of mothers who must return to work 2-3 months post partum, infant formulae have continued to play a major role in infant nutrition [7]. Although the use of evaporated milk and whole cow's milk has decreased because of recent recommendations to avoid them before 1 year of age [10], the use of infant formulae have increased [11, 12].

The increased administration of infant formulae was a sufficient as a supplement but some radical and life-threatening situations have begun to appear. In Sub-Saharan Africa, United Nations (UN) agencies estimated that approximately 40% of children under 5 years of age were stunted; about 30% underweight; and about 7% wasted [13]. In South Africa, the South African Vitamin A Consultative Group (SAVACG) reported stunting of children aged 6-71

months to be about 23%, with about one in every 10 of these children underweight (SAVACG, 1996). These figures represent only part of the problem. Many children are likely to be malnourished and grow poorly but have not been officially classified as malnourished [13]. One of the most common causes of child morbidity and mortality in developing countries is diarrhoea. According to Moterjemi et al., (1993) it is estimated that across the world but excluding China, 1400 million episodes of diarrhoea occur annually in children under the age of 5 years. He also stated that 70% of the diarrhoeal episodes could be due to pathogens transmitted through food [14]. In 1990 over three million children died as a result of diarrhoea [14]. This disease exposes the sick child to other secondary health complications, and ultimately to death, if not properly managed. This happens in various ways: growth stunting of the child, dehydration, malnutrition and anemia as well as rendering the child more susceptible to other bacterial and parasitic infections such as malaria [14, 15, 16].

These facts underline the importance of studying weaning practices within the community, especially within populations with a high infant mortality rate. The Republic of South Africa (RSA) has a population with a large variety of different cultural backgrounds. Each of these cultures has different views regarding the weaning of infants. Socio-economic status, as well as regional and urban-suburban factors, also plays a major role within the outcome of these views [17]. The total number of deaths in the Free State for 1999 was approximately 6870 [18], of this total 3644 (53.0%) were male and 3226 (47.0%) female deaths, according to the Department of Health (Free State). The mortality rate for infants in the age group 0-5 years in the Free State comprises 15.6% (1072) of this total. Deaths in this age group are considered to be an accurate indicator of access to essential child health and environmental health services, the level of provision of antenatal services and delivery care. When looking at the top three causes of death in this age group, it becomes clear that deaths due to conditions originating from the perinatal period are the most common and accounted for 352 (43.7%) of the total infant deaths. The patterns for girls and boys differ, e.g. 193 (54.8%) were boys while 159 (45.2) were girls [18]. Furthermore, conditions originating from the perinatal period are followed by deaths due to respiratory disorders, namely 216 (26.8%). The most common respiratory disorder for boys and girls is pneumonia (134

;162.0%). Infections were the next most common (142; 17.6%) cause of death in children under five years old, with diarrhoea and gastro-enteritis as the most common causes. The contribution of weaning malpractice to this figure is unknown. Although it is a very important aspect, it has not received much attention in the past. Limited data is available for at least the past two decades regarding weaning practices within South Africa. As indicated by the mortality rate, figures associated with diarhoeal disease indicated that diarrhoea might be a large contributing factor towards infant mortality [18].

The aim of this study is to investigate the level of contamination and nutritional inadequacies present in weaning feeds obtained from a randomly selected population in the communities of Namibia, Joe Slovo, Phahameng and Bochabela in the Mangaung region of Bloemfontein. The degree of malnutrition will also be estimated by the calculation of the nutritional content of the feeds. The standard plate, total coliform and *E. coli* counts present in the feeds will be used to estimate the level of contamination. The information gathered will assist in establishing intervention methods to improve the standard of health education amongst caregivers, and ultimately contribute to a decrease in mortality and morbidity associated with poorly prepared infant feeds.

CHAPTER 2

LITERATURE REVIEW

2.1. HISTORICAL OVERVIEW

The earliest reference to weaning foods is from Isaiah 7:15. When revealing the birth of Christ, Isaiah said "He will eat curds and honey." In Daniel 1:8-16, the first prospective, controlled study in nutrition is described [1]. Daniel suggested to the guards that instead of the royal food and wine that the king had ordered for him and his companions, only vegetables and water be provided. Henceforth, the servants would feast on the royal food and wine and at the end of 10 days, they would compare their appearances [1].

Nutritional information gathered from ancient times revolves primarily around breast-feeding, and later, the use of a wet nurse. In classical Greece, where wet nurses were popular, artificial feeding was widespread; as evidenced by the large number of baby bottles found in children's graves during this period [1, 19]. Classical Rome followed the customs of the later Grecian period [1].

One of the most popular physicians of the time was Soranus of Ephesus, who established himself in Rome during the first century. He wrote a set of recommendations for newborn care, which included withholding food from the infant for 2 days after birth. After this period boiled honey was to be fed, but no butter, because it was considered bad for the stomach. Human milk was not to

be fed for the first 20 days after birth because it was presumed to be bad for the infant – thick, cheesy, and difficult to digest. Instead, a wet nurse was to be made available [1]. The feeding recommendations of Soranus alone on newborn feeding may explain the decline and fall of the Roman Empire. Soranus also introduced the fingernail test to ascertain human milk quality. A drop of milk would be placed on one's fingernail, and quality would be judged according to its colour, consistency, and so on. This test gained such a wide popularity that it was still used by physicians some 15 centuries later. This demonstrates how difficult it is to shake non-scientific dogma [1].

The most influential writings for the next 15 centuries were those of Avicenna. In his popular book, *The Canon of Medicine,* he advised that mothers chew food before feeding it to the baby. Putting aside the infectious consequences of such a practice, there is a physiologic basis to his advice [1]. Mixing food with saliva containing oral amylase results in pre-digestion of the food. Today, the custom of pre-chewing food for infants still exists in many primitive societies [1, 20].

During the Middle Ages, infant mortality was rampant. For the next few centuries, physicians commonly relied on non-scientific remedies that were not only useless but often dangerous. These remedies explain the very high infant mortality rate in some of the royal families of Europe during that era, and why their children often experienced higher mortality than children of the common people. The most tragic example of this is Queen Anne of England (1665-1714), the last Scottish ruler, who lost 18 children in infancy due to "spoon feeding and wet nursing" [1, 21].

In Spain, G. Marañón, the Spanish physician and historian masterfully described the dangers of royal medicine. King Philip IV and his wife Isabel, the daughter of Henry IV of France and Maria DeMedici, had 10 children. Four were premature

and died at birth. Four others died either at birth or shortly thereafter. Only two of their children survived childhood. The King, who was a great womaniser, had a large number of illegitimate children; all of them survived. Two became bishops; one, an artillery general; one, a Mother Superior of a convent; and one, a well-known friar [1]. This is how Marañón described it:

> To be fair, the explanation of these deaths is the unfortunate intervention of the royal physicians with their pedantic care. These colleagues of mine were true Herod kings of the royal families. On the other hand, the illegitimate were sent to different villages under the care of faithful servants. In this way, they were escaping the deadly influence of the physicians at the royal chambers [22].

The feeding of children was first described in 1472 in Pietro Baggalardo's book, entitled *The Aegtitudinibus Infantum* [19]. The first descriptions of weaning foods appeared between 1500 and 1800. The two most popular weaning foods during this time were pap and panada [23]. Pap dates back to the mid-15th century; panada, even earlier. Pap was a mixture of bread or flour and/or milk, not very different from baby cereals today. Panada was a mixture of broth or milk, with cereals, oil or butter, and occasionally eggs. Sometimes, beer or wine was added to these mixtures, adding nothing of nutritional value, but it is quite possible that the babies slept better! Eventually, pap and panada meant the same thing [20].

The earliest printed information concerning weaning in America was found in Culpepper's English Physician, published in Boston in 1708 [24]. He recommended weaning when the teeth erupt, weaning the stronger baby first; the weaker, later. During early colonial life, there was very little progress in the care and feeding of children. By the very end of the 18th century, in 1799, in the fourth edition of Underwood's *Treatise on the Diseases of Children*, the chemistry of

milk was mentioned for the first time. This marked the beginning of the scientific approach to infant feeding [24]. In 1867, the German chemist, Justus von Liebig, attempted to market an infant food. This was the origin of the infant food industry. He humbly called it " the perfect infant food" [24]. It was made of wheat flour, cow's milk, and malt flour. In the beginning, it was made in liquid form but this did not keep well in suspension. A few years later, a powdered form was widely advertised in America as the most perfect substitute for modern milk. Several proprietary foods containing starches, malt extracts, and diverse cereals were marketed [1].

At the beginning of the 20th century, infants in this country were not fed solid foods until they were about 1 year old. Jacoby, the father of American paediatrics, advised that before 2 years, no solids must be fed [1]. In 1895, J.B. Crozer-Griffith, in his *Care of Baby*, written for nurses and parents, ordinarily advised weaning at 10 to 12 months [24]. Early in this century, Finklestein of Berlin published an outstanding monograph on the nutritional care of foundlings. He thought that alimentary fever and diarrhoea were due to sugar fermentation, and this led him to develop protein milk, or Eiweissmilch. It gained great popularity in Europe and was widely used as late as the 1950s to treat acute gastro-enteritis. It was the first therapeutic formula to be used to treat gastrointestinal disease [1]. Rosenthal, a friend of Finklestein's, described to Radbill how he had invented such milk [25]. He had been made despondent by the number of babies dying in the hospitals from acute diarrhoea and decided to take a walk. He observed a constipated dog defecating, constipation, being common in dogs, and concluded that dogs are constipated because they eat a diet consisting almost entirely of protein. This was the scientific basis for a once very popular Eiweissmilch.

No historical review of infant feeding should bypass one of its most remarkable personalities. Truby King [26] of New Zealand was an extraordinary demagogue.

After graduating from medical school at the University of Edinburgh, he returned to his native New Zealand to work in a mental hospital. At the age of 47 he made a career change by starting a home for abandoned children.
His famous decalogue on infant feeding is as follows:

1. I am the repository of infant lore.
2. Thou shalt harken unto others in this field.
3. Honor and love thy child.
4. Remember the breast-fed is the best fed.
5. Observe the 4-hour routine to keep it holy.
6. Thou shalt not overfeed.
7. Thou shalt not push protein.
8. Thou shalt not permit constipation.
9. Thou shalt not feed except with precision.
10. Thou shalt not permit solid food too early.

He developed two infant formulas and, in 1913, published a book on how to prepare them. He was knighted by the Queen of England in 1952. The main feature of his ideas was total rigidity and self-discipline in the feeding and rearing of children. His recommendations had a positive impact on New Zealand, where infant mortality started to drop [26].

One of King's primary concerns, common in his time, was his obsession with the regularity of infant bowels. He insisted on placing babies on pots at regular intervals during the day. He was very concerned about overfeeding and went so far as to say that protein was the cause of nephritis. He was a total master in the science of coprology – he was one of the master stool gazers of his day. He listed nine types of normal stools and championed that dreadful enemy of children everywhere, the enema. He was convinced that babies were poisoned by the retention of excreta [1].

In the beginning of the 20th century, infants were not fed solid food until the age of one. In 1925, McLean and Fales wrote that even if many paediatricians were opposed to introducing solids until the second year of life, others were convinced that better results were obtained by giving them after 6 months [24]. From roughly 1920 to 1970, vast positive changes occurred in infant-feeding practices. Cereals and strained foods, such as fruits and vegetables, were added to the infant's diet to supply energy, iron, and vitamins. In 1954, to establish guidelines for infant feeding practices and to advise paediatricians on infant-feeding matters, the American Academy of Paediatrics created the Committee on Nutrition. It was through this body that infant-feeding practices were to be defined and reviewed periodically for paediatricians [27].

In most circumstances babies have incredible resilience and strength. They survive the whims of their caretakers. Most of the world's babies often receive foods other than breast milk within the first few weeks of life. This may explain why acute gastro-enteritis is prevalent, even in the breast-fed infant [1].

Today, most paediatricians agree that the proper age to introduce weaning foods is 4 to 6 months. Nevertheless, it is our impression that many mothers, with or without their paediatrician's blessing, introduce solids much sooner, perhaps as early as the first or second month of life. Parents, caretakers, and unjustly maligned grandmothers everywhere seem to obtain a great deal of pleasure from spoon-feeding an alert and eager young infant [1].

2.2. CURRENT WEANING PRACTICES

2.2.1. Introduction

Weaning recommendations are based on nutritional need, physiological maturation, and the behavioural and developmental aspects of infant feeding. Inadequate energy and protein intake and deficiencies of iron, zinc, Vitamin A, and Vitamin D are the most commonly observed nutrient deficiencies during infancy and weaning recommendations have focused on their prevention [28].

The importance of the question is underscored when one considers the consequences of inappropriate weaning [29, 30]. Too early initiation of weaning carries the risk both of increased morbidity due to diarrhoea and food allergies, as external challenges are introduced into the immature digestive tract, and of infant malnutrition due to the normal decrease in maternal milk production as the baby is withdrawn from the breast. Weaning too late can lead to faltering growth, decreased immune protection, and again, increased diarrhoeal disease and malnutrition when exclusive breast-feeding becomes inadequate [28].

Inappropriate choice of weaning foods can lead to protein-energy malnutrition and an array of micro-nutrient deficiencies. The first year of life is characterised by rapid growth and changes in body composition: most healthy infants double their birth weight in six months and triple it in one year. The progression from breast milk/infant formula to solid food is based not only on the infant's nutrient requirements, but also on developmental maturation and environmental influences [31]. Therefore correct weaning practice cannot be emphasised enough as it contributes greatly to the person the infant will become later in life. Will he/she be able to lead a prosperous life without suffering from any long-term effects caused by inappropriate weaning?

2.2.2 Weaning Recommendations

Despite broad cultural diversity [29, 30], published recommendations for weaning are remarkably consistent world-wide [32-42]. The Food and Nutrition Board defines the Recommended Daily Allowance (RDA) as follows: RDA is the level of intake of essential nutrients considered, in the judgement of the Committee on Dietary Allowances of the Food and Nutrition Board on the basis of available scientific knowledge, to be adequate to meet the nutritional needs of practically all healthy persons [51].

World Health Organisation (WHO) and United National Children's Fund (UNICEF), Weaning Recommendations

The current policy of the WHO/UNICEF is to recommend the introduction of solid food to breast-fed infants between four and six months [31, 37, 44], the admonition "and not later than six months" has been added repeatedly by others [28, 45].

The Innocenti Declaration (WHO/UNICEF 1990) states that

all women should be enabled to practice exclusive breast-feeding and all infants should be fed exclusively on breast milk from birth to six months of age.

During the first 5 to 6 months the breast-fed baby who is gaining weight at a normal rate receives all the nutrients needed with a few exceptions. The following supplements are generally recommended [46]:

- Vitamin K - is usually given parenterally shortly after birth.

- Vitamin D - 10 μg (400IU) as a water-miscible preparation, beginning a week to 10 days after birth.
- Iron - (not more than 15 mg) at 4 to 6 months; it may be given as an iron-fortified cereal or as iron drops.
- Vitamin B_{12} - if the mother is a strict vegetarian [46].

When the supply of milk declines or breast-feeding must be terminated for any reason, the baby is gradually weaned by offering a formula for a single feeding. After 4 to 5 days the baby is offered the bottle for the second feeding, and so on [46]. Beginning at four months and no later than six months, the infant is gradually introduced to weaning foods. Iron, zinc, vitamin D, and vitamin A rich-foods should be emphasized. Initially, complementary foods are given once a day, then gradually the frequency is increased so that the infant is eating two to four meals per day by about six months of age. From six to 12 months the infant should consume meals and snacks about four to six times a day in addition to breast-feeding. From 12 months the child must start to feed himself and by 24 months should be able to consume a varied diet from each of the food groups [28].

With all this in mind a great deal of difficulty is experienced in the implementation of these recommendations because South Africa is a land of contrasts, climatic, geographic and socio-political. In the latter context, communities vary from third to first world with distinct cultural differences for infant feeding and the diet is often modified by economic factors [47]. In a study conducted by Ladzani et al., (1998) on the feeding practices of Pedi women in six semi-rural areas of the Northern Province, 90% mothers breast-fed their infants [48]. Fifty-eight to sixty

percent of the mothers breast-fed their infants for a period longer than twelve months and four to eight percent breast-fed for less than seven months. The first solid food introduced to infants was maize meal porridge (86%) and 7% gave water with traditional herbs. Seventeen percent of the mothers introduced porridge on the first day of birth and 52% of the infants received porridge before the age of three months [48].

In a preliminary study conducted by myself on infant weaning practices in the Mangaung region in the Free State the results differ a great deal from those obtained by Ladzani et al., (1998) [48]. Seventy-six percent of the mother's breast-fed their babies and 24% did not breast-feed at any time. Of the mothers practising breast-feeding, 59% did so for less than four months and only 41% practised it for more than four months. Solids, predominantly (maize meal) porridge, were introduced by 50 % of the mothers before the age of four months and 50 % of the mothers introduced solids after 4 months.

There is a remarkable difference in the weaning techniques practised by the mothers in these two provinces. This illustrates that geography, urban and rural communities, socio-economic, cultural, and climatic factors make it impossible to apply the weaning recommendations set by the WHO strictly without any deviation. According to the rising mortality rates amongst infants, mostly due to malnutrition and infection, it is believed that current weaning practices in SA are under a great deal of scrutiny.

Table 2.2.1 Food and Nutrition Board, National Academy of Sciences – National Research Council Recommended Daily Dietary Allowances [49].

Nutrient	Dietary Allowance				
	0-6 Months	6-12 Months	Human Milk per 1,000 ml+	Cow's Milk (Whole) per 1,000 ml	Milk-Based Formula Per 1,000 ml
Weight, kg Lb	6 13	9 20			
Height, cm In	60 24	71 28			
Water, ml			897	894	875
Energy , kcal	kg x 115	Kg x 105	718	620	670
Protein, g	kg x 2.2	Kg x 2.0	10.6	33.4	15-16
Fat, g			44.9	33.9	33-37
Carbohydrate, g			70.6	47.3	70-72
Vitamin A, RE IU	420 1,400	400 2,000	656 2,470	315 1,279	340-500 1,700-2,500
Vitamin D, µg	10	10		10ϕ	10
Vitamin E, mg TE	3	4	1.3-3.3	5.7	5.7-8.5
Ascorbic Acid, mg	35	35	51	10	55
Thiamin, mg	0.3	0.5	0.14	0.39	0.4-0.7
Riboflavin, mg	0.4	0.6	0.37	1.65	0.6-1.0
Niacin, mg NE	6	8	2.0	0.85	7-9
Vitamin B-6, mg	0.3	0.6	0.11	0.43	0.3-0.4
Vitamin B-12, µg	0.5	1.5	0.46	3.63	1.5-2.0
Folacin , µg	30	45	51	51	50-100
Calcium, mg	360	540	328	1,208	550-600
Phosphorus, mg	240	360	144	945	440-460
Sodium, mg	115-350*	250-750*	141	498	250-390
Potassium ,mg	350-925*	425-1,275*	523	1,544	620-1,000
Magnesium, mg	50	70	31	132	40-50
Iodine, µg	40	50	30-100		40-70
Iron, mg	10	15	0.3	0.5	1.4-12.5#
Zinc, mg	3	5	1.8	3.9	2.0-4.0

❑ Food and Nutrition Board: Recommended Dietary Allowances, 9th ed. National Research Council National Academy of Science, Washington, D.C., 1980.
\+ One litre of human milk = 1.025g; 1 litre of cow's milk = 1.017g.
ϕ Assumes fortification of cow's milk with 10µg vitamin D.
* Allowances for sodium and potassium are ranges considered to be safe and adequate.
\# Values for formula not fortified and fortified with iron.

2.3 NUTRITIONAL CONTENT

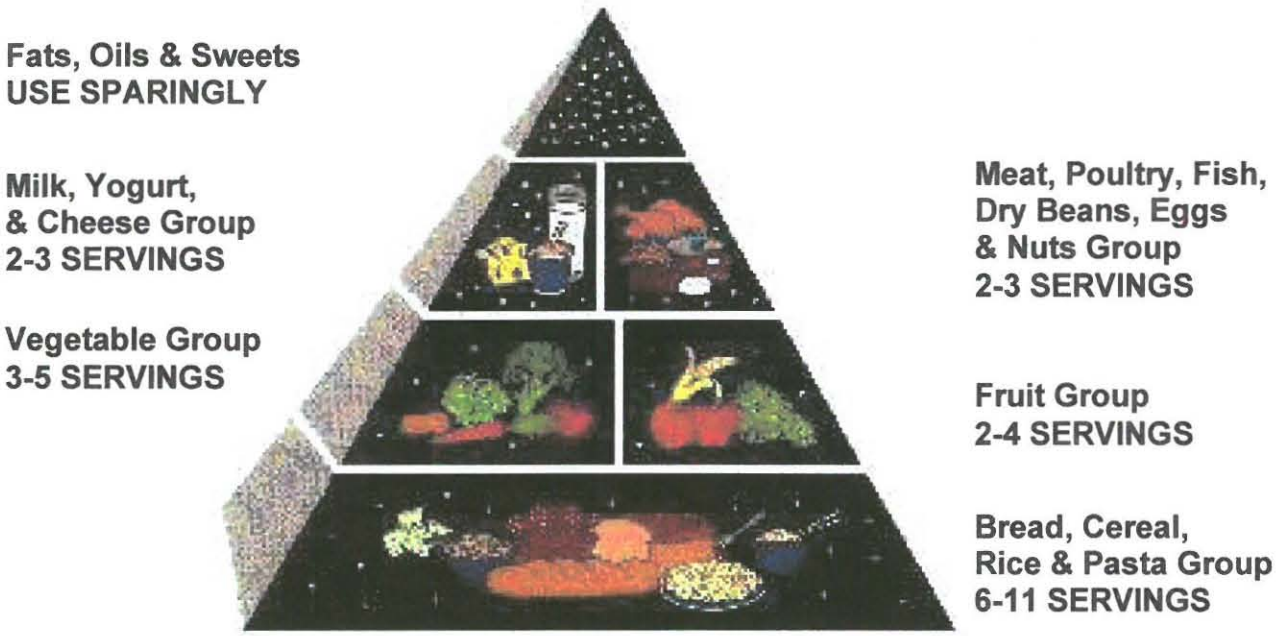

Figure 2.1 **A healthy diet consists of a variety of foodstuffs. This diagram illustrates the typical content of a healthy balanced diet. (Adapted from http://www.nal.usda.gov:8001/py/pmap.html.)**

2.3.1 Minerals

2.3.1.1 Introduction

The function and requirements of trace minerals represent one of the most exciting and dominant topics of current nutrition research [50]. Minerals are those elements that remain largely as ash when plant or animal tissues are burned. About 4% of the body weight consist of mineral matter. Seven macro-nutrients - that is, those occurring in appreciable amounts – account for most of the body

content of minerals. Calcium and phosphorous account for three-fourths of all mineral matter. Some fifteen to twenty elements are present in such minute amounts that they are generally referred to as trace elements or micro-nutrients. Many of these are known to be essential for humans, whereas the essentiality of others has been demonstrated only in animals. Minerals once considered contaminants in foods are now known to be essential. Furthermore, the number of these minerals is expected to increase. Research related to minerals has been facilitated by the development of new analytical instruments and techniques that make it possible to detect trace concentrations or, as with the use of stable isotopes, to follow more precisely the utilization of the mineral in the human body. The findings of such research have emphasized the importance of the interrelationships in function that exist among all minerals as well as among other nutrients [50].

Mineral elements are present in organic compounds such as phosphoproteins, phospholipids, haemoglobin and thyroxine; as inorganic compounds such as sodium chloride and calcium phosphate; and as free irons. They enter into the structure of every cell of the body. Hard skeletal structures contain the greater proportions of some elements such as calcium, phosphorous and magnesium, and soft tissue contains relatively higher proportions of potassium. Mineral elements are constituents of enzymes such as iron in the catalyses and cytochromes; of the hormones such as iodine in thyroxine; and of vitamins such as cobalt in vitamin B_{12} and sulphur in thiamin. Their presence in body fluids regulates the permeability of cell membranes; the osmotic pressure and water balance between intracellular and extracellular compartments; the response of nerves to stimuli; the contraction of muscles; and the maintenance of acid-base equilibrium [50].

Recommended daily allowances have been established only for calcium, phosphorous, magnesium, iron, iodine and zinc. An "estimated safe and adequate daily dietary intake" has been set for several additional minerals; expression of recommended intake in this way emphasises that these minerals are required but that sufficient data is not available to establish traditionally recommended allowances [51]. Thus, the safe and adequate intakes are more tentative and suggested ranges of intakes for maintaining health. The upper limit set on these ranges emphasises the risk of possible toxicity of these nutrients. For many minerals the range between what is necessary and what becomes toxic is quite narrow. Although this danger is not widespread, it can become very real through the use of highly fortified products and supplements [50, 51].

2.3.2 The importance of iron, magnesium, zinc and calcium during infancy

2.3.2.1 Iron

2.3.2.1.1 Introduction

Iron is probably the mineral element that requires the most emphasis in infant feeding, especially for the infants of lower socio-economic groups [52]. Iron is present in all body cells and approximately 70% of the iron is in the haemoglobin, 5% is held as myoglobin, 5% is present in cellular constituents including the iron-containing enzymes, and 20% is stored as ferritin or haemosiderin by the liver, spleen, and bone marrow. Haemoglobin is the principal component of the red blood cells and accounts for most of the iron in the body. It acts as a carrier of oxygen from the lungs to the tissues and indirectly aids in the return of carbon dioxide to the lungs. Myoglobin is an iron-protein complex in the muscle which

stores some oxygen for immediate use by the cell. Enzymes such as catalases, the cytochromes in hydrogen iron transport, and xanthine oxidase contain iron as an integral part of the molecule, and iron also acts as a cofactor for many other enzymes [53].

2.3.2.1.2 Functions and Recommended Daily Allowances

Dietary iron is compromised of non-haem and haem iron, the latter being absorbed by a separate pathway and more efficiently than non-haem iron [54]. Dietary iron is required for (1) replacement of the daily losses of all individuals; (2) an expanding blood volume and increasing amounts of haemoglobin in growing children; (3) replacement of varying losses through menstruation; (4) development of the foetus and to avoid anaemia in pregnant and lactating women; and (5) as an iron reserve that is available when blood loss occurs from any cause whatsoever [53]. The daily dietary allowance (National Academy of Science, 1990) of Fe is 10-12mg for children, 10mg for men, 15mg for women, and 30mg for women in pregnancy. Additionally, a recent recommendation is that the allowance for menstruating women should be raised to 20mg daily [55]. While allowances must not be confused with physiological needs, it would appear that in Western populations, the intakes of a large proportion are low, and need supplementation. In developing populations, understandably, the proportion with low intakes is higher; moreover, in such populations there is a need for additional Fe in areas where infections that involve blood destruction, as in the case of malaria, and blood loss with helminths, such as hookworm, are endemic [57].

2.3.2.1.4 Iron Overload

Strachan, (1929), was the first to describe Fe overload in the African population of South Africa [62]. It is now believed that it results primarily from excessive intake of dietary Fe in a highly bio-available form [63, 64]. Recently it has been put forward that the overload in part may be genetically determined, and accordingly is the result of combined effects of environmental and host factors [65, 66]. Iron imbalance involving excessive rather than deficient iron occurs in haemosiderosis and haemochromatosis, two disorders of iron metabolism in which there are large deposits of iron in the liver, pancreas, and other iron storing organs. The term haemosiderosis is generally reserved for excess iron stores without tissue damage. In haemochromatosis the excess iron produces complications such as cirrhosis of the liver or diabetes. One form of haemochromatosis appears to be a congenital disorder in which iron is absorbed much more efficiently than normally [53]. There are different viewpoints regarding the significance of Fe overload to the health of Africans. On the one hand, the conclusion has been reached that siderosis is an important public health problem, that steps should be taken to reduce the intake of adventitious Fe, and, furthermore, that those who are severely affected by Fe overload should be treated with phlebotomy [67]. Yet on the other hand, as noted, there is evidence that many factors additional to Fe intake have a bearing on the development of hyperferritinaemia and siderosis: these factors include a genetic factor, infections, inflammation, cancer and alcohol intake. As regards to Fe stores and cancer, it has been stated that the relationship remains controversial, and that further investigation is needed to determine whether Fe does indeed play a pathogenic role [68, 69].

2.3.2.2 Zinc

2.3.2.2.1 Introduction

About 2 to 3 g zinc is present in the human body and is widely distributed unevenly in all tissues. High concentrations are found in the eye, especially the iris and the retina, as well as in the liver, bone, prostate and prostatic secretions, and in the hair [70]. Zinc is mainly needed for growth and is therefore very important during infancy [71]. In less developed, non-industrialised countries, there have been many reports suggesting low intakes of zinc [72] or zinc deficiency [73] during childhood. In the United States (USA), there have also been reports on infant growth responsiveness to zinc supplementation [74]. Even under conditions of "normal" growth, zinc supplementation has proved to be beneficial to infants [75].

2.3.2.2.2 Functions

Zinc is essential for all living organisms and because of its variety of functions it is essential to human life. As already mentioned, zinc's main function is for growth during infancy. However, some other functions include the transportation of carbon dioxide to the lungs, the interconversion of pyruvic and lactic acid in the glycolytic pathway, bone metabolism, and it serves as a co-factor in the synthesis of DNA and RNA, thus they are proteins. Zinc also functions in the mobilisation of vitamin A from the liver to maintain normal concentrations of zinc in the blood circulation, and it is essential in normal cellular immune functions and spermatogenesis [70]. The essential role that zinc plays in growth results in an infant's requirements being higher than those of older children and adults [76]. At the beginning of lactation, when growth is at its peak, the zinc:calorie ratio is at its highest [77, 78]. Absorption of zinc takes place primarily from the duodenum and jejenum. The presence of a zinc–binding compound found in pancreatic

secretions and human breast milk facilitates absorption [79]. Ionic zinc absorption can be impaired by high intakes of calcium, vitamin D, phytate, and possibly dietary fibre. The normal zinc serum concentration is about 80-140 μg per dl [70].

2.3.2.2.3 Recommended Daily Allowances

As the importance of zinc in human nutrition has become better understood, increasing research is being conducted to determine the zinc requirement. The present recommended allowance is 3 to 5 mg for infants [51]. In spite of the available information on the concentration of zinc in human milk, we know very little about the concentration of these elements in milk diets consumed by infants. Although there are great differences in concentrations of nutrients between bottle and breast-milk, it seems that for minerals further differences can be expected when availability is taken into account. Bio-availability of zinc in cow's milk or formulae is lower than in human milk. Besides specific proteins present in human milk that may help zinc absorption, casein of cow's milk is estimated to comprise of 50-75% zinc [80]. Although it is known that the zinc concentration can be low in cow's milk or in infant formulae available in many parts of the industrialised world [81, 82], its impact on infant nutritional status has not yet been fully evaluated.

2.3.2.2.4 Zinc Deficiency and Excess

Severe complications can be present when zinc intake is inadequate or excessive but to a lesser extent when it is excessive. The first descriptions of human zinc deficiency were reported from Iran and Egypt [83]. In addition to growth failure, other abnormalities include hypogonadism in males, liver enlargement, severe anaemia, mental lethargy, delayed wound healing, and an increased susceptibility to infection [70]. With the addition of zinc there was a

considerable improvement in growth and the development of the sexual organs. Problems in zinc nutrition have been identified in patients with malabsorption, kidney diseases, pancreatic insufficiency, sickle cell anaemia, and inflammatory bowel disease. Acrodermatitis enteropathica is a rare genetic disorder occurring in infants, characterised by severe dermatitis, chronic diarrhoea, emotional disturbances and growth retardation. The condition appears to be due to a defect in zinc absorption [70]. On the other hand there is no evidence to support the value of increased zinc intake in persons with adequate zinc nutrition. Zinc supplements have become popular for a variety of purposes, including the treatment of acne and the improvement of sexual function. Zinc salts in very large amounts, 60-120 times the recommended allowances, will induce vomiting, cramps, and diarrhoea within 3 to 12 hours, but the symptoms soon diminish [70]. Therefore it is evident that an excessive intake of zinc is of less importance than an inadequate intake of zinc, for it can leave the patient with devastating long-term effects.

2.3.2.3 Magnesium

2.3.2.3.1 Introduction

The amount of magnesium in the body is much smaller than that of calcium and phosphorus. Of the 25 to 35 g in the body, about 60% is present as phosphates and carbonates, mainly at the surface of the bones. Most of the remaining magnesium is within the cells, where it ranks next to potassium in magnitude. Extracellular fluids account for about 2% of the body's magnesium. The normal concentration of magnesium in blood serum is 2 to 3 mg per dL, about 80 % of this being ionised; the remainder is bound to protein [84].

2.3.2.3.2 Functions

Magnesium is essential for all living cells. In addition to its function in the skeletal structures, magnesium is a catalyst in numerous metabolic reactions. It is involved in protein synthesis through its action on the aggregation of ribosomes. It is an activator for enzymes involved in the oxidative phosphorylation of adenosine diphosphate (ADP) to adenosine triphosphate (ATP), and also for all enzymes that bring about the conversion of ATP to cyclic adenosine monophosphate (AMP), which in turn regulates parathyroid hormone secretion. These reactions are essential whenever energy is expended, as in active transport across cell membranes, and the accomplishment of physical work. Therefore, it is clear that magnesium is essential during the development stage, thus during infancy. Magnesium, together with calcium, sodium, and potassium, must be in balance in the extracellular fluids so that the transmission of nerve impulses and the consequent muscle contraction can be regulated. Magnesium is also involved in muscle relaxation and thus has a function opposite to that of calcium [84].

2.3.2.3.3 Recommended Daily Allowances

The Food and Nutrition Board has recommended a daily allowance from 50 to 70 mg during the first year of life and thereafter the allowance gradually increases from 150 mg for the toddler [51]. Magnesium is absorbed by active transport and competes with calcium for carrier sites. Thus, a high intake of either element interferes with the absorption of the other. Many factors enhance calcium absorption, such as acidity. Other factors that interfere with calcium absorption, such as oxalic and phytic acids, also affect the absorption of magnesium. Neither vitamin D nor the parathyroid hormone is believed to influence magnesium absorption [84].

2.3.2.3.4 Magnesium Deficiency and Excess

Under normal conditions of health and food intake, magnesium deficiency is not likely to occur. Unlike calcium, magnesium is only slowly mobilised from bone. Therefore, a generally poor intake of magnesium, if it is also accompanied by increased excretion, leads to rapid lowering of the plasma magnesium concentration. The ionic imbalance thus produced in extracellular fluid, upsets the regulation of nervous irritability and muscle contraction. Characteristic symptoms of magnesium deficiency include muscle tremor, paresthesias, and sometimes convulsive seizures and delirium, prominent in infants. These symptoms are characteristic of hypocalcemic tetany and may be the consequence of the hypocalcemia that also frequently accompanies magnesium deficiency. Administration of calcium without magnesium, however, will not correct the condition. [Among the conditions under which magnesium deficiency is encountered during infancy, are these: sprue, kwashiorkor, severe vomiting, prolonged use of magnesium-free parenteral fluids, and diabetic acidosis [84].] The conditions mentioned above are some of the most common causes of mortality during infancy and emphasis is placed on adequate magnesium intake during infancy [18]. In most of these instances the deficiency has occurred because of curtailment of food intake or lowered absorption or both. The loss of magnesium from the body is increased during diuretic therapy, and also in diabetic acidosis. High intakes of certain magnesium salts have a laxative effect but generally are not toxic unless kidney function is impaired. Symptoms of high blood concentrations of magnesium include extreme thirst, a feeling of excessive warmth, marked drowsiness, a decrease in muscle and nerve irritability, and atrial fibrillation. The early stages of hypermagnesemia are readily corrected by the administration of calcium gluconate [84].

2.3.2.4 Calcium

2.3.2.4.1 Introduction

Of the approximately 1,200 g of calcium in the body, 99% is combined as the salts that give hardness to the bones and teeth. The bones not only provide the rigid framework for the body, but they also furnish the reserves of calcium to the circulation so that the concentration in the plasma can be kept constant at all times. The remaining 1 % of the calcium in the body – about 10 to 12 g – is distributed throughout the extracellular and intracellular fluids [85]. The concentration of calcium in the plasma is kept within the narrow range of 9 to 11 mg per dL. About 40 % of calcium are bound to plasma protein and 60 % is diffusible. The plasma calcium level is regulated by (1) the active form of vitamin D synthesised by the kidney, (2) the parathyroid hormone, and (3) calcitonin, a hormone secreted by the thyroid gland [85].

2.3.2.4.2 Functions

Because of the large percentage of calcium present in the human body calcium fulfils several very important functions. Calcium activates a number of enzymes including pancreatic lipase, adenosine triphosphatase, and some proteolytic enzymes. Calcium is required for the synthesis of acetylcholine, a substance necessary for the transmission of nerve impulses. Furthermore, calcium increases the permeability of cell membranes, thereby aiding in the absorptive processes. Another absorption process in which calcium acts is in the absorption of vitamin B_{12} from the ileum. Calcium regulates the contraction and relaxation of muscles, including the heartbeat, and catalyses several steps in the clotting of blood. Body need is the major factor governing the amount of calcium that is

absorbed by the gastro-intestinal tract. During growth the absorption of calcium is increased in order to take care of the increase in the size and hardness of the skeleton. Thus, children absorb proportionally more calcium than adults do. Several mechanisms control the amount of calcium that is absorbed. The two most important of these involve vitamin D and the parathyroid hormone (PTH). PTH is secreted when the blood calcium concentration decreases. One of its functions is to stimulate the kidney to synthesise calcitriol (1,25-dihydroxyvitamin D_3), the active form of vitamin D. This metabolite stimulates increased absorption from the intestine and, together with PTH, enhances mobilisation of bone stores of calcium. The presence of ascorbic acid and certain amino acids may facilitate calcium absorption, and the lack of vitamin D seriously impairs the absorption of calcium. The shortage may arise from inadequate exposure to sunlight or failure to ingest vitamin D in some form [85].

Calcium's best known function during infancy is in the formation of the infant's bones and teeth:

Bone

The formation of bone is initiated early in foetal life with the development of the cartilagenous matrix. During the latter part of pregnancy some mineralization of the foetal skeleton takes place so that the infant at birth has a body calcium content of about 28 g. During growth the addition of mineral to bone exceeds the amounts that are removed. The bone hardening is achieved by a gradual addition of minerals by a process referred to as mineralization or ossification. During foetal development and the first few months after birth the bones achieve sufficient mineralization so that the skeleton can support the weight of the baby when he or she walks. Throughout childhood and adolescence the bones increase in length and diameter. This increase in size is dependent upon adequate protein as well as mineral elements. The hardness of bones increases

throughout the first 20 years – sometimes longer. About 165 mg calcium is added to the skeleton daily during the early growing years. During adolescence the retention is as high as 300 mg per day, causing a yearly increase of up to 90 g in bone mass. Bone is the principal reserve of calcium and phosphorus in the body. In the well-nourished individual the readily available stores of calcium are in the ends of the long bones, and are known as trabeculae. In the absence of trabeculae calcium is withdrawn from the shaft of the long bone to provide in the calcium needs of the infant's body [85].

Teeth

Like bones, teeth are complex structures consisting of a protein matrix (keratin in the enamel, and collagen in the dentin) and mineral salts, principally calcium and phosphorus as hydroxyapatite. In the foetus the development of teeth begins by the fourth month and calcification proceeds during the growth of the foetus. Prenatally and during infancy and childhood, tooth development requires adequate supplies of many dietary factors, including not only calcium and phosphorus, but also vitamins A and D, as well as protein. The deciduous teeth of the infant are fully mineralised by the end of the first year of life, but the calcification of permanent teeth is completed at various times during childhood and adolescence; for some teeth the mineralization is not completed until early adult years. The turnover of calcium in teeth is very slow, but, unlike calcium in bone, once the calcium in teeth is lost it cannot be replaced. Thus, any factor that increases the solubility of mineral salts at the tooth surfaces will lead to decay: for example, the acids produced by microbial activity when sugars stick to the teeth. On the other hand, the presence of fluoride in the salts of tooth enamel increases the hardness, thereby reducing decay. Because of the slow rate of turnover of calcium in teeth, the foetus does not take much calcium from

the mother's teeth, and the popular notion of the loss of "a tooth for every child" is false [85].

2.3.2.4.3 Recommended Daily Allowances

The recommended daily allowance for children aged 1 to 10 years is 1,200 mg and 360 to 540 mg during the first year of life [51]. Before optimal calcium intakes for lactating women can be identified, it is necessary to know whether there are physiological compensations for conserving calcium during lactation and to what extent these physiological compensations can offset the calcium secreted in breast milk [86]. The recommended allowance for calcium is based on about 60 mg calcium per kg body weight, the amount supplied to breast-fed infants [51]. Breast-fed infants retain about 50 to 60 percent of the total calcium intake, whereas bottle-fed infants retain 25 to 30 percent of a cow's milk formula. Since formulas contain a much higher proportion of calcium, the net retention is approximately the same. The calcium-to-phosphorous ratio during the first year should be 1.5 to 1 in order to offset the tendency toward hypercalcemia that sometimes occurs with a high phosphorous intake [52].

2.3.2.4.4 Calcium Deficiency

Evidence regarding the effect of a low intake of calcium on deficiency symptoms is contradictory. If a low calcium intake alone could cause deficiency, one would expect to see much more deficiency throughout the world than actually exists. There are, however, a number of disturbances of calcium metabolism that have serious consequences [85; 89; 90]. Bone resorption is increased during immobilisation from illness or injury. The loss of calcium from bone occurs almost immediately, as demonstrated in the early space flights of astronauts [88]. Failure to provide vitamin D by exposure to sunshine or in the diet reduces the

absorption and utilisation of calcium. Eventually this leads to rickets in the young. Osteomalacia involves a reduction in the mineral content of the bone without reduction in bone size. In malabsorption disorders such as celiac disease, large amounts of fat are excreted. The unabsorbed fat combines with calcium in the intestinal lumen to form insoluble soaps. The absorption of calcium as well as fat-soluble vitamins is greatly decreased. Hypocalcemia, tetany, and osteoporosis are frequently seen in these cases. Chronic renal disease has long been recognised as contributing to hypocalcemia, osteitis, and osteomalacia. The cause of the metabolic disorder is the failure of the malfunctioning kidney to synthesise the metabolically active Vitamin D_3. Tetany is a condition characterised by a decrease in blood calcium, increased excitability of the nerves, and uncontrolled contractions of the muscles. It is not caused by a dietary lack of calcium alone, although low intakes in combination with impaired absorption may lead to its occurrence. Tetany is often the consequence of lowered parathyroid function. Administration of parathyroid hormone brings the blood calcium level back to normal [85].

2.3.2.4.5 Calcium Excess

In the case of an excessive amount of calcium intake a condition named hypercalcemia results. A number of conditions can cause hypercalcemia or increase in the blood calcium. It is accompanied by increased deposition of calcium in the soft tissues and increased calcium excretion in the urine. A high intake of calcium is not, in itself, a causative factor. One of the situations in which hypercalcemia occasionally occurs is the milk-alkali syndrome in which patients with peptic ulcers have used excessive amounts of readily absorbed alkalis together with large amounts of milk over a period of years. Vomiting, gastrointestinal bleeding, and an increase in blood pressure accompany the hypercalcemia in these patients. Hypercalcemia occurs in persons who ingest

Early classifications listed two groups of vitamins: fat soluble and water soluble. This classification is still used although within each of the classes the vitamins differ widely in their properties, functions, and distribution. Vitamins were first named for their curative properties and were given a convenient letter name according to the order of their discovery; for example antiscorbic vitamin or Vitamin C [91].

2.4 THE IMPORTANCE OF VITAMINS A, C AND E DURING INFANCY

2.5.1 Vitamin A

2.5.2.1 Introduction

The ancient Egyptians, and later the Greeks, treated nightblindness by applying the juice squeezed from cooked liver to the eyes. However, the active component, vitamin A, was not identified until the beginning of the nineteenth century [92, 93]. In 1913 McCollum and Davis of the University of Wisconsin [94] and Osborne and Mendel of Yale University [95] independently discovered that rats consuming purified diets with lard as the only source of fat failed to grow and developed soreness of the eyes. When butterfat or ether extract of egg yolk was added to the diet, growth resumed and the eye condition was corrected. The term fat-soluble A was applied by McCollum to the organic complex present in two ether extracts that were necessary for normal growth. A few years later Steenbock, at the University of Wisconsin, [96] demonstrated that the yellow pigments in plants, the carotenes, had vitamin A activity. Because these carotenes and certain other carotenoid compounds can be converted to vitamin A in the body, they are now often referred to as precursors of vitamin A or as

provitamin A [97]. In its pure form vitamin A is a pale yellow crystalline compound. It is soluble in fat and fat solvents. It is insoluble in water, but water-miscible forms are available for use in pharmaceutical products and food fortification. Vitamin A is relatively stable to heat and has an alkaline characteristic. It is unstable to light and acid and is easily oxidised. Rapid destruction occurs with exposure at high temperatures in the presence of air, with ultraviolet irradiation, or in rancid fats. The ultimate source of all vitamin A is the carotenoids, which are synthesised by plants. Animals, in turn, and humans as well, convert a considerable proportion of the carotenoids in the foods they eat into vitamin A. The carotenoids are dark red crystalline compounds that give a deep yellow coloration to plants such as carrots and sweet potatoes [97].

2.5.2.2 Function

Although the existence of vitamin A has been known for over 60 years, its function has not been fully explained. Retinyl esters, retinol, and retinaldehyde are readily converted from one form to the other, but retinoic acid cannot be converted to other forms. Retinoic acid appears to be the active form of the vitamin in some tissues but it cannot function in the visual cycle or support reproduction in most species. Retinoic acid also cannot be stored in the body. The best understood function of vitamin A is related to the maintenance of normal vision in dim light. Retinol and retinoic acid have a marked effect on the differentiation and proliferation of cells [92, 97]. Data from many studies suggest that vitamin A influences the expression of genes or gene products involved in these processes [98]. Vitamin A is required for healthy epithelium, whether covering the body externally or lining the mucous membranes. Because of the rapid turnover of epithelial cells, the need for vitamin A may be related to its role in cell differentiation and proliferation. Another way in which vitamin A influences epithelial tissue is through its role in the synthesis of constituents of mucus, such

as the mucoproteins. The mucus secretions maintain the integrity of the epithelium, especially the membranes lining the eyes, the mouth, and the gastrointestinal, respiratory, and genito-urinary tracts. These membranes provide resistance to bacterial invasion, and tissues weakened by a lack of vitamin A are more susceptible to infection. However, large intakes of vitamin A exceeding amounts needed for normal functions, will not confer additional protective benefits against infection [92, 97, 99, 100, 101]. Vitamin A is essential for normal skeletal and tooth development. With a deficiency of vitamin A bones do not grow in length and the normal remodeling process does not take place. The precise function of vitamin A in these processes is not known but may relate to its role in the synthesis of glycoproteins, in cellular differentiation and proliferation, and in maintenance of the stability of cellular membranes [97].

2.5.2.3 Recommended Daily Allowances

Recommended dietary intakes of vitamin A vary around the world. These are often misinterpreted as being requirements. In fact, they are set higher than known requirements to allow for individual variability, and the allowance varies among countries. The intakes recommended by the WHO/FAO in 1988 for various groups, are 350 μgRE for infants under the age of 1 year, 400 μgRE for children aged 1 to 6 years [102]. Maternal vitamin A levels decrease as blood volume expands during pregnancy. If intake and stores are low, the mother may develop mild eye symptoms which disappear after delivery [103]. However, the foetus is relatively protected from maternal deficiency since serum retinol levels are maintained until hepatic stores are almost fully depleted, and hence the placental supply is usually maintained unless severe deficiency occurs. Retinol stores in the foetal liver increase late in pregnancy [104] but are not large even if maternal vitamin A status during pregnancy was sufficient [103]. Colostrum contains more vitamin A than mature breast milk but the level declines by more

than 50% over several days [103, 105]. Breast milk is the only source of vitamin A for the fully breast-fed infant. The breast-feeding mother clearly needs her own normal requirements plus the amount excreted in her breast milk. Breast milk concentrations of vitamin A are less in developing countries than in developed countries, in some cases being only half of what they should be [103, 105]. Therefore encouraging prolonged breast-feeding is important for general infant health and vitamin A status [92].

2.5.2.4 Vitamin A deficiency

In Africa, vitamin A deficiency is reported to affect 1.3 million children younger than five years of age, and due to low dietary intakes of vitamin A, it is a common problem in developing countries world-wide [99, 106]. The earliest symptom of deficiency is night blindness. More severe deficiency may cause dryness (xerosis) of the conjunctiva and later the cornea, leading to ulceration and scarring with consequent blindness (xerophthalmia) [92]. In some countries, notably India, Bangladesh, Indonesia and the Philippines, it is endemic, while in other countries clinical cases occur only occasionally [92]. It is estimated that throughout the world 124 million pre-school age children are deficient, that this deficiency causes 1.3 to 2.5 million deaths annually, and that the ocular manifestations of vitamin A deficiency cause blindness in half a million children annually [107]. Vitamin A supplementation plays an important role in the prevention and treatment of measles [92]. The joint WHO/UINICEF [108] statement recommends that children diagnosed with measles in areas where vitamin A deficiency is a recognised problem or areas where the case fatality rate is greater than 1%, be given vitamin A immediately. Vitamin A reduces the incidence, severity and complications of measles. It appears that adequate vitamin A status may reduce the severity rather than the incidence of infectious diseases [109]. One mechanism for vitamin A's influence in infectious diseases is that it seems to enhance the

immune function. It has long been known that severe infections, such as measles, can precipitate deficiency signs. Several placebo-controlled studies have indicated that vitamin A supplementation leads to enhanced immune, cellular and humoral responses [92]. Furthermore, a number of studies have found an association between vitamin A levels and hypochromic anaemia. Unlike iron deficiency anaemia, vitamin A-related anaemia does not occur with lowered serum ferritin levels [92]. Vitamin A may affect haemopoesis directly, either by enhancing the differentiation of the erythrocyte or the mobilisation of iron stores, or alternatively vitamin A may be increasing resistance to infection and thus preventing infection related impairment of haemopoesis [110].

2.5.2.5 Vitamin A Excess

On the other hand vitamin A is toxic if ingested in excessive amounts, though, apart from polar bear and seal liver, no food contains concentrations of retinol high enough to cause toxicity. The consequence of acute hypervitaminosis A is a raised intracranial pressure. The resulting symptoms are severe headaches, restlessness or drowsiness, bulging of the fontanelles in infants, anorexia, nausea and vomiting. The symptoms of chronic hypervitaminosis A include headaches, anorexia, pruritis, hepatomegaly and often alopecia, cheilosis and bone pain. Symptoms take several months to develop fully, but are reversible [92, 111, 112]. The adverse effects appear to be due to the excessive quantities of unbound retinyl esters in the blood resulting in non-specific delivery of retinol to the cell surface and subsequent damage to the lysosomes. Reported doses that lead to acute toxicity in infants are 30 mg RE or more in adults [111].

2.5.2 Vitamin C

2.5.2.1 Introduction

Ascorbic acid (Vitamin C) was identified more than 50 years ago as the component in foods that cured scurvy, but much remains to be learned about the extent and manner of its involvement in chemical reactions taking place in the body. Controversial claims about the importance of ascorbic acid in preventing or treating the common cold and cancer have led to new questions regarding the desirable dietary intakes of the vitamin as well as the value and safety of supplements [113]. Scurvy has been known as a diminishing disease since ancient times. In 1747, Dr James Lind, a British physician, tested six remedies on 12 sailors who had scurvy. He found that oranges and lemons were curative, but it took another 50 years before the British navy required rations of lemons or limes on the sailing vessels. During the same period Captain Cook was able to reduce the incidence of scurvy on his seagoing voyages by stocking up on fresh foods and vegetables whenever he was in port and also by including sauerkraut as part of the rations. The sauerkraut kept well and was a good preventive of scurvy. The scientific era of vitamin C began in 1907 when two Norwegian scientists, Holst and Frölich, produced scurvy in guinea pigs. The isolation and chemical nature of vitamin C, or ascorbic acid, was accomplished by Dr. Charles G. King and his co-workers at the University of Pittsburgh and by Dr. Szent-Györgyi of Hungary, in the early 1930's [113].

Ascorbic acid is a white crystalline compound of relatively simple structure, and closely related to monosaccharide sugars. It is synthesised from glucose and other simple sugars by plants and by most animal species. It can be prepared synthetically at low cost from glucose. Vitamin C activity is possessed by two

forms: L-ascorbic acid (the reduced form) and L-dehydroascorbic acid (the oxidised form). The latter is oxidised further with complete loss of activity. Isoascorbic acid, a compound often used as a preservative in foods, appears to have little to no biologic value in humans. Of all vitamins, ascorbic acid is the most easily destroyed. It is highly soluble in water. Heat, light, alkalis, oxidative enzymes, and traces of copper and iron accelerate the oxidation of ascorbic acid. Oxidation is inhibited to a marked degree in an acid reaction, and when the temperature is reduced [113].

2.5.2.2 Functions

Vitamin C has many important functions which have recently be well summarised by Gershoff et al., (1993) [114]. One of the principal functions of ascorbic acid is the formation of collagen, an abundant protein that forms the intracellular substance in cartilage, bone matrices, dentin, and the vascular epithelium. In the synthesis of collagen, ascorbic acid is necessary for the hydroxylation of proline and lysine to hydroxyproline and hydroxylysine. This function helps explain the importance of vitamin C in wound healing and the ability to withstand the stress of injury and infection [113, 115]. Vitamin C significantly increases neutrophil chemotaxis and this suggests a possible role for vitamin C in neonates with suspected sepsis. Vohra et al., (1990) pointed out that vitamin C also improves the migration of neutrophils in situations where abnormalities of neutrophil function occur, including Chediak-Higashi syndrome, chronic granulomatous disease and lazy leukocyte syndrome [116]. Ascorbic acid may also play an important role in other hydroxylation reactions. Conversion of tryptophan to serotonin, an important neurotransmitter and vasoconstrictor, and formation of norephinephrine from tyrosine, involve hydroxylation reactions that may utilise ascorbic acid. These reactions may explain some of the abnormalities in vascular and neurologic activity, observed in persons deficient in the vitamin.

Conversion of cholesterol to bile acids is another hydroxylation reaction that may require vitamin C [113]. Ascorbic acid is an important antioxidant and thus has a role in the protection of vitamins A and E and polyunsaturated fatty acids from excessive oxidation. Ascorbic acid enhances iron absorption by reducing ferric iron to ferrous iron, the form which is absorbed most efficiently. It may also bind with iron to form a complex that facilitates transfer of iron across the intestinal mucosa. Evidence also exists that vitamin C plays a role in biosynthesis of mucopolysaccharides, microsomal drugs metabolism, leukocyte function and synthesis of anti-inflammatory steroids by the adrenal glands [47]. It does not appear that vitamin C functions as a coenzyme in these reactions or the hydroxylation reactions described above [113].

2.5.2.3 Recommended Daily Allowance

As little as 10 mg ascorbic acid will prevent scurvy. This level may be regarded as a minimum requirement, but it does not ensure fully satisfactory tissue levels. Each day the adult male removes about 30 mg ascorbic acid from body stores. The recommended allowances have been set at 35 mg for infants and 45 to 50 mg for children [51]. During infections such as tuberculosis, rheumatic fever, pneumonia and severe stress as in the case of burns, the ascorbic requirements are greatly increased [113]. Human milk will meet the ascorbic acid needs of normal infants. Formula-fed infants require supplementation if the formula itself is not fortified. When increased amounts of protein are given, infants require additional amounts of ascorbic acid for the metabolism of tyrosine [117].

2.5.2.4 Vitamin C Deficiency and Excess

In a Nation-wide Food Consumption Survey the average intakes of ascorbic acid were considerably higher in 1977 than in 1965 due to the fortification of beverages and other foods with vitamin C, and an increased consumption of citrus fruits and juice [118]. In spite of high average intakes, substantial percentages in a few sex-age categories had intakes of ascorbic acid that might be considered low. Intakes were poorest in women aged 19 to 22 years, with 43% of the group consuming less than 70% of the recommended allowance for vitamin C [119]. A deficiency of ascorbic acid results in the defective formation of the intercellular cement substance. Fleeting joint pains, irritability, retardation of growth in the infant or child, anaemia, shortness of breath, poor wound healing, and increased susceptibility to infection are among the signs of deficiency, but none of these can establish a diagnosis. A dietary history, the concentration of ascorbic acid in the blood plasma and in the white blood cells, and a measure of the excretion of a test dose in the urine help to establish the diagnosis [113]. On the other hand high concentrations of vitamin C can also be harmful. For instance, Silvers et al., (1994) [120] inhibited the use of vitamin C supplements to improve leukocyte function in pre-term infants. A high concentration of vitamin C can lead to potentially harmful pro-oxidant side effects.

2.5.3 Vitamin E

2.5.3.1 Introduction

Evans and Bishop established the fact that a fat-soluble factor was necessary for reproduction in rats. They showed that absence of vitamin E, or the anti-sterility factor, as it was designated, led to irreparable damage of the germinal epithelium in male rats, and female rats which had diets deficient in vitamin E were unable

to carry their young to term. In severe deficiency the foetus dies and is reabsorbed completely. In the female the damage is not permanent; that is, normal reproduction could again take place if the diet is once more adequate in this factor. The name tocopherol was suggested for this factor based on the Greek words *tokos* meaning "*birth*" and *phero* "*to carry*". The ending –ol indicates that the substance is an alcohol [121]. The biological importance of vitamin E is largely related to its important antioxidant properties. Vitamin E (alpha-tocopherol) is the body's principal lipid soluble non-enzymatic antioxidant [122].

Vitamin E is a generic term for a group of lipid-soluble compounds, the tocopherols and tocotrienols that possess varying degrees of vitamin activity. Alpha-tocopherol is the most active of these compounds. The tocopherols and tocotrienols differ in the chemical structure of their side chains. High temperatures and acids do not affect the stability of vitamin E, but oxidation takes place readily in the presence of rancid fats or lead and iron salts. Decomposition however takes place in ultraviolet light [121].

2.5.3.2 Functions

The metabolic roles of vitamin E are poorly understood. Its principal role appears to be as an antioxidant. By accepting oxygen, vitamin E helps to prevent the oxidation of polyunsaturated fatty acids and phospholipids, thereby helping to maintain the integrity of cellular membranes. As a constituent of the enzyme glutathione peroxidase, selenium shares a role with vitamin E in preventing destruction of lipids by oxidation. In animal experiments selenium has been shown to prevent some of the symptoms associated with vitamin E deficiency. Vitamin E has a sparing effect on vitamin A by protecting it against oxidation [121].

2.5.3.3 Vitamin E and Pre-term infants

One of vitamin E's most important functions is in conditions occurring in pre-term low-birth-weight infants. Pre-term infants are thought to be relatively vitamin E deficient at birth due to their low plasma vitamin E levels. This is thought in part to be due to the placental barrier, which exists for the lipids and thereby also for fat-soluble vitamins. In addition, the infant has low concentrations of low-density lipoporoteins (LDH) at birth and therefore a reduced capacity to transport vitamin E to the tissues. However, while serum levels may be low, vitamin E is primarily tissue bound and may be present in adequate amounts in cell membranes, at least initially [122]. Miyake et al., (1991) have looked at the peroxidizability of red cell membranes in the term neonate using as a model the red cell membrane depleted of iron (a potent pro-oxidant) [123]. Tocopherol levels were similar in cord, adult and maternal red cell membranes providing effective protection from oxidation. Once tocopherol is depleted, however, peroxidation proceeds at a faster pace in foetal cells because of high levels of polyunsaturated fatty acids (PUFAs), arachidonic acid and docosahexanoic acid, in neonatal cell membranes. Mino, (1992) hypothesised that a combination of lower levels of tocopherol, high levels of PUFAs in cell membranes and exposure to oxygen radicals in infants on mechanical ventilation make the pre-term infant highly susceptible to oxidative stress [124]. The retina has certain features which make it particular susceptible to oxidative damage such as a plentiful blood supply and a high rate of oxidative metabolism. Also, the membranes of the round outer segment contain at least 65% PUFAs, the highest concentration of PUFAs in any tissue. The pre-term infant also moves from a relatively hypoxic to a relative oxygen rich environment at birth, and is frequently given supplemental oxygen for long periods of time. Oxidative damage to sensitive migrating spindle cells is thought to prevent the formation of normal retinal vasculature and to lead to retinopathy of prematurity (ROP). Penn et al., (1992) found that maternal dietary

manipulation with vitamin C did not reduce retinal vascular damage, but supplementation with vitamin E did increase the levels in serum and this was associated with less retinal vaso-obliteration [125]. Despite good theoretical reasons for postulating that vitamin E supplementation will prevent ROP, this has been difficult to establish in practice.

Law et al., (1990) [126] estimate that at best prophylactic vitamin E would prevent neurological disability in at most 2.5% of all treated infants. Vitamin E supplementation is also recommended for heterozygous or, indeed, homozygous infants for alpha-1-antitrypsin deficiency. Deficiency of vitamin E in the pre-term neonate can cause a haemolytic anaemia with trombocytopenia [122]. So, just by naming a few functions of vitamin E in pre-term low-birth-weight infants it is clear that vitamin E play an important role in infant health. Many other functions have been proposed for vitamin E as well but they have not received wide acceptance.

2.5.3.4 Recommended Daily Allowances

Intakes of Vitamin E recommended by the Food and Nutrition Board are expressed as alpha-tocopherol equivalents in which 1 mg d-alpha-tocopherol = 1α-TE [51]. The recommended intakes of vitamin E are 3 to 4 mg α-TE during the first year of life [121]. The plasma vitamin E content of newborn infants is low but rises rapidly to normal levels by the end of the first month [117]. The need for vitamin E is normally met by human and cow's milk. Vitamin E supplementation may be required if formulas have an increased level of polyunsaturated fatty acids and are also fortified with iron. The presence of iron increases lipid peroxidation, reduces the available level of vitamin E and leads to anaemia, reticulocytosis, and thrombocytosis. The need for vitamin E is higher when the intake of polyunsaturated fatty acids increases. Since the principal source of vitamin E is from vegetable oils and margarines, the increased intake of linoleic

acid from these fats accompanies the satisfactory intake of vitamin E. At the present time there is no fixed ratio of vitamin E to polyunsaturated fatty acids that can be recommended [127].

2.5.3.5 Vitamin E Deficiency

Vitamin E deficiency is extremely rare. Changes occurring in severe deficiency include increased haemolysis of red blood cells, creatinuria, and deposition of brownish ceroid pigment in smooth muscle. Recent evidence established that vitamin E deficiency is a cause of the impaired neuromuscular function sometimes seen in patients with disorders that interfere with absorption or transport of the vitamin [128, 129]. Symptoms include poor reflexes, impaired locomotion, and decreased sensation in the hands and feet, and changes in the retina. This evidence indicates that vitamin E has an important role in the maintenance of normal neurologic structure and function [121]. Premature and low-birth-weight infants show an extremely low level of tocopherol in the serum and increased haemolysis of red blood cells. When such infants were fed a diet high in polyunsaturated fat and low in vitamin E, they developed a syndrome characterised by oedema, skin lesions, and haemolytic anaemia. These abnormalities disappeared when vitamin E supplements were given [130]. Evidence of vitamin E deficiency has also been observed in children with cystic fibrosis. Vitamin E has been recommended for such widely varying conditions as heart disease, muscular dystrophy, acne, ulcers, habitual abortion, disorders of the menopause, and sexual impotence. Objective studies, however, have failed to support most of these exaggerated claims. Vitamin E treatment may lead to improvement in some patients with intermittent claudication, a condition that causes pain in the calves of the legs while walking. Administration of vitamin E also appears to be beneficial in preventing retinal damage in premature infants

receiving oxygen therapy [121]. However, further research is needed to evaluate these and other therapeutic uses, which have been proposed for vitamin E [128].

2.6 PROTEINS

2.6.1 Introduction

In 1938 a Dutch chemist, Mulder, described certain organic material that is "unquestionably the most important of all known substances in the organic kingdom. Without it no life appears possible on our planet. Through its means the chief phenomena of life are produced". Berzelius, a contemporary of Mulder, suggested that this complex nitrogen-bearing substance be called protein from the Greek word meaning to "take the first place" [131, 132].

Protein is now retained as a group name to designate the principal nitrogenous constituents of the photoplasm of all plant and animal tissues: proteins are necessary for the synthesis of all body tissues and for innumerable regulatory functions [131]. Proteins are extremely complex nitrogenous organic compounds in which amino acids are the basic structure. They contain the elements carbon, hydrogen, oxygen, nitrogen, and, with few exceptions, sulphur. Most proteins also contain phosphorus, and some specialised proteins contain very small amounts of iron, copper, and other inorganic elements. The presence of nitrogen distinguishes protein from carbohydrate and fat. Proteins contain an average of 16% nitrogen and have a molecular weight that varies from 13, 000 or less to many millions. Thus, the protein molecule is much larger than those of carbohydrates and lipids. The large protein molecules from colloidal solutions do not readily diffuse through membranes [131]. Some estimates place the number of functioning proteins in the human body at more than 100,000. Each protein is synthesised to perform a specific function, and another generally cannot assume

that function. Haemoglobin, insulin, albumin, myosin, keratin, collagen, retinene, and carboxylase are only a few examples of proteins that differ widely in their structure, properties, and functions. Moreover, specific proteins of one species differ from those of another. For example, insulin's from pigs, horses, and sheep are distinct because of differences in one or two amino acids in the peptide chains [131].

The body requires 20 or so amino acids for the synthesis of its proteins. In 1915 Osborne and Mendel observed that rats failed to grow or even survive if some amino acids were omitted from the diet, but that the elimination of other amino acids had no such harmful effects [131]. Later work by others, especially Dr. William C. Rose [133], established that this was also true for humans. Thus, amino acids came to be classified as essential or indispensable and nonessential or dispensable. Essential amino acids are those that cannot be synthesised in the body at a rate sufficient to meet body needs. Histidine, for which the requirement by adults has long been uncertain, is now believed to be essential [134]. Thus, humans require nine essential amino acids. Methionine, an essential amino acid, can be converted to cystine, but cystine cannot be converted to methionine. Likewise, phenylalanine can be converted to tyrosine, but tyrosine cannot be converted to phenylalanine. When cystine and tyrosine are present in the diet, the requirements for methionine and phenylalanine are reduced. Thus, cystine and tyrosine are sometimes classified as semi-essential. Nonessential or dispensable amino acids are those that the body can synthesise from an available source of nitrogen and a carbon skeleton. Typical mixed diets contain ample amounts of both essential and nonessential amino acids. Based on their content of amino acids, foods are often classified as sources of complete, partially complete, or incomplete proteins [131].

2.6.2 Functions

a) Maintenance and Growth

The average weight gain in the first year of infancy is 7.0 kg, with approximately half of it occurring during the first four months of life [135]. To achieve this dramatic growth spurt, an estimated 61,000 kcal is required between birth and four months [135]. About one third of this calorie consumption is expended solely for growth [135, 136]. At an age of about five months, the infant enters a transitional period characterised by a decreased rate of growth, increased level of caloric expenditure for physical activity, developmental readiness for transitional foods, and digestive capacity. Although total calorie and nutrient requirements continue to increase as a result of growth, the decreasing need for energy and protein per unit of body weight reflects the progressive slowing of the growth rate [137]. Proteins constitute the chief solid matter of muscles, organs, and endocrine glands. They are major constituents of the matrix of bones and teeth; skin, nails, hair; blood cells and serum. In fact, every living cell and all body fluids, except bile and urine, contain protein. The first need for amino acids, then, is to supply the materials for the building and the continuous replacement of the cell proteins throughout life, therefore proteins play an important role throughout infancy where the main aim of the infant is to get "big and strong" [131].

b) Regulation of Body Processes

Body proteins have highly specialized functions in the regulation of body processes. Some of these can be classified as follows [131]:

Nucleoproteins contain the blueprint for the synthesis of all body proteins. **Catalytic proteins**, the enzymes, facilitate each step of digestion, absorption,

2.6.3 Daily Recommended Allowances

On a weight basis, it will be noted that the infant requirements are several times higher - a fact that one would expect in view of the high rate of tissue synthesis during infancy. For adults, only 20% of the total nitrogen requirement need to be supplied by essential amino acids [131]. The infant adds about 3.5 g protein daily to his or her body during the first 4 months and thereafter about 3.1 g per day for the rest of the year. This results not only in a net increase in body size but also in an increase of the percentage of body protein from 11 to 14.6 [51, 138]. For infants, essential amino acids should furnish about 35 % of the total nitrogen requirement [51]. The protein equivalent for this nitrogen loss is 0.34 g per kg (0.054 x 6.25). To allow for individual variability, an intake of 0.45 g protein per kilogram should cover the daily nitrogen losses assuming the intake of protein of maximum biologic availability [138]. The allowances for infants are based on human milk as the source of protein [131]. Human milk furnishes about 2 to 2.4 g protein per kg per day during the first month of life, but by the sixth month this has fallen to 1.5 per kg per day. In the recommended allowances this lower level has been adjusted to 2.0 g per kg for the second 6 months in order to allow for less deficiency of protein in a mixed diet [138]. An additional 30 g protein per day during pregnancy will take care of the growth of the foetus and of maternal tissues. During lactation, an increase in the protein allowance of 20 g is satisfactory for the production of an upper limit of 1,200 ml milk per day [131].

2.6.4 Protein Excess

In the case of a liberal protein intake, most people in the United States far exceed the recommended allowances, and there is little evidence that such intakes by healthy persons are harmful. Arctic explorers, who consumed diets consisting mainly of meats for several years, showed no pathologic effects.

Consumption of a high-protein diet is wasteful, however. Since the body does not store protein in the sense that it stores other nutrients, the excess amino acids are de-aminized and then enter the common pathway of metabolism for fat and carbohydrate. Protein foods thus entail more work for the liver and kidneys and also cost more in the marketplace. High-protein diets are undesirable in some circumstances. As the protein intakes increase, the urinary losses of calcium also increase. With an increase in nitrogenous wastes there also is an increased need for water to excrete them. Premature and very young infants do not have the ability to excrete the additional nitrogenous wastes incurred from high-protein formulas. Persons who have chronic renal failure also have less ability to excrete nitrogenous wastes, and blood urea levels are elevated when protein is fed in excess of synthetic needs. Diets that provide a high proportion of protein from animal sources also furnish considerable amounts of saturated fats and cholesterol, two factors that may lead to elevation of serum cholesterol and low-density lipoproteins [131].

2.6.5 Protein deficiency

Results obtained in both the Nation-wide United States Food Consumption Survey and the first Health and Nutrition Examination Survey (HANES) showed that the mean intakes of protein for each age-sex category exceeded the recommended allowances [139, 140]. In the Nation-wide United States Food Consumption Survey, 3 % of the population had protein intakes of less than 70 % of their respective recommended intakes. The HANES data showed that protein intakes were below standard for 12 % of children aged 1 to 5 years. The protein intake per 1,000 kcal was 38 to 40 g and varied little regardless of income. Thus, the failure to ingest the recommended amount of protein appeared to be related to the inadequate quantity of food. The lower intakes of protein were not correlated with lower blood protein levels. In fact, not a single case of severe

protein deficiency showing clinical signs and a serum albumin below 2.5 g was found in the HANES survey. Clinicians sometimes do encounter persons with protein deficiency. Among these are young children who have had diets grossly deficient in protein and calories because of parental abuse or ignorance. A protein intake that fails to meet the individual requirements leads first to depletion of tissue reserves and then to a lowering of the blood protein levels. Nutritional oedema is a clinical sign, but it does not appear until substantial depletion of tissue reserves has taken place and the serum albumin level is decreased. It must be differentiated from oedema that is caused by fluid-electrolyte imbalance in cardiac failure. Protein deficiency sometimes becomes abruptly evident when an infection, injury, or surgery occur [131].

2.6.6 Protein-Calorie Malnutrition (PCM)

On a world-wide basis the shortage of protein is second only to the shortage of calories. Protein-calorie malnutrition, also known as protein-energy malnutrition (PEM), is a broad term that encompasses kwashiorkor and marasmus together with milder stages of these diseases. Literally millions of infants and young children are victims of these diseases in Asia, Africa, Central America, the West Indies and South America. Many of the children who survive are unable to achieve their full physical growth and development. Even more serious is the danger that the most severely malnourished may be retarded in their mental development, and that this retardation may be irreversible [131]. Kwashiorkor (meaning "the displaced child") occurs in children shortly after weaning, usually between the ages 1 and 4 years, and is characterised by growth failure, skin lesions, oedema, and changes in hair colour. The liver is extensively infiltrated with fat. The principal dietary defect is a lack of good quality protein in the foods available to the child when he or she is weaned. Marasmus (from a Greek word meaning "withering") is usually seen at a somewhat earlier age than kwashiorkor

and is caused by a deficiency of both protein and calories. Growth failure is even more severe than in kwashiorkor, but oedema is usually absent.

2.7 CARBOHYDRATES

2.7.1 Introduction

In our culture "starchy foods" and "sugars" are regarded by some as undesirable or unnecessary dietary components that must be eliminated or avoided if a person wishes to lose weight or practise good nutrition. For most people in the world, however, high-starch cereal grains not only provide most of the energy in the diet but supply an appreciable portion of the protein as well. From 45 to 80 % of the energy requirement of people throughout the world is met by consumption of starches and sugars stored in the leaves, stems, fruits, seeds, and roots of plants. The ability of plants to harness solar energy in the form of usable carbohydrates is basic to the continuation of life by all species [140].

Carbohydrates are simple sugars or polymers of sugars such as starch that can be hydrolysed to simple sugars by the action of digestive enzymes, or by heating with dilute acids. Like thousands of organic compounds, they contain carbon, hydrogen and oxygen. Generally, but not always, the hydrogen and oxygen are in the proportions to form water, hence the term carbohydrate [140].

2.7.2 Classification of Carbohydrates

The simplest forms of carbohydrates are the *monosaccharides*, or simple sugars. Although naturally occurring simple sugars may contain three to seven carbon atoms, only the hexoses are of dietary importance. The most commonly known monosaccharides are glucose, galactose, fuctose, and mannose. The

polysaccharides are carbohydrates known as complex compounds composed of many molecules of simple sugars. They have a relatively high molecular weight, are amorphous rather than crystalline, are not sweet, are insoluble in water, and are digested with varying degrees of completeness. Starches, dextrins, glycogen, and several indigestible carbohydrates are of nutritional interest [140]. The group of carbohydrates relevant in this study is the *oligosaccharides*. This group of carbohydrates is composed of 2 to 10 monosaccharides joined together. The most common oligosaccharides are sucrose, lactose and maltose. These substances are disaccharides, or double sugars, formed from the combination of two hexoses with loss of one molecule of water. They are water soluble, diffusible, and crystallizable and vary widely in their sweetness. They are split into simple sugars by acid hydrolysis or by digestive enzymes [140]. The most important oligosaccharide in this study is lactose and most emphasis will be placed on this.

2.7.2.1 Lactose

2.7.2.1.1 Introduction

Lactose, or milk sugar, is produced by mammals and is the only carbohydrate of animal origin of significance in the diet. It is about one sixth as sweet as sucrose and dissolves poorly in cold water. The concentration of lactose in milk varies from 2 to 8 %, depending on the species of animal [140].

2.7.2.1.2 Functions

Lactose has several functions in the gastrointestinal tract, which are most important during infancy. It promotes growth of desirable bacteria, some of which are useful in the synthesis of B-complex vitamins. Lactose also enhances

the absorption of calcium. It is undoubtedly no accident of nature that milk, which is the outstanding source of calcium, is also the only source of lactose [140].

2.7.2.1.3 Daily Recommended Allowance

The low-carbohydrate diet of Eskimos and the high-carbohydrate diet of many people in Far Eastern countries indicate that humans can be healthy with wide variations in carbohydrate intake. This wide variation is compatible with health because of the interrelations with fatty acids and amino acids in meeting the energy needs of the body. The minimum requirement for carbohydrate is not known, but at least 50 to 100 g of carbohydrates daily is desirable to prevent ketosis [51]. Intakes considerably above this level are customary and desirable. The revised Dietary Goals recommend that complex carbohydrates and naturally occurring sugars provide about 48 % of the total caloric intake and that refined sugars provide no more than 10 % of the energy requirement [141]. Furthermore, milk is the only animal food that contributes to the daily carbohydrate intake [140].

2.7.2.1.4 Lactose Intolerance: Malabsorption syndrome

The lactose tolerance test is used in suspected lactase deficiency. Administration of lactose, 2 g per kg body weight, or a maximum of 50 g, is followed by determination of blood glucose levels for 2 hours [142]. Lactose malabsorption is indicated if the blood glucose level rises less than 26 mg per dl. Symptoms of abdominal distension, cramping, and diarrhoea may occur following ingestion of lactose in persons with lactose intolerance [142]. The inability to utilize lactose may be due to lactase deficiency or may be secondary to conditions that produce alteration in absorptive surfaces. In the absence of lactase lactose is not hydrolyzed to glucose and galactose. The accumulation of lactose in the intestine causes fermentation, abdominal pain, cramping and diarrhoea.

Failure to gain weight is an important symptom in infants. Congenital lactose intolerance is a rare disorder characterized by absent brush border lactase activity. Symptoms occur following ingestion of milk by the infant. A strict lactose-free formula is used, several commercial products being available. All products containing lactose in any form whatsoever are rigidly excluded [143]. Intestinal lactase activity is normally high during infancy but declines after weaning, to low levels in adults. The decline in lactase activity is determined by autosomal recessive mechanism and is not influenced by dietary lactose intake [144]. Throughout most of the world the majority of adults are unable to digest lactose, and they develop symptoms of distension, cramping, and diarrhoea following its ingestion. These individuals, who have no history of gastrointestinal disease or childhood intolerance to milk, are described as having primary lactose intolerance. In the United States, from 60 to 95 % of adult blacks, American Indians, Jews, Mexicans, Americans, and Orientals are lactose malabsorbers compared to 5 to 15 % of whites [143].

Several hypotheses have been proposed to explain the differences in ability to utilize lactose among various ethnic groups. One theory holds that a genetic mutation occurring as a result of some selective advantage may permit high levels of lactase to persist into the adult years in certain populations, primarily those from northern and western Europe [145]. Adults with primary lactose intolerance can usually tolerate the amounts of milk in many prepared foods such as bread, lunchmeats, and even cream soups and cream sauces provided that the lactose source is spaced through the day. Those who experience classical symptoms following excessive milk or lactose ingestion, can be kept asymptomatic by limiting their intake of milk products. A controlled lactose diet that restricts only obvious sources of lactose is used. The quantity of lactose allowed is a matter of individual tolerance [143]. Several studies with lactose

malabsorbers indicates that subjects experience significantly fewer symptoms following ingestion of lactose-hydrolyzed milk than ingestion of regular milk [145, 146]. A commercially available enzyme which, when added to milk, hydrolyzes about 75 % of the lactose, permits intake of a larger quantity of milk without provoking symptoms. Secondary lactose intolerance is often observed following gastrectomy or extensive small bowel resection, and in celiac disease, sprue, colitis, enteritis, cystic fibrosis, kwashiorkor and malnutrition. In these conditions it may be necessary to omit obvious sources of lactose initially, but a strict lactose-free diet is usually not required [143].

2.8 LIPIDS

2.8.1 Introduction

Fats are the most concentrated source of energy in foods and often supply two fifths or more of the total energy intake in the typical American Diet [147]. Fats (glycerides, sterols and phospholipids) constantly contain fatty acids (FAs), which dictate the structural and metabolic characteristics of the compounds in which they are inserted. The knowledge of their functions represents the basis to define children's needs during the first year of life. The role of some types of FAs, and the advantages of their intake has been documented for pre-term infants, but there is less information on their role in term babies [148]. Infants and young children are a vulnerable group with regard to nutrition, because growth velocity and nutritional demands are highest at this age. Along with these demands, enormous changes in the diet take place during this period; from an exclusive breast milk or formula diet to the customary family food and from the high-fat diet of early infancy to the lower-fat diet of children and adolescents [149].

Lipids are a heterogeneous group of substances that includes fats, oils, and fat-like substances that share the characteristic of being soluble in certain organic solvents such as ether, alcohol and benzene. Like carbohydrates, fats are organic compounds made up of carbon, hydrogen and oxygen, but the resemblance ends there. Fats have a much smaller proportion of oxygen than do carbohydrates and differ in important ways in their structure and properties. Some lipids also contain carbohydrates, phosphates, or nitrogenous components [147].

2.8.2 Classification of Lipids

The major constituent of many lipids is fatty acids (FAs). Fatty acids can be divided into short, medium and long-chain fatty acids. Furthermore they can be "saturated" or "unsaturated". Unsaturated fatty acids can be divided into monounsaturated fatty acids consisting of a single double bond, and polyunsaturated fatty acids consisting of two or more double bonds [147]. The basic classification of lipids include three major groups:

a) Simple Lipids: These are generally esters of fatty acids and alcohol's, although free fatty acids sometimes are also included in this group. The most common esters are combinations of fatty acids with glycerol, a 3-carbon alcohol with three hydroxyl groups. These compounds, also referred to as neutral fats, may contain one fatty acid (monoglyceride or monoglycerol), two fatty acids (diglycerides) or three fatty acids (triglycerides) combined with glycerol. A simple triglyceride is one in which the three fatty acids are the same. A mixed triglyceride is one in which at least two fatty acids are different. Mixed triglycerides account for 98 % of fats in foods and over 90 % of fat in the body [147].

b) ***Compound Lipids:*** These are esters of glycerol and fatty acids, with substitution of other components such as carbohydrate, phosphate, and/or nitrogenous groupings. Phospholipids such as lecithin and cephalin contain a phosphate and nitrogen grouping replacing one of the fatty acids in the molecule. Glycolipids such as cerebrosides contain a molecule of glucose or galactose. Lipoproteins include a variety of lipid molecules bound to protein molecules in order to facilitate transport in the aqueous medium of the blood [147].

c) ***Derived lipids:*** These include alcohol's (glycerol and sterols such as cholesterol); carotenoids; and the fat-soluble vitamins, A, D, E, and K [147].

2.8.3 Functions

All body cells contain some fat and with that in mind it is not strange that fat has such a large variety of functional applications in the human body [147].

2.8.3.1 Body composition

Adipose tissue, which consists principally of triglycerides, is stored in the subcutaneous tissues and in the abdominal cavity. It also surrounds the organs and is laced throughout muscle tissue. The predominant form of adipose tissue is white. Brown adipose tissue occurs in small amounts in the interscapular and axillary regions and at the nape of the neck. It is relatively more abundant in the newborn infant. Cell membranes contain lipids that facilitate the transfer of nutrients [147].

2.8.3.2 Insulation and Padding

The subcutaneous layer of fat is an effective insulator that reduces losses of body heat in cold weather. Excessive layers of subcutaneous fat, as in obesity, interfere with heat loss during warm weather, thereby increasing discomfort. Vital organs such as the kidneys are protected against physical injury by a padding of fat [147].

2.8.3.3 Energy

The primary function of fat is to supply energy. Each gram of fat when oxidised yields approximately 9 kcal, or more than twice as much energy as a gram of carbohydrate or protein. The high density and low solubility of fats make them an ideal form in which to store energy. In fact, not only are fats as such stored in adipose tissue but any glucose and amino acids not promptly utilised are also synthesised into fats and stored [147].

2.8.3.4 Satiety

Closely related to the provision of energy is the satiety value of fats. It is speculated that because fats reduce gastric motility and remain in the stomach longer, the onset of hunger sensations is delayed. Diets that contain generous amounts of fat are sometimes described as "sticking to the ribs," "rich", or "satisfying"; that is, they have high satiety value [147].

2.8.3.5 Palatability

Fats lend palatability to the diet, whether as butter or margarine on bread, meats, poultry, fish and the oils in fruit. They are responsible for the flavours that we enjoy [147].

2.8.2.6 Carriers of Fat-Soluble Vitamins

Dietary fat is a carrier of the fat-soluble vitamins A, D, E, and K. Some fat is also necessary for the absorption of vitamin A and its precursor, carotene [147].

2.8.3.7 Fatty acids, Phospholipids and Cholesterol

In the first year of life FAs have fundamental functions as regards energy, metabolism and structural requirements. Some molecules serve more than others in the elective achievement of these requirements, but it is still not clear to what extent genetic factors and the intrinsic balance of FAs in the diet govern the biological fate of the different molecules. Saturated and monosaturated FAs are mainly responsible for energy, although essential C18 polyunsaturated FAs (EFAs) may take this path too. The medium-chain FAs are a rapidly absorbed available source of energy [150], but may be converted also to longer chain derivatives (such as 16:0) before storage in adipose tissue [151]. The C16-18 molecules, on the other hand, whether exogenous (directly from the diet) or endogenous (synthesised in the liver from shorter chain precursors or from carbohydrates), are taken up mainly into triglycerides and end up in fat deposits [148].

The ratio between saturated and unsaturated molecules affects the lipoprotein metabolism and catabolism. Saturated C12-14 FAs also play a direct role in this

regulation, through their hypercholesterolemic effect [153]. Essential fatty acids (18:2 n-6 and 18:3 n-3) cannot be synthesized in the human body, and must be supplied preformed in the diet. The importance of EFAs lies in their metabolites. The long-chain polyunsaturated essential fatty acid (LCP) derivatives (C20 or longer) play the most important part in membrane quality and hence also in tissue development. The LCPs of the n-3, n-6 and n-9 series accumulate markedly in central nervous system tissues in the first 18-24 months of life [154]. These FAs are not only inserted passively in membrane phospholipids, affecting their fluidity, but also have a vital active role in short and medium-term intra and extracellular homeostasis, modulating neurohormonal information and the synthesis of molecules involved in vasomotor and inflammatory responses. It has been suggested that immune reactions too are influenced by the quality of available polyunsaturated FAs [155]. Indeed, LCP levels were found to be low in the milk of mothers of breast-fed children with atopic eczema [156]. Unlike the pre-term infant, term infants receive increasing amounts of LPCs through the placenta and the umbilical cord in the last weeks of pregnancy [157], and can also count on maturer enzymatic systems. However, in the natural course of events the supply of LCPs would continue in maternal milk. It has been seen experimentally that the enzymatic activity of delta-6 desaturase in brain cells, the rate-limiting step in the synthesis of LCPs, drops as myelinization peaks, while in parallel this enzymatic activity increases in the liver [158]. It is still not clear whether this happens because a continued flow of exogenous molecules is expected or because of endogenous synthesis from the essential precursor in the liver [159]. In any event it seems to be part of the body's overall economy to permit faster uptake into the nerve tissue to cope with rising demand.

Much debated over recent years is the question of cholesterol supply; this is a basic component of the cell membrane and other biologically active molecules. Experimentally, most of the cholesterol employed in forming brain tissue is

synthesized rapidly in situ starting from acetate [160]. The central nervous system of the 7-week human embryo already synthesizes cholesterol [161], and by 3 months of pregnancy only about 20 % still appears to come from maternal sources [162]. Endogenous synthesis thus appears to cover structural requirements fairly promptly, but there are still questions about how exactly the cholesterol supply is involved in short and long-term regulation of the lipoprotein metabolism [163]. A new interpretation based on recent data suggests that exogenous cholesterol serve to facilitate LCP transport to nerve cell membranes as cholesterol esters [164]. Furthermore, cholesterol is a component of cell membranes and furnishes the nucleus for the synthesis of provitamin D, adrenocorticol hormones, steroid sex hormones, and bile salts [147].

2.8.4 Recommended Daily Allowances

The Food and Nutrition Board has not set precise recommendations for either the quality or type of fat that should be included in the diet. Since the energy value of the diet is derived mainly from fat and carbohydrate, any drastic restriction in the one means that the other must be increased in order to maintain caloric equilibrium [147]. During infancy, the growing infant obtains its first fats from its mother's milk or, if this is not available, from a commercial formula. Later, during weaning, the child is gradually introduced to solid foods and fats in condiments. Fats provide 50% of the total calories in milk-fed babies and about 30% after weaning [51]. Each gram of fat deposited is the equivalent of 9 calories of energy compared with a 4-calorie equivalent for each gram of protein or carbohydrate. Thus, during periods of maximum fat deposition, such as the late foetal period, early infancy, and adolescence, the caloric demand is greatest, reflecting the high-energy cost of growth associated with increased fat deposition [147].

The desirable level of cholesterol intake is not known. Human milk contains significantly more cholesterol than commercial formulas made with vegetable oils. Cholesterol is required for the synthesis of bile salts, the development of the central nervous system, and the elaboration of enzymes that control the body's synthesis of cholesterol [165].

2.8.4.1 Recommended Daily Allowance of Essential Fatty Acids

Each diet must provide some linoleic acid, an essential fatty acid. The signs of deficiency are prevented when 1 to 2 % of dietary calories are provided by linoleic acid. For infants, a formula that supplies 3% of calories as linoleic acid is recommended. This level of linoleic acid is also satisfactory for persons who have a relatively low fat intake (less than 25% of calories).

2.8.5 Conditions caused by excessive Fat intake

2.8.5.1 Obesity

Some studies have shown that infants who remain in the 90th percentile of weight during the first year are more likely to become obese in later life than are infants whose weight remains within the 25th to 75th percentile. According to these studies, adipocytes increase at an abnormal rate until adolescence. Thereafter, the number of cells remains fixed throughout life, but the size of each cell increases enormously [166]. When weight is lost the size of the cells decreases but the cells themselves remain intact. A recent study showed a close correlation between excessive weight gains at 6 weeks, 3 months, and 6 months, and overweight and obesity at 6 to 8 years [167]. More recently it has been suggested that the fat cell theory is speculative, and that fat infants will not necessarily remain fat [168].

Infantile obesity must be approached individually [169]. It is not necessarily caused by bottle-feeding instead of breast-feeding, or the early introduction of solid foods. Nor is it caused by a mother's inability to heed the baby's satiety signals. Some mothers, however, may urge the baby to finish the bottle, thinking that the baby will be undernourished if the amounts recommended by the nutritionist or pediatrician are not consumed. Other mothers may give food to their babies whenever they cry to keep them quiet. Babies who are inactive will have lower energy requirements, and their caloric intake needs to be monitored. Non-fat milk formulas should not be used in the first year. Any solid foods that are given should be rich in nutrients but restricted in the amounts of fat that they contain. The obese infant should be monitored for gains in height and weight, the objective being to allow the baby to "slim out" and to maintain a more moderate rate of gain. Inactivity in an infant is a tendency to be watched in the forthcoming years when the child needs special encouragement to activity [166].

2.8.5.2 Coronary Heart Disease

The diet-heart disease controversy has existed for about a quarter of a century and is destined to continue for many more years [147]. Atherosclerosis is a disease process that begins early in life with the formation of cholesterol-containing plaques on the inner walls of the arteries. Smooth muscle fibres and connective tissue infiltrate these plaques. Gradually the lumen through which the blood flows is narrowed, and the heart must work harder to pump the blood through the arteries. If the condition is severe, the lumen can be closed depriving the given tissue of its blood supply. All children and young adults have fatty streaks in the walls of the aorta. Under some circumstances not yet fully understood these fatty streaks progress to form the disease lesions. A serum cholesterol level above 225 mg per dl is an increased risk for coronary heart disease [147].

Dietary factors may be important in atherosclerotic cardiovascular disease because of effects other than alteration of blood cholesterol levels. It is speculated that diets containing large amounts of marine oils decrease blood clotting and platelet aggregation and may explain the low prevalence of coronary heart disease in Greenland Eskimos. The major factor in the oil contributing to these effects is believed to be eicosapentaenoic acid, a 20-carbon fatty acid having five double bonds, with the first double bond at the third carbon [170, 171]. Dietary modification has been widely used for patients who have been identified as being at high risk, such as obese patients. The results have not always been as successful as anticipated. But one may argue that dietary adjustments, especially a reduction in fats, if they are to be effective, must begin early in life, not after pathologic changes have occurred [147].

2.9 MICROBIOLOGICAL CONTENT

2.9.1 Microbiological Contamination of Weaning Feeds

2.9.1.1 Introduction

The true complexity of our surroundings was unappreciated until the existence of micro-organisms was first revealed with the microscope. Indeed, the use of microscopy has helped to define the relationships among a diversity of organisms, ranging from the smallest viruses consisting of a few proteins and minimal genetic information to multicellular parasites almost 10 metres in length. Microbiology, the study of living organisms, is traditionally subdivided into the examination of viruses, bacteria, fungi, and parasites. As the science of microbiology increased in sophistication, the characterisation of organisms evolved from morphological descriptions to analysis of their phenotypical and genotypical properties. Despite these advances, the initial recognition and

identification of organisms are still determined most commonly by the morphological appearance of the microscopic cells and macroscopic colonies [172].

The fruit or vegetable is harvested, milk is drawn, eggs are gathered, fish and other products are obtained from natural waters, and animals are collected and slaughtered, all carrying contaminating micro-organisms from natural sources. In most instances, with the commencement of human handling further contamination begins, and it continues while the product is being handled and processed. The processor attempts to clean and sanitise equipment coming in contact with food to reduce contamination from that source and to employ packaging materials that will not add significant contamination. As has been stated, the term "sanitise" is used rather than "sterilise" because, although an attempt is made to sterilise the equipment, i.e. free it all of all living organisms, sterility is seldom attained [172]. Many foods are a rich medium for micro-organisms, encouraging their growth and eventually the production of toxins. Under favourable conditions, a single bacterium can multiply to 500 million bacteria in 10 hours. Bearing in mind that the minimum infective dose of pathogens varies from a few (10 or less) to as many as 10^4 or 10^6, the survival of even a small number of pathogens in freshly prepared food can become health threatening, particularly if the food is stored at ambient temperatures for several hours or overnight, as is often the case. For some micro-organisms, cooked food is an even more favourable milieu than raw foods, since cooking reduces the number of competitive flora. If food is contaminated by such micro-organisms after cooking, e.g., by contaminated hands, and is then stored at inappropriate temperatures for an extended period of time ($\geq$ 4 hours), it is more likely to cause disease [14].

Infants and young children are very susceptible to food-borne diseases and, if they consume contaminated foods, are likely to contract infections or

intoxications leading to illness and often death. While food-borne diseases may be caused either by chemical or biological agents, those of biological origin are of specific interest, since they are responsible for a considerable proportion of diarrhoeal diseases. However, it should be noted that infants and children are sensitive to various chemical contaminants of foodstuffs, e.g. lead, and such contamination is also a major public health concern in several communities [173].

Food-borne diseases can cause severe and/or long-lasting damage to health, including acute, watery and bloody diarrhoeas (leading to severe dehydration or ulceration), meningitis, as well as chronic diseases affecting the renal, articular, cardiovascular, respiratory, and immune systems [174, 175, 176]. However, the most serious implications of food-borne infections are their effects on nutritional status. The association between diarrhoeal diseases and malnutrition has been the subject of extensive studies [177]. Despite the complex nature of the interaction between infectious diseases and malnutrition it is generally accepted that infectious diseases can affect children's growth once weaning is initiated [178, 179, 180].

2.9.1.2 Sources of contamination

The sources of food contamination are numerous: nightsoil, polluted water, flies, pests, domestic animals, unclean utensils and pots, dirty hands, a polluted environment caused by the lack of sanitation, domestic animal droppings, dust and dirt, etc. Raw foods themselves are frequently the source of contaminants, since some foodstuffs may naturally harbour pathogenic agents or have been obtained from infected animals. Unclean pots, cooking utensils, baby bottles, teats, etc., are potential sources of contamination. Although the risk factors of food-borne diseases are well known, their prevention may be impeded or hampered by many social and cultural constraints. Social infrastructure,

ignorance, incorrect beliefs and practices, taboos, poverty, insufficient food, lack of safe water and sanitation, shortage of fuel and time are some of the many factors that aggravate the situation [14].

Evidence that flies contribute to the transmission of the dairrhoeal disease agents has been reviewed. Many pathogens that cause diarrhoea in humans, including *V. cholerae*, *Shigella* spp., campylobacter, *E. coli*, poliovirus, and *Entamoeba histolytica*, can be recovered from flies. Many pathogens can be recovered from flies, and many pathogens can survive on the integument of flies for up to 10 days. Pathogens can also be carried in the gut of flies and deposited when they regurgitate or deposit excreta [14]. Several studies have also reported the presence of infected domestic animals on household premises [181], presenting an additional risk factor for the contamination of food. Touching food with contaminated hands has been the cause of many outbreaks of food-borne diseases. For those pathogens that have a low minimum infective dose and for which the human body is the main reservoir, e.g., *Shigella* spp., *S. typhi*, contaminated hands are a particularly important risk factor. Nevertheless, the washing of hands after defecation or changing nappies, and prior to the preparation of food, is frequently neglected or ignored. Contamination of weaning food with faecal matter has been frequently reported, and lack of basic sanitation certainly is a contributing factor. Water used for the preparation of food itself is a source of pathogenic agents, and in rural areas water is very often contaminated. Some pathogens exist naturally in the environment, e.g., earth, and are consequently endogenous contaminants of food. One example is *B. cereus*, the spores of which are often found in foods, such as rice and dried milk [14]. The seasons of the year also play an important role in the level of contamination. For instance, children born in spring and during the hot dry season, shortly before and at the height of diarrhoeal prevalence had the lowest death rates for diarrhoeal disease during the first year of life. They were

predominantly breast-fed at the time of major risk. Children born in autumn, with weaning beginning in the hot dry season at the time of greatest risk, had the highest death rates of any cohort determined by month of birth [182].

Pathogenic agents can therefore contaminate food in many different ways, and at various stages in the food chain, including during the preparation of food. Under the favourable conditions that exist in many countries, especially in slum and rural areas, the risk of contamination of weaning foods during their preparation is even greater. However, in terms of the causes of food-borne diseases, the most critical factors are the following: the preparation of food several hours prior to consumption, combined with inadequate storage conditions; and insufficient cooking or re-heating of stored food [183]. Whatever, the source of the food contamination, food-borne pathogens and some of their toxins can be destroyed by appropriate heat treatment, and adequate cooking or re-heating can reduce their numbers to safe levels. However, contrary to popular belief, normal cooking does not necessarily eliminate all the micro-organisms. In the preparation of porridge or gruels, for example, prolonged cooking is often avoided, since sustained cooking produces a food that is too glutinous and viscous for young infants to consume. Consequently, depending on the extent of the initial contamination and the duration of cooking, a number of pathogens may survive the cooking process [14]. However, there are some traditional practices that are advantageous from the food safety point of view. For example, in many African countries it is customary to give infants fermented cereal products such as ogi (Nigeria), ugi (United Republic of Tanzania, Uganda, Kenya) and mahewu (South Africa, Zimbabwe). As a result of fermentation by lactic bacteria and yeasts, the pH of the food decreases to ≤ 4.3, at which levels micro-organisms associated with spoilage or disease cannot multiply [14]. Several studies in Africa have demonstrated the importance of this traditional technology in controlling and improving the microbiological quality of weaning food [184, 185]. Mensah et al.,

(1990) showed that there were lower numbers of contaminated fermented porridge than unfermented, and that after several hours of storage the level of contamination was significantly lower in fermented porridge [184].

The chemical contamination of food also needs to be discussed, since many outbreaks of chemical intoxication have arisen as a consequence of errors of ignorance or negligence made by those handling food. For example, in a number of instances, foods have been contaminated because of unsafe packing and leakage of pesticides during the storage or transport or because food was stored in containers that previously contained pesticides, but which were not adequately washed before being re-used. In addition, seeds intended for planting and which had been treated with fungicides have been consumed; fish have been caught in ponds where rice treated with pesticide was growing; or cereals have been harvested too soon after being treated with pesticide. The problems presented by intoxication with marine biotoxins are also increasing in many parts of the world, and if seafood constitutes a part of the diet of infants and children, they too will be affected if such food is contaminated [14].

2.9.2 Standards set by the World Health Organisation regarding the quality of milk

2.9.2.1 Introduction

The coliform group of bacteria comprises all aerobic and facultatively anaerobic, Gram negative, non-spore-forming rods able to ferment lactose with the production of acid and gas at 32° or 35°C within 48 hours. One source of these organisms is the intestinal tract of warm-blooded animals; certain bacteria of non-faecal origin are also members of this group. Typically, these organisms are classified in the genera Esherichia, Enterobacter, and Klebsiella. A few lactose-

fermenting species of other genera are also included in the coliform group. In proportion to the numbers present, the existence of any of these types in dairy products is suggestive of unsanitary conditions or practices during production, processing, or storage [186].

Application of the test for coliforms is not intended to detect faecal pollution but, rather, to measure the quality of the practices used to ensure proper processing and to minimise bacterial contamination of processed dairy products. Coliform tests are conducted following pasteurisation primarily to detect bacterial recontamination of milk and other processed dairy products. Results of tests on raw samples are to be interpreted differently from those obtained when testing pasteurised milk [186]. Coliforms in small numbers may enter raw milk and cream under normal conditions of production and handling. Some laboratories perform coliform estimates on raw milk to determine the degree of contamination during milk production. For both raw and pasteurised samples, however, in the absence of a storage interval and temperature record for milk and cream before testing, it is impossible to conclude from positive tests alone to what degree bacterial growth following initial contamination may have contributed to test results. For cultured products, some limitations on the age of the sample before testing seem necessary because of the rapid and marked reductions encountered in coliform counts within 24 hours of processing [186, 187].

2.9.2.2 Regulations for Milk stipulated by the Foodstuffs, Cosmetics and Disinfectants Government Regulation no 1555/1997, Act 54, 1972 [188]

No person shall sell-

(a) pasteurised milk, pasteurised reconstituted (prepared) milk, pasteurised skimmed milk, pasteurised reconstituted (prepared) skimmed milk or pasteurised cream which-

(i) contains the following:

(aa) Antibiotics or other antibacterial substances in amounts that exceed the maximum residue levels stipulated in the Maximum Limits for Veterinary Medicine and Stock Remedy Residues Regulations or which by virtue of a presumptive test (for example the Kundrat test) is suspected to contain antibiotics or other antimicrobial substances in amounts that exceed such maximum residue levels;

(bb) Pathogenic organisms, extraneous matter or any inflammatory product or other substance which for any reason whatsoever may render the product unfit for human consumption;

(ii) has been shown by the Aschaffenburg and Mullen phosphatase test described in paragraph 3 of Annex A or any other test, provided its accuracy equals that of the aforementioned test, to yield the equivalent of 10 micrograms or more of p-nitrophenol per 1,0 mℓ;

(iii) (aa) on execution of the VRB MUG agar method or dry rehydrated film is found to contain more than 10 coliform bacteria in

1,0 ml milk or 1,0 semi-solid product; or [Reg. 6 (a) (iii) (aa) corrected by G.N.R. 1278 dd. 29.10.1999.]

(cc) on execution of the modified Eijkmann test, the VRB MUG agar method or the dry rehydrated film method described in paragraphs 2, 5 and 11, respectively, of Annex A, is found to contain any Esherichia coli in 1,0 mℓ of milk or 1,0 g of semi-solid product;

(iv) gives a standard plate count of more than 50 000 colony forming units (CFUs) per 1.0 ml of fluid or per 1.0 g of semi-solid product when subjected to the tests described in paragraph 7 or 10 of Annex A; [Afrikaans text corrected by G.NR. 1278 dd. 29.10.1999]

(v) is not packed in a hermetically sealed container when sold to the ultimate consumer: provided that in cases where the consumer supplies his or her own empty container to be filled from a bulk tank or container, the filled container need not be hermetically sealed;

2.9.3 Diseases associated with Bacterial Contamination

2.9.3.1 Diarrhoea

2.9.3.1.1 Introduction

The term "weanling diarrhoea" was introduced nearly 30 years ago to describe the increased risk of diarrhoea associated with the transition in an infant's diet from exclusive breast-feeding to an adult pattern, the period of highest risk being the first 3 months after breast-feeding stops [189]. Diarrhoeal disease is still one of

the most prevalent and important public health problems in so-called developing countries, despite improvements in knowledge, understanding and management that have occurred over recent years [190, 191, 192, 193]. The first estimate of worldwide morbidity and mortality from diarrhoeal disease was based on active surveillance data collected from 24 selected longitudinal studies of children undertaken over 3 decades, and was published in the early 1980s [194]. This was a landmark report which showed that, based on 1980 population estimates, there were 744 to 1000 million episodes of diarrhoea and 4.6 million deaths each year from diarrhoeal disease in children under 5 years of age in Africa, Asia (excluding China), and Latin America. A decade later improved case management had helped improve survival rates so that global mortality was lower (3.3 million deaths per year; estimated range = 1.5 to 5.1 million), but the incidence of diarrhoea (2.6 episodes per child per year) was virtually unchanged [195]. This raises important questions about the causes of infectious diarrhoea, its transmission, the epidemiology of diarrhoeal infections, and the effectiveness of public health measures to overcome these illnesses.

Infantile diarrhoea is a worldwide problem [196]. Underdeveloped and developing countries have a greater incidence and prevalence than do developed countries. A significant number of diarrhoeal episodes result in serious retardation of growth and development of affected children. In some of those in whom diarrhoea persists, other complications occur which may ultimately lead to death. It was estimated that 5 million children under 5 years of age died in 1980 from intractable diarrhoea. At this rate, 10 young children die from chronic diarrhoea every minute of the day, an abstract figure that exemplifies both the magnitude and devastation of its effect. In the majority of cases, the etiology of the diarrhoeal morbidity and mortality is not identified. Irrespective of the cause, a variety of bacteria, viruses and parasites have been shown to precipitate the onset of intractable diarrhoea. When malnutrition is associated with episodes of

diarrhoea, excessive morbidity and mortality have been recorded. Environmental factors, including poor sanitation, contaminated water supply, improper waste disposal, inadequate food processing, storage and handling, and underlying poor nutrition, have been considered to be the initiating and perpetuating factors of chronic infantile diarrhoea. The complexity of the problem has only recently been recognized [196]. Based on a study performed by Lebenthal (1984), the majority of cases indicated that the final common pathway of intractable diarrhoea of infancy is that of prolonged small intestinal mucosal injury [197]. The mucosal injury is caused, intensified and perpetuated by the associated factors, namely, protein-energy malnutrition, deficient enteric hormones, increased absorption of native foreign protein, ineffective villous repair, bacterial overgrowth, infection and malabsorption of nutrients. Diarrhoeal syndromes are closely associated with severe malnutrition. It is difficult to distinguish the cause from the effect, but it is generally accepted that diarrhoea and severe malnutrition form an integrated cyclic event [196].

2.9.3.1.2 Classification of Diarrhoea

Diarrhoea can be classified as acute or chronic. However, there is no universal definition for acute and chronic diarrhoea. Generally speaking, **acute diarrhoea** is described as the expulsion of stools or fluid in excess of normality, usually more than three evacuations per day, which resolves within 5 to 6 days. The presence of foetid stools and blood will also characterise acute diarrhoea; dysenteri-form diarrhoea may last longer. **Infectious diarrhoea** is that in which an enteric pathogen is demonstrated or isolated in the bowel or the stools of the diseased individual. **Chronic diarrhoea** is described by some authors as an episode lasting for more than 3 weeks, and by others as one lasting more than 6 weeks. Definitions are established by clinicians working in hospitals, who often lack an adequate history of the child's total background. Therefore, the

prospective study of the natural history of enteric infection and of diarrhoea reveals no clear distinction between repeated acute diarrhoeal episodes and the "chronic recurrent diarrhoea syndrome" observed under poverty and unhygienic conditions. Chronic and chronic recurrent diarrhoea are used interchangeably. The "intractable" chronic diarrhoea that establishes itself in the first 3 months of life is not seen among children who are exclusively breast-fed for several months in rural communities of developing countries [198].

2.9.3.1.3 Most Important Causes of Infantile Diarrhoea

2.9.3.1.3.1 Bacterial Pathogens

Various pathogens have been identified as causing diarrhoeal diseases [14]. Some of these include bacteria such as *Escherichia coli*, *Shigella* spp., *Salmonella* spp., *Vibrio cholerae* O1 and *Campylobacter jejuni*; protozoa such as *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium* spp.; and also enteric viruses such as rotavirus. In addition, *Bacillus cereus*, *Staphylococcus aureus*, *Clostridium perfringens*, and helminths are common food-borne pathogens that cause diseases frequently accompanied by diarrhoea [199, 200, 201]. *Escherichia coli* is found throughout the distal small intestine and colon. However, only certain strains of *E. coli* seem to be capable of producing diarrhoeal disease in humans. Several pathophysiological mechanisms have been described including enterotoxigenic, enteroinvasive, enteropathogenic, and enteroadherent *E. coli*. The enteroinvasive organisms tend to fall within certain serotypes. They produce disease by invading the epithelial cells of the terminal ileum and colon, thus producing symptoms of diarrhoea with blood, mucus, and fever. The syndrome is indistinguishable from shigellosis. Sheets of leukocytes may be identified by Wright stain of fecal mucus [197, 202].

atrophy, disordered arrangement of enterocytes, sub-nuclear vacuolization of crypt cells, and a moderately increased number of plasma cells, lymphocytes, eosinophils, and Paneth cells. The bacterial attachment factors (adhesions) that have been described seem to reside in their hair-like pili or fimbria. An adherence function may also reside in the bacterial capsule [197]. They may only be expressed under certain conditions [208]. These and other bacterial properties, including chemotaxis and motility may determine whether or not a particular pathogen is swept away by the secretions, colonizes the mucus layer, or penetrates the mucus layer and glycocalyx to attach to the cell membrane [197].

Other bacterial pathogens may also cause illness in infancy. *Camphylobacter* may affect infants, children and adults and thus develop the associated features of fever, abdominal cramps, and presence of blood and mucus in the stools. *Yersinia enterolitica* may be invasive or enterotoxigenic. In addition to diarrhoeal disease, it has also been associated with mesenteric adenitis with abdominal pain mimicking acute appendicitis. *Salmonella* and *Shigella* invade intestinal epithelial cells as a prerequisite to initiating diarrhoeal disease. Enterotoxins produced by some strains of *Shigella* and *Salmonella* [197] may be involved in pathogenesis following invasion [203]. Other bacterial enteric pathogens, including *Bacillus cereus* , noncholera *Vibrio* and *Aeromonas hydrophilia*, have been reported to cause diarrhoea [197, 209].

2.9.3.1.3.2 Diarrhoea Caused by Viruses

Until 1972, viral enteritis was only a hypothesis. Support for the suspicion was lent by transmission experiments in which bacteria-free stool filtrates from patients with diarrhoea induced diarrhoea in human volunteers or experimental animals [210, 211]. With the development of electron microscopy and other new technologies [212, 213], the identification of two groups of viral agents capable of

causing acute nonbacterial gastro-enteritis was accomplished. Rotavirus was found to be the major cause of episodic diarrhoea in infants and young children whereas the Norwalk agent (parvovirus) appeared to be the responsible agent in outbreaks of diarrhoea in adults [197]. Many other viruses have been identified in stools, but at present none have met the criteria of Koch for acceptance as true agents of diarrhoeal disease [214]. The characteristic clinical features of rotavirus gastro-enteritis have been well described. The incubation period ranges between 48 and 72 hrs. Fever and vomiting usually precede the diarrhoea and last 1 to 3 days; the diarrhoea lasts for 4 to 7 days. The illness can be severe and can lead to death from dehydration in all age groups. The lower prevalence of symptomatic infection in infants under 6 months of age may be related to the presence of maternal antibodies [214]. In addition, breast-feeding has been found to offer protection. Rotavirus antibody has been found in colostrum and may be present in breast milk for up to 2 years [215]. The protective effect of breast-feeding has been demonstrated in experimental animals [216]. Breast-fed babies have a lower incidence of rotavirus infection than do bottle-fed infants and excrete fewer virus particles per gram of faeces [217]. Rotavirus infection is accompanied by a sero-conversion with persistence of high antibody titre for at least 2 years. Complement-fixing antibodies to rotavirus have been found in approximately 70 to 80% of newborn studies. This antibody is most probably of maternal origin, declines to 10 to 15% of early concentrations in infants by 6 months, and is then followed by a rise to adult concentrations in 50 to 90% of 3 year-old patients studied [218]. The decline in rotavirus infection in older children may therefore be related to the acquisition of the antibody. Re-infection has been recorded only rarely, but it has been well documented that rotavirus infection can occur despite the presence of circulating rotavirus antibodies. This implies that other factors (e.g., intestinal secretory IgA in the host or strain differences in the virus) are important in protection or infection [214]. Mucosal biopsy specimens obtained from infected infants have exhibited rotavirus particles, detectable on electron

microscopy, within the cisternae of the endoplasmic reticulum. Only the absorptive cells appear to be infected. Studies of jejunal mucosa in piglets infected with human rotavirus reveal that the diarrhoea occurs at a time when the intestinal villi are populated by immature cells that have failed to differentiate into mature absorptive cells during an accelerated transit from the crypts [197].

The progression of an acute viral illness to one of chronic and protracted diarrhoea may be related to the above pathogenic features of rotavirus. Electron microscopy of faeces during acute infectious diarrhoea has revealed a number of additional possible viral agents. The role of these agents in acute and chronic diarrhoea remains to be clearly established. The agents suspected include adenovirus, astrovirus, coronavirus, calcivirus, and "mini reo" virus [197, 219].

2.9.3.1.3.3 Diarrhoea and Acquired Immunodeficiency Syndrome (AIDS)

The acquired immunodeficiency syndrome (AIDS) resulting from infection by the human immunideficiency virus (HIV) will become the most deadly epidemic that has affected humankind throughout history. It is estimated that 10-12 million adults and two million children are currently affected, and that the number of fatalities by the year 2000 will be more than 30 million adults and 10 million children. AIDS is caused by the RNA cytopathic human retrovirus HIV-1, and less commonly by HIV-2, which are particularly trophic for T-helper (CD4) lymphocytes and other cells, such as macrophages, that have CD4 receptors. Binding of the virus to these receptors leads to its replication within the host cell. The net biological effect of this infection is the progressive decline of CD4 cells and a decrease in the CD4/CD8 (T-helper/T-suppresser) cell ratio, causing the host to become more susceptible to an increased risk of both common and opportunistic infections [220].

In paediatric HIV disease, multiple mechanisms can cause growth retardation, malnutrition, and further immunologic impairment, leading to a worsening of the child's quality of life. Children with AIDS and diarrhoea are implicitly at risk of developing recurrent or protracted diarrhoea and subsequently malnutrition, which can further compromise their immunologic status. Unfortunately, a rigorous approach to the HIV-infected child with diarrhoea is made difficult by a number of factors. Firstly, it is difficult to distinguish at a young age a child who is truly HIV infected from one who is exposed to HIV but not infected. Secondly, compared with the adult population, there is significantly less data on the yield of current diagnostic tests in HIV-infected children. Thirdly, extrapolation from experience with adults with HIV infection may be misleading because the course of an infection depends on the concentration and virulence of the pathogen, the susceptibility of the host, and the nature and power of the host's response – all of which may be quite different in children, whose instrinsic susceptibility to infection and whose developing immune systems are not comparable to those in adults. Fourthly, children experience a different course of illnesses. For example, they experience higher rates of infection with bacterial enteric pathogens and higher rates of bacteremia. Finally, environmental exposures differ, as many childhood infections are primary rather than reactivations. For example, Epstein-Barr virus (EBV), herpes simplex virus, and cytomegalovirus (CMV), which are frequent pathogens in adult HIV patients, are primary infections in children over the first 5-19 years of life. Rotaviruses also peaks in childhood, as already mentioned [220].

2.9.3.1.3.4 Diarrhoea Caused by Parasites

The association between chronic and acute diarrhoea and the presence of intestinal parasites has been well established. *Giardia lamblia* is considered to be the most common parasite causing intestinal disease. This organism may affect infants, children and adults and may be associated with clinical and/or

laboratory features suggestive of malabsorption, in extent of which is variable [221]. *Giardia lamblia* (duodenalis) is a binucleate flagellate. Infestation follows after ingestion of cysts contained in contaminated water or food. Excystation then occurs in the acidic environment of the stomach. Subsequently, the trophozoites colonise the proximal small intestine, where conditions for survival are optimal (pH 6.4-7.0). The mechanisms related to the resultant pathogenesis of malabsorption in giardiasis are still evolving and have been recently summarised by Poley [221]. The variety of mechanisms postulated includes mucosal injury mechanical destruction, bacterial overgrowth, and mucosal invasion, increased cell turnover and increased secretion of mucus. Often, no pathogenic changes are identifiable by either light or electron microscopy. The more severe pathologic changes, i.e. flat mucosa, appear to occur only in the immunocompromised host. A transient lactase deficiency is thought to be related to an increased cell turnover, allowing immature cells to occupy the highest cell positions [197].

Giardia has also been demonstrated to invade the epithelium; they have been demonstrated in villus and crypt cells and in the lamina propria. Invasion, however, is usually insignificant, and other than rare isolated cases there are no evidence that invasion of the mucosa by *Giardia* is a major contributor to malabsorption [197].

Cryptosporidium is a relatively recent pathogen in the scenario of diarrheal disease and is now thought to be a very common agent of acute gastro-enteritis world-wide [222]. The parasite has been found throughout the intestinal tract of humans, and its pathogenesis is still under investigation. Recent data supports a secretory effect due to an enterotoxic activity found in the faeces of infected calves [223], consistent with the secretory type of diarrhoea found in nine infected patients in whose stools enterotoxic activity was seen in epithelial cells in

vitro [224]. From a clinical viewpoint, Cryptosporidium infection typically runs a mild course in immunocompetent children, whereas in children and adults with acquired immunodeficiency syndrome (AIDS), it is known to be a very harmful pathogen, leading to chronic, aggressive, and sometimes devastating illness. However, it is now clear that Cryptosporidium may also lead to prolonged diarrhoea in immunocompetent children from both developing and developed countries [225]. In the latter setting, an impressive 50% of 64 children admitted to the hospital with Cryptosporidium infection alone had prolonged diarrhoea, and in several of them an enteropathy was shown [226].

Some other parasites that have also been identified as causing diarrhoea include: *Entamoeba histolytica, Balantidium coli, Isospora beli, Sarcocystis hominis* and *Cyclospora* [227].

2.9.3.2 Malnutrition

2.9.3.2.1 Introduction

It is estimated that about 780 million people in developing countries do not have access to enough food [228]. Approximately more than 500 million people are chronically malnourished and more than 13 million children under the age of five years die annually because of infection and as a direct or indirect result of hunger and malnutrition. The WHO Global database confirms that more than a third of pre-school-age children, or more than 200 million, are affected by the consequences of malnutrition. A total of 80% of the affected children live in Asia (mainly Southern Asia), 15% in Africa and 5% in Latin America [228].

The causes of malnutrition and death are described as immediate, underlying and basic. Immediate causes include inadequate dietary intake and disease,

while underlying causes imply inadequate or improper education, particularly of women, leading to insufficient household food security, inadequate maternal and child care, insufficient health services and an unhealthy environment. Basic causes include ecological conditions and economic structures which are influenced by the political and ideological superstructure. The main causes of malnutrition and growth retardation are also seen as deeply rooted in poverty and lack of education. Growth retardation occurring in childhood limits the biological and intellectual abilities which cause diminished working capacity in adulthood. Diminished working capacity not only perpetuates the vicious circle of poverty but also leads to enormous waste of human potential. Following protein-energy malnutrition (PEM), iron deficiency anaemia, vitamin A and iodine deficiencies are regarded as the major nutritional problems with increasing public health significance. In South Africa the major nutritional deficiency abnormalities that have been identified are associated with protein-energy, vitamin A, niacin, riboflavin, folate, vitamin C, vitamin D, iron and calcium [228].

In the initial stages of development a deficiency is so mild that physical signs are absent and biochemical methods generally cannot detect the slight changes. As tissue depletion continues the biochemical changes can be measured in body fluids and tissues. With further depletion the physical signs become apparent until finally the full-blown signs of the predominating classic deficiency can be recognised. Nutritional deficiencies rarely occur singly inasmuch as an inadequacy of food almost always reduces the intake of more than one nutrient. Moreover, the metabolic interdependence of nutrients means that lack of one will interfere with the proper utilisation of another. Primary nutritional deficiencies are those caused by inadequate or imbalanced intake of food. These conditions are the result of many environmental factors. Secondary deficiencies are those that result from some fault in digestion, absorption, and metabolism so that tissue needs are not met even though the ingested diet would be adequate in normal

circumstances. Thus, the restoration and maintenance of good nutrition are important concerns in clinical nutrition. Classic deficiency diseases are diagnosed relatively easily because the physical and biochemical findings are prominent and specific [229].

2.9.3.2.2 Factors Contributing to Malnutrition

The causes of malnutrition are complex. They include conditions that pre-exist within the individual – the host, the quality of the environment and the specific agents that provoke the problem [229]. These include the following:

(a) Susceptibility of Individuals

Within a given environment some individuals are more susceptible than others to malnutrition. Among the vulnerable groups are infants and pre-school children, whose nutritional requirements are high during rapid growth. When nutrients are not available for a given stage of development, the physical or mental rehabilitation may be delayed or may be unattainable. During pregnancy, inadequate diets compromise the development of the foetus as well as the mother's own nutritional status and her freedom from the complications of pregnancy. The groups include the elderly and sick people. The vulnerability of these groups to malnutrition must be considered when priorities are assigned to food assistance and to educational programs [229].

(b) Environmental Factors that Lead to Malnutrition

In the United States and throughout the world, poverty and ignorance are leading causes of malnutrition. Lack of available food is a principal cause of malnutrition

in the underdeveloped and developing countries of the world, but not in Northern America, Europe, and Oceania [229].

Poverty: Two billion people live in about 100 poor underdeveloped countries in the world. Two thirds of these people live in Asia. Their per capita income ranges from moderate to extreme poverty. The word poverty implies too little money to spend on food; competition between food and other necessities of life as well as things that give personal satisfaction but are beyond the available income; lack of food-storage and preparation facilities; inability to purchase foods under the most favourable price conditions; and crowded, often unsanitary housing [229, 230]. Poverty results in a vicious cycle: poverty➡inadequate diet➡malnutrition➡illness➡inability to work➡poverty [229, 231, 232, 233, 234].

Population Growth: The population of the world in 1984 was estimated to be 4.76 billion [235]. It is increasing by 80 to 90 million per year (about 10, 000 every hour of every day). By the end of this century about 6.1 billion persons will share the earth's resources – but not equally. By the end of the century food production will need to increase by more than one fourth to meet the present-day inadequate levels. Any improvement in nutritional status would require far greater improvement in food production. Population control is a possible answer but this involves religious, political, economic, and personal values [229].

Inadequate food supply: Within a 40-year time span world grain production increased from 631 million metric tons in 1950 to 1620 million metric tons in 1981 [236]. This remarkable increase was accounted for primarily by a doubling of the yield per acre of land. During this same period population increased at a rapid rate so that the grain available per person improved at a somewhat slower rate (251 kg grain per person in 1950 to 365 kg per person in 1981). Since 1972-73 there have been major food crises throughout the world. Natural disasters

such as floods in Bangladesh and India, severe and prolonged droughts in the Sahel, region in Africa, and political upheaval such as in Campuchea, have severely curtailed food production, leading to starvation for millions of people. The tragedy of famine in several countries of Africa is causing millions of deaths. Moreover, economic and political factors as well as severe problems of food distribution further exacerbate the problem. In developing countries governments are usually unable to finance irrigation programs needed for crops, industrial plants for food processing and storage, and roads for food distribution [229, 232].

Urbanisation: Jelliffe (1970) described the flood of rural dwellers to the cities that is now occurring throughout the entire world as "de-urbanisation" because the influx is too rapid to accommodate people in terms of employment, housing, food, and services [237]. The shanty towns and ghettos provide surroundings that are often worse than rural areas left behind [231]. Because they need cash to purchase food, people find themselves with diets that are more meagre than their rural fare. Infants and children suffer most from this trend. Infants are often weaned early, particularly because the mother seeks employment and partly because she is trying to emulate the women of the Western world who do not breast-feed their babies [234]. Unfortunately the substitute feedings for the baby are insufficient in quantity, often poor in quality, and likely to be grossly contaminated with bacteria [229].

Cultural Factors: Malnutrition sometimes results because people refuse to eat foods prohibited by religious beliefs or taboos and superstitions, those that lack prestige value, and those that are unfamiliar. The taking of life is prohibited by some religions and no animal-based foods may be eaten. For some this restriction excludes even eggs and milk. Social customs, taboos and superstitions interfere with adequate food intake, especially by vulnerable groups. In some developing countries the head of the family and other men in

the family eat first and are given the choicest share of food. When food is scarce, women and children may get less that they need. Many primitive people believe that foods are endowed with specific qualities that can influence the personality of the unborn child or that can mark him or her physically. Thus, animal-based foods in particular may be taboo for pregnant and lactating women [229].

Lack of Education: People of all income classes and at all educational levels lack knowledge regarding the essentials of an adequate diet. Those who are ignorant concerning nutrition are particularly susceptible to food faddism, superstition, and nutritional quackery. A limited education exacts a particularly severe toll from those who are also poor. It is, for many of them, the cause of their poverty inasmuch as people with minimal education and technical skills are unable to secure employment, to spend every cent more carefully, but they have too little consumer information to help them. Moreover, inasmuch as the amount and quality of food available to them are limited, they need to employ the best techniques in food preparation to preserve nutritive values – but they lack the facilities and skills to do so [229, 234].

Misinformation and Faddism: A fad is a fashion of the moment – here today, gone tomorrow. Fads sometimes disappear, only to reappear some years later in a new form. The lack of nutritional education, the influence of peers and the pressures of advertising may be influential. Certain code words are widely used by food faddists and quacks to characterise foods, supplements, and devices. The "good" words include health food, prevent, cure, pep and energy, organic, natural, herbal, fibre, supplement (vitamin, mineral, protein), vegetarian, and many others. The "bad" words include additive, chemical, devitalised, pesticide, preservative, processes, synthetic. With a liberal use of "good" and "bad" words, the faddist develops a rationale for a given product or practice that manages to

sway the uninformed consumer. In addition to claims for health maintenance, the faddist makes exaggerated and untrue statements regarding the prevention and cure of numerous diseases and conditions: arthritis, cancer, diabetes, hypoglycemia, hyperactivity, obesity, retardation of ageing, maintenance of sexual virility, skin disorders, and so on. Many fads and myths related to food are probably harmless. But if the consumer adopts a particular food, diet, supplement, or device as a treatment for disease without consulting a physician, the harm can be serious indeed. A second danger of food faddism is economic. About 1.5 billion dollars is spent annually on "natural, " "organic," and "health" foods. Such foods are neither more nor less nutritious than their counterparts available in supermarkets, but they are usually much more expensive. For persons with limited incomes the additional expenditure for such foods could mean the elimination of other essential foods [229].

2.9.3.2.3 Diseases Associated with Malnutrition

In 1980, approximately 39% of the world's pre-school children, 141 million in all, suffered from some degree of malnutrition. Fifty-nine percent of these children lived in Southeast Asia. It is estimated that in India alone 56 million and in Africa and the Middle East 18 to 20 million pre-school children are less than 80% of weight-for-age [238]. Although primary protein-energy malnutrition (PEM) is not common in certain states, physicians are becoming increasingly aware of malnutrition secondary to disease states such as AIDS [239], renal, hepatic, and cardiopulmonary diseases. Secondary nutritional deficits must also be considered when evaluating the nutritional status of children throughout the world. It is important to recognise that the child who is malnourished as a result of inadequate intake or recurrent infections has deficiencies in protein, energy, vitamin, and mineral stores. In recognition of this fact, it is proposed that the

term "the malnourished child" replaced the term "protein-calorie" or "protein-energy" malnutrition when referring to the undernourished child [238].

In 1959, Jelliffe used the term "protein-calorie malnutrition" to include the whole spectrum of nutritional disorders, which include marasmus, marasmic-kwashiorkor and kwashiorkor [240]. These three states of malnutrition can be differentiated most clearly on the basis of clinical findings. Marasmus, which predominates in infancy, is clinically characterised by severe weight reduction, gross wasting of muscle and subcutaneous tissue, marked stunting and no detectable oedema. Children often develop marasmus as a result of severe deprivation of protein, energy, vitamins and minerals, which often results when there is a significant decrease or absence of breast-feeding. The hair and skin changes and hepatomegaly resulting from fatty infiltration of the liver, which are seen in kwashiorkor, are not usually found in marasmus. The marasmic child is often psychologically irritable and apathetic . The most striking characteristics found in the marasmic child are the marked deficit in weight relative to height and the significant degree of stunting. The child with marasmic-kwashiorkor has clinical findings of both kwashiorkor and marasmus. Characteristically, the child with marasmic-kwashiorkor has oedema and gross wasting and is usually stunted. These children usually have mild hair and skin changes and a palpable fatty-infiltrated liver [241]. Kwashiorkor, which is found predominantly in older infants and young children, results from a combination of a diet with an inadequate protein intake and/or superimposed infection. The clinical picture is characterised by oedema, skin lesions, hair changes, apathy, anorexia, a large fatty liver, and a decreased serum albumin [238]. Although kwashiorkor is not defined by a decrease in energy intake, weight loss is usually seen. The diet of the child with kwashiorkor usually consists of a decreased protein and increased carbohydrate intake. This has led to the term "sugar baby." The oedema of kwashiorkor can only be partially explained by low serum albumin; other

contributing factors include increased cortisol levels and an inability to inactivate antidiuretic hormone [238].

While these forces of malnutrition are operative in patients of all ages, the accelerated rate of growth normally occurring in children and adolescents serves to accentuate existing deficiencies and needs. If permitted to develop undetected and untreated, secondary PEM will predictably lead to an increased risk of infection, impaired wound healing, perioperative complications, possibly a suboptimal response to therapy or a restriction of therapy of certain primary diseases, and ultimately, an increase in overall morbidity and mortality [242, 243, 244, 245, 246].

CHAPTER 3

METHODOLOGY: MATERIALS AND METHODS

3.1 STUDY DESIGN

The study was a randomised descriptive design. Both qualitative and quantitative data were collected.

3.1.2 Population and Sample size

The study population included a randomly selected population, from four of the neighbourhoods in the Mangaung region, proportional to the population size of the particular neighbourhood (Fig. 3.1). Mangaung is one of the traditionally black townships in Bloemfontein, the capital city of the Free State. The Department of Bio-statistics, Free State University, determined the method of sampling. Samples were obtained randomly, according to geographical information gathered from the Bloemfontein municipality. A total of two hundred caregivers of weaning infants were each requested to provide a sample of the infants' bottle feeds. The four neighbourhoods included were Phahameng with a sample size of 41 [(n=41) Fig 3.1 yellow area], Joe Slovo 32 [(n=32) Fig 3.1 red area], Botchabelo 55 [(n=55) Fig 3.1 green area], and Namibia 72 [(n=72) Fig 3.1 blue area]. Twelve of the participating infants had to be eliminated because they did not meet the standards set by the inclusion criteria for the study. Each caregiver or mother was asked to provide 50 ml of the infant's bottle feed for analyses. There was a great variety of feeds which included cereal mixtures,

infant formula feeds, gruels, pasteurised and unpasteurised cow's milk, tea, coffee and fruit juices.

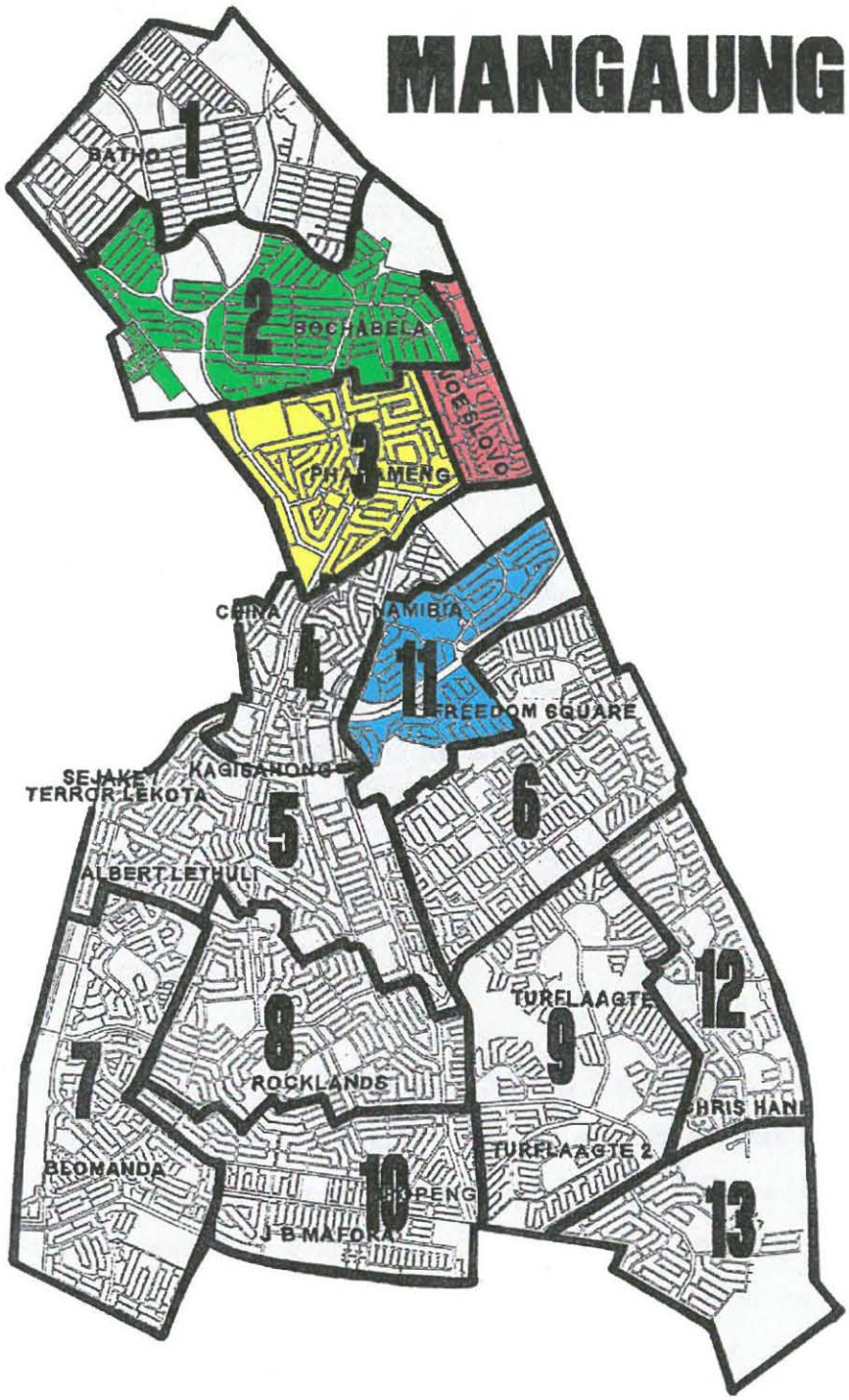

Figure 3.1 A schematic presentation of the Mangaung area

3.1.2 Inclusion and Exclusion Criteria

3.1.2.1 Inclusion Criteria

- All black infants between the ages of 0-24 months, receiving bottle feeds.
- The caregivers of these infants must be the persons responsible for preparing the infant's food for more than 5 days per week.

3.1.2.2 Exclusion Criteria

- Infants without bottles or older than 24 months.
- If the caregiver does not prepare food for the infant for at least 5 days per week, the infant does not qualify for the study.

3.2 SAMPLING

3.2.1 Research Group

A research group consisting of previously trained fieldworkers was responsible for the visits to each of the households. Only one fieldworker was involved in the completion of the questionnaire (Addendum A), ensuring more reliable results as only one person's objectivity and discretion was used. The study leader of this protocol was responsible for obtaining the samples, as well as for applying quality control measures during sampling. A different researcher was responsible for the relevant questionnaires and another was responsible for the anthropometric measurements of the mother and child. The reliability of the results was ensured as each person was trained beforehand.

3.2.2 Sample collection and preparation

Each caregiver was asked to supply a total of 50 mL of the infant bottle feed. Samples were collected in standard sterilised 50 mL sampling bottles. The samples were immediately placed in a dark container filled with ice. The samples were kept on ice and transported to the laboratories of the Technikon Free State. The samples were then processed into aliquots. One aliquot was immediately sent to the Laboratories of Diary Belle Bloemfontein for analyses. The vitamin A, C and E determinations were performed on the fresh samples. Samples for the mineral analyses were frozen at –20 ^{O}C. All microbiological counts were performed immediately. A commercially available milk sample was used as external control sample. The manufacturers of the different diagnostic kits used throughout the study supplied internal control samples.

3.3 BACKGROUND INFORMATION

Every participating mother completed a questionnaire (Addendum A). Information regarding previous breast-feeding and bottle-feeding practices, the incidence of previous or current diarrhoea and a total clinical history regarding the infant's weight and height were obtained to ascertain the infants general health. The information also included the household's socio-economic status, educational level, parity and other relevant particulars.

3.4 BIOCHEMICAL MEASUREMENTS

Chemical analysis performed on the bottle feeds included:

- Vitamins A, C and E
- Lactose
- Fat

- Protein
- Solids
- Calcium
- Magnesium
- Zinc
- Iron

3.4.1 VITAMINS A, E, and C

3.4.1.1 Vitamin C

The vitamin C in the bottle feeds was measured by using an enzymatic colorimetric method obtained from Roche/Boehringer Mannheim GmbH, Germany, (Cat. No. 409677).

L-ascorbic (L-ascorbate) and other more reducing substances (x-H_2) reduced the tetrazolium salt MTT [3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] in the presence of the electron carrier PMS (5-methylphenazinium methosulfate) at pH 3.5 to a formazan. In the cuvette with the sample the sum of the reducing substances was measured. Samples were prepared according to the prescribed instructions supplied by the manufacturer. For the specific determination of L-ascorbate, in the sample blank determination, only the L-ascorbate fraction as part of all reducing substances present in the sample was oxidatively removed by ascorbate oxidase (AAO) in the presence of oxygen. The dehydroascorbate formed did not react with MTT/PMS. The absorbance difference of the sample minus the absorbance difference of the sample blank was equivalent to the quality of L-ascorbate in the sample. The MTT-formazan was the measured parameter and was determined by means of its light absorbance in the visible range at 578nm on a Shimadzu UV-1201 spectrophotometer, (Shimadzu

Company, Japan). Thereafter it was mixed and incubated for 6 minutes at 37 °C. While incubating the contents of the sample blank, the cuvette was mixed with a spatula every 2 minutes for about 5 seconds. After the removal of the ascorbate oxidase spatula, the absorbances of the sample blank and sample (A_1) were red. After the addition of PMS solution the change in absorbance was measured again at 578 nm. The absorbance differences < A for both sample and sample blank were then determined.

Calculation

The calculation was carried out with the aid of the absorption coefficient of MTT-formazan. According to the general equation for calculating the concentration, the L-ascorbate acid concentration was determined by using the following equation:

$$C = \frac{2.700 X 176.13}{16.9 X 1.00 X 0.100 X 1000} X \Delta A = 0.2814 X \Delta A$$

$$[\text{g L-ascorbic acid} / \text{l sample solution}]$$

3.4.1.2 Vitamins A and E

An adapted method was standardised for the determination of vitamins A and E within our own Laboratories at the Technikon Free State. This method was previously used for the determination of vitamins A and E in serum/plasma, but was applied successfully to milk specimens. The vitamins A and E were determined by using high-performance-liquid-chromatography (HPLC) Chomsystems Test Kits, obtained from SEPARATIONS, München (Cat. No. CS34000).

A simple isocratic system with a HPLC pump, injector and UV-detector, is all that was needed for the vitamin A/E analysis. As the absorption maxima of the vitamins A/E are different, the detection wavelength has to be changed after vitamin A has been eluted from the HPLC-column in order to detect vitamin E which elutes later. Therefore, a programmable UV-detector is recommended to switch the detection wavelength automatically. Vitamin A is measured at a wavelength of 325 nm and Vitamin E at 295 nm.

Before beginning quantitative analysis, it is recommended that a calibration chromatogram, containing all the compounds of interest in the sample mixture, be constructed. After the baseline had stabilised, 50 µl of the prepared calibration standard (Cat. No. 34004) was injected, and separation was awaited. To ensure that the system was in equilibrium, the process was repeated until two successive chromatograms yielded identical values for retention time, peak resolution, area and height. The last of these test runs was used to calibrate the evaluation system.

The precision and correctness of the analyses were checked by including additional controls (SEPARATIONS CHOMSYSTEMS München, Vitamin A/E Serum Controls, Cat. No. 00032, -36, -37) in the analyses. If the values obtained did not lie within the given range, the system was checked and, if necessary, recalibrated. The coefficient of variation for Vitamins A and E were 1.81 and 1.52 respectively.

3.4.2 LACTOSE, PROTEIN, FAT, and SOLID CONTENT

All of these analyses were performed within the laboratory of Dairy Belle, Bloemfontein. Samples were analysed on an ultraviolet MilkO-Scan 104 Type 19900 automatic analyser.

The MILKO-SCAN 104 type 19900 series instrument is a semi-automatic instrument for the detection of fat, protein, lactose and water in milk and other dairy products. The basic operation is similar to that of an infrared spectrometer, insofar as it involves an infrared beam that is focused to pass through the sample and strike a detector, after which the energy detected is amplified and converted to a readout. The Coefficient of Variation for protein was 0.71, lipids were 0.32 and lactose was 1.17.

The values obtained from these measurement were also used for the determination of the total calorie content of each sample.

3.4.3 MINERALS

Adapted methods were standardised for the determination of the minerals, calcium, magnesium, zinc and iron in milk samples. All determinations were performed on a BOEHRINGER MANNHEIM, Hitachi 902 Automatic analyser, Tokyo, Japan.

3.4.3.1 Calcium

Calcium was determined by using a colorimetric method obtained from RANDOX, Crumlin, United Kingdom (Cat. No. CA2637). Arsenazo III specifically binds to calcium and forms a coloured complex at 600nm.

Ca^{++} + Arsenazo III → Coloured complex.

The amount of calcium present in the sample is directly proportional to the intensity of the coloured complex formed. The unit of measurement is mg/dL. The Coefficient of Variation for this method was 0.90.

3.4.3.2 Magnesium

Magnesium was determined by using a colorimetric method obtained from RANDOX, Crumlin, United Kingdom (Cat. No. MG533).

The magnesium ions react with xylidyl blue in an alkaline medium to form a water soluble purple-red chelate, the colour intensity of which is proportional to the concentration of magnesium in the sample. The unit of measurement is mg/L. The Coefficient of Variation for this method was 5.34.

3.4.3.3 Zinc

Zinc was determined by using a colorimetric method obtained from RANDOX, Crumlin, United Kingdom (Cat. No. ZN 2341).

Zinc present in the sample is chelated by 5-Br-PAPS 2-(5-bromo-2-pyridylazo)-5-(N-propoyl-N-sulfopropyl-amino)-phenol in the reagent. The formation of this complex is measured at a wavelength of 560nm. The unit of measurement is μg/dL. The Coefficient of Variation for this method was 1.88.

3.4.3.4 Iron

Iron was determined by using a colorimetric method obtained from RANDOX, Crumlin, United Kingdom (Cat. No. SI255).

Ferric iron is dissociated from its carrier proteins in an acid medium and simultaneously reduced to the ferrous form. The ferrous iron is then complexed with the chromogen, a sensitive iron indicator, to produce a blue chromophore which absorbs maximally at 595nm. The unit of measurement is mg/dL. The Coefficient of Variation was 0.51

3.5 MICROBIAL ANALYSES PERFORMED ON BOTTLE FEEDS

Microbiological analyses were performed according to the procedures as described in Annex A of Regulation 1555 of 1997 of the Foodstuffs, Cosmetics and Disinfectants Act, Act 54 of 1972 [188]. Counts recorded were minimum counts as no provision was made for fastidious bacteria. Procedures rely only on indicator organisms to show (indicate) specific problem areas.

The microbiology analyses performed on the bottle feeds included the following:

- Standard plate count
- Total *E. coli* count
- Total coliform count

Standards for milk as in the Foodstuffs, Cosmetics and Disinfectants Act, 1972 [188] were used for interpreting the bacterial counts obtained from the samples investigated.

3.5.1 STANDARD PLATE COUNT

Dilutions were prepared for the total bacterial counts by means of making a 10-fold dilution series in Ringer's solution (Oxoid, Unipath Limited, Basingstoke, Hampshire, England) to 10^{-4} for each sample.

Total counts were determined using Petrifilm™ Total aerobic count –3M (St. Paul, MN, USA). From the 10^{-2} and 10^{-3} dilutions 1 ml was plated from each dilution onto the Petrifilm. When high counts were expected, 1 ml each of a 10^{-3}

and 10^{-4} dilution were plated. The sample was evenly distributed with the flat side of the spreader provided by the company. The inoculated Petrifilm was incubated in the horizontal position (stack not exceeding 20) at 35°C for 48 h after which counts were recorded.

For the standard plate counts all the red colonies were counted. Film with 25 – 250 colony forming units (CFUs) give the most reliable results. An estimated count was made on films counting more than 250 CFUs. The presence of very high concentrations of CFUs resulted in the entire growth area becoming pink and they were recorded as too numerous to count (TNTC).

3.5.2 TOTAL *E.coli* COUNTS

Dilutions were prepared for the *E. coli* counts by means of a 10-fold dilution series made in Ringer's solution (Oxoid, Unipath Limited, Basingstoke, Hampshire, England) to 10^{-4} for each sample.

The total *E. coli* counts were determined using Petrifilm ™ Total *E. coli* by plating 1 ml of the undiluted sample and 1 ml of a 10^{-1} dilution on the Petrifilm. The sample was evenly distributed and the inoculated Petrifilm incubated at 35°C for 24 h.

For the total *E. coli* counts all the blue colonies associated with gas were counted. Film with counts between 15 – 150 colony forming units (CFUs) give the most reliable results. An estimated count was made on films counting more than 150 CFUs.

3.5.3 TOTAL COLIFORM COUNTS

The Total coliform count was determined by using Chromocult Coliform Agar (Merck KgaA 64271 Darmstadt, Germany). A volume of I ml of the milk sample was placed into a petridish, thereafter about 20 ml of the choromocult agar was added to the sample and left to gel. After the agar was solidified it was incubated at 35°C for 24 ± 2 hours. The characteristic enzyme for coliforms, β-D-galactosidase cleaves the Salmon-GAL substrate and causes a salmon to red colour for all the coliform colonies. Thereafter the coliforms were counted according to the instructions supplied by the manufacturer.

The same method can be used for the identification of *E. coli*. This method was used only to confirm the results obtained from the Petrifilm™ Total *E. coli* count. *E. coli* cleaves both Salmon-Gal and X-glucuronide, so that the positive colonies take on a dark blue to violet colour. These are easily distinguished from the other coliform colonies which have a salmon to red colour. If a positive colony was found *E. coli* detection was confirmed by coating the dark blue to violet colonies with a drop of KOVACS' Indole (SAARCHEM PTY LTD) reagent. If the reagent turns to a cherry-red colouring after some seconds, a positive indole formation confirms the presence of *E. coli*.

3.6 STATISTICAL ANALYSIS

The data is described by frequencies and percentages for categorical data and means and standard deviations or medians and percentiles for continuous data for the whole group as well as for the different beverage groups. The measured intake of each respondent is categorised and described as percentages of the RDA for each nutrient. Categories of the less than 67% of the RDA and greater

than or equal to 67% of the RDA were used. The analyses were done using the SAS-statistical package [247].

CHAPTER 4

RESULTS

4.1. INTRODUCTION

Results are given in tables. The results are first discussed for the group as a whole, and then the results are separated according to the different types of bottle contents consumed by the infants, for instance milk, coffee, tea etc.

4.2 BACKGROUND

One hundred and eighty-eight samples were randomly collected as determined by the Department of Biostatistics, UOFS. Out of the Mangaung region four areas were selected, and a trained fieldworker collected the bottle feeds on a daily basis. The study was difficult to conduct because it was community dependent. Many problems were encountered that caused delays, mostly with sample collection. The sample collection was hampered because the door-to-door method could not be used and a random study design was applied to ensure the reliability of the results. Sample collection was limited to daytimes because of security reasons. Due to daytime limitation it was difficult to interview the mothers because of their work obligations. Some of the mothers were not eager to co-operate in the study, because they were scared that they might encounter problems with Welfare. According to the WHO babies must be breast-fed for the first four to six months only [31, 37, 44]. Some of the mothers weaned their infants very early on a gruel named "suurpap" (sour porridge) because they believe that the milk did not supply enough energy. The gruel could not be

analysed because we encountered some problems as it tended to clog some of the analysers used.

A combination of tests was performed on the bottle feeds in order to obtain more information regarding the nutritional quality of the feeds. It is important to note that only a limited number of nutritional markers were measured due to practical, as well as financial implications. For this reason, we tried to measure a spectrum broad enough to make a reasonable assessment. The CODEX standard for Infant Formulas [248] was used to see whether the milk received by the infants met these standards set by CODEX. This standard applies to infant formulas in liquid or powdered form intended for use, where necessary, as a substitute for human milk in meeting the normal nutritional requirements of infants. It also provides a standard for formulas intended for infants with special nutritional requirements, except for certain provisions which must be modified to meet those special requirements [248].

4.3 RESULTS FOR THE GROUP AS A WHOLE

Table 4.3.1 indicates the adapted CODEX standard according to the kilojoule content of all the bottle feed samples analysed.

Table 4.3.1 The adapted nutritional CODEX standards for all the bottle feeds analysed per 100 kilojoules (n=188)

VARIABLES	AMOUNTS PER 100 KILOJOULES	
	MINIMUM	MAXIMUM
Vitamin A (mg/L)	0.323	0.664
Vitamin C (g/L)	0.0341	N.S.
Vitamin E (mg/L)	0.401	N.S.
Calcium (mg/dL)	21.54	N.S.
Magnesium (mg/dL)	25.13	N.S.
Iron (mg/dL)	0.449	N.S.
Zinc (μg/dL)	215	N.S.
Protein (g/100ml)	0.772	1.72
Fat (g/100ml)	1.44	2.69
Carbohydrates (g/100ml)	2.82	6.57

N.S. = Not specified

The nutritional variables measured on the bottle contents as a whole are shown in Table 4.3.2. In comparison with the CODEX standards the nutritional content of vitamins A, E, fat, protein and lactose were more than the standards set by CODEX. All the other nutritional variables did not meet the standards set by CODEX [248].

Table 4.3.2 The nutritional content for all the bottle feeds analysed (n=188)

VARIABLE	n	$\bar{x}$	MEDIAN	σ_{n-1}	% OF CODEX STD
Vitamin C (g/L)	188	0.01	0.001	0.02	23.46
Vitamin A (mg/L)	188	0.45	0.125	0.62	139.00
Vitamin E (mg/L)	188	0.64	0.00	1.54	159.60
Calcium (mg/dL)	188	2.56	2.99	1.40	11.88
Magnesium(mg/dL)	188	9.89	8.43	7.40	39.07
Zinc (µg/dL)	188	98.04	76.00	92.67	45.51
Iron (mg/dL)	188	0.27	0.20	0.32	60.13
Fat (g/100mL)	188	1.83	1.66	1.06	127.08
Protein (g/100mL)	188	1.31	1.15	0.99	169.69
Carbohydrates (g/100mL)	188	5.16	4.92	2.44	182.98
Solids (g/100mL)	188	8.83	8.79	3.64	-

n = number of samples analysed; $\bar{x}$ = Mean; σ_{n-1} = Standard deviation

4.4 THE MICROBIAL ANALYSES PERFORMED ON ALL THE BOTTLE FEEDS ANALYSED

The level of contamination can be determined by counting the number of organisms present as well as the number of coliforms. The standard plate counts for all the bottle feeds can be seen in Table 4.4.1. The Foodstuffs, Cosmetics and Disinfectants Act 54, 1972 [188] states that the standard plate counts must not exceed 50 000 organisms/ml. The results from the study revealed that 76.60% exceeded 50 000 organisms/ml with only 23.40% less than 50 000 organisms/ml.

Table 4.4.1 The standard plate counts performed on the bottle feeds as a whole (n=188)

STANDARD PLATE COUNT	FREQUENCY
0 – 50 000	44
50 001 – 100 000	5
100 001 – 500 000	28
500 001 – 1 000 000	12
1 000 001 – 1 500 000	17
1 500 001 – 2 000 000	24
TNTC	58

Standard plate count ≤ 50 000 organisms/ml = Fit for human consumption; > 50 000 organisms/ml = Unfit for human consumption; TNTC = Too numerous to count

The total coliform counts for all the bottle feeds analysed can be seen in Table 4.4.2. According to the Foodstuffs, Cosmetics and Disinfectants Act 54, 1972 [188], the total coliform count may not exceed 10 coliform organisms per millilitre. Of the one hundred and eighty-eight samples analysed 37% had counts of less than 10 organisms/ml and 63% had counts of more than 10 organisms/ml.

Table 4.4.2 The total coliform counts performed on the bottle feeds as a whole (n=188)

TOTAL COLIFORM COUNT	FREQUENCY
0 – 10	70
11 – 20	9
21 – 30	5
31 – 40	4
41 – 50	6
> 50	94

Total coliform count ≤ 10 coliforms/ml = Fit for human consumption; > 10 coliforms/ml = Unfit for human consumption.

Table 4.4.3 summarises Table 4.4.1 and Table 4.4.2. In Table 4.4.3 the standard plate count and the total coliform count are expressed as fit or unfit for human consumption. According to the standards set by the Foodstuffs, Cosmetics and Disinfectants Act 54, 1972 [188], 84.57% of the samples analysed were unfit for human consumption, with only 15.43% fit for human consumption.

Table 4.4.3 The standard plate counts and total coliform counts for the whole group expressed as fit or unfit for human consumption (n=188)

VARIABLE	FIT FOR HUMAN CONSUMPTION(n)	UNFIT FOR HUMAN CONSUMPTION(n)
Standard plate count	44	144
Total coliform count	70	118

Standard plate count ≤ 50 000 organisms/ml = Fit for human consumption; > 50 000 organisms/ml = Unfit for human consumption. Total coliform count ≤ 10 coliforms/ml = Fit for human consumption; >10 coliforms/ml = Unfit for human consumption.

E. coli present in milk is mostly associated with infantile diarrhoea [197, 202]. The percentage of positive *E. coli* was 15.4% as shown in Table 4.4.4. Before *E. coli* can be positively identified it needed to be confirmed by the Indole test. The Indole test was positive in only 12.23% of the bottle feeds, showing the percentage of samples positive for *E. coli* as 12.23%. When the *E. coli* test was positive and the Indole test negative, the *E. coli* was recorded as negative.

Table 4.4.4 *E. coli* identified and confirmed by the Indole test for the bottle feeds as a whole (n=188)

VARIABLE	POSITIVE (n)	NEGATIVE (n)
E. coli	29	159
Indole	23	165

E. coli positive = unfit for human consumption; *E. coli* negative = fit for human consumption; Indole positive = confirms *E.coli;* Indole negative = confirms absence of *E. coli*

Diarrhoea is one of the major causes of infant morbidity and mortality [18]. Table 4.4.5 shows the number of diarrhoeal incidences present in the infants during the two weeks before sample collection. The percentage of the mothers reported that the infant had a loose tummy during the two weeks before sample collection was 11.7. The percentage of mothers that were unsure whether the infant had diarrhoea the previous two weeks was 3.7, therefore showing that the mothers can't distinguish between a loose stool and diarrhoea.

Table 4.4.5 The presence of a loose tummy in the infants two weeks previous to sample collection (n=188)

VARIABLE	YES	NO	UNSURE
Loose tummy previous 2 weeks	22	159	7

4.5 ANTHROPOMETRY

Table 4.5.1 shows all the anthropometrical variables to give a background regarding the age of the infants and mothers as well as the birth and current weight and body mass index of the infants. Only 184 of the babies' ages were known to us with a mean age of 0.9 and a median of 0.86 of a year, thus 10.8 and 10.2 months respectively. Of the 188 respondents interviewed only 156

were mothers, others included grandmothers, sisters, aunts or other family members. Two of the respondents did not know the baby's birth weight and four values were absent regarding the current weight of the infants. The body mass index was then determined by dividing the weight of the infant (kg) with by height^2, (m).

Table 4.5.1 Ages of the participating infants, mothers and respondents as well as the birth weight, current weight of the infants

VARIABLE	n	$\bar{x}$	MEDIAN	σ_{n-1}
Baby's age (months)	184	0.90	0.86	0.53
Age of Mother (years)	156	28.15	28.00	7.90
Birth Weight (g)	186	2918.5	2919.5	527.7
Current Weight (g)	184	8548.9	8500.0	3128.2

n = number of samples analysed; $\bar{x}$ = Mean; σ_{n-1} = Standard deviation

Table 4.5.2 states the most important socio-economic facilities as required for this type of project. The electricity available in the households is divided into seven codes that indicate exactly what kind of cooking method is applied in the different households. Running tap water was present on only 16.49% of the premises and 77.13% of the households received water from another source like a dam, a communal tap on neutral ground, or from a neighbour. Six point three eight percent of the respondents could not give a definite answer as to where the household obtained its water from. Table 4.5.2 also focuses on the sanitary conditions and is divided into three classes such as a bucket, a water-closet and none. Ninety seven point three four percent of the households had a water-closet available to them, 2.13% had a bucket toilet and only one household, 0.53%, did not have any sanitary facilities available. Although 97.34% of the households had water-closets available, it is not necessarily in a working

condition. We suspect that only the 16.49% of the households with running tap water available on the premises have water-closets in a working condition.

Table 4.5.2 Socio-economic status with regard to running tap water, electricity, and sanitary conditions

SOCIO-ECONOMIC VARIABLES	PRESENT	ABSENT	UNKNOWN
ELECTRICITY			
Primus/Electric stove/plate	156		
Open fire	1		
Electricity & open fire	12		
Primus & open fire	18		
Unknown	1		
WATER			
Running tap water on premises	31	145	12
SANITARY CONDITIONS			
Bucket	4		
Water-closet	183		
None	1		

The high level of microbiological contamination causes great concern. It is very important to find possible explanations for the origin of these high levels of contamination. Evaluation of the socio-economic determinants supplies little evidence to support the belief that these factors are solely responsible for the frequency of contamination. A percentage of 99.0 households either used an electric stove or primus for cooking purposes. If applied correctly, these facilities should be sufficient to keep the number of viable organisms to a minimum. The water availability was quite a different scenario. Only 16.5% of the households had running tap water available on the premises. Six point four percent of the caregivers were uncertain as to where the water was collected. The high

percentage of inadequate water facilities increases the risk of water contamination. Long distances have to be travelled from the point of water collection to the households. Different types of water buckets are used, varying from open to sealed containers. Exposure to air increases the risk of contamination. Sanitary conditions were acceptable within 97% of the households. Only 2.1% of the households used a bucket as a toilet facility. Only one household (0.5%) had no sanitary facilities available.

Houses in the area under discussion had flush toilets installed and water lines were provided. At the same time the bucket service to the specific area was stopped. People have however to pay to have the service activated. Only the 16.5%, that have running water in their homes, have done so. This boils down to the fact that in only 16.5% of the homes the flush toilets are definitely working. As for the rest, it depends on them carrying water in buckets to their homes for those toilets.

4.6 THE NUTRITIONAL CONTENT FOR THE BOTTLE FEEDS CONTAINING DIFFERENT INGREDIENTS:

Having given the results as a whole for all the bottle feeds analysed, we divided the bottle contents into the four groups that made up the 188 samples. The four groups consist of coffee (4.79%), fruit juice (1.06%), milk (78.72%) and tea (15.43%). Both nutritional and microbiological analyses were performed on these samples. However, it is very difficult to draw specific conclusions regarding the nutritional content of these samples. This is mainly due to the fact that these foods are not accepted as "traditional" weaning foods. However, in order to show that these foods do not meet nutritional standards, or otherwise, the CODEX was adapted according to the energy content of the foodstuffs [248]. We underline the fact that this action was only taken in order to determine the

value of these foods as weaning preparation. On the other hand the contamination levels present in the bottle feeds could be directly compared with the standards set by the Foodstuffs, Cosmetics and Disinfectant Act 54, 1972 [188] and therefore the microbiological data can be used in a more credible capacity.

4.6.1 COFFEE

In order to compare the nutritional content of the coffee with the CODEX standard of milk, the expected contents as determined by CODEX were adapted accordingly to the kilojoule content of coffee [248]. The adapted CODEX standard is given in Table 4.6.1.1. It should be noted that most coffee samples were a mixture of milk and coffee. No black coffee samples, prepared using only water and coffee, were obtained.

Table 4.6.1.1 The adapted nutritional CODEX standard for bottle feeds containing coffee per 100 kilojoules (n=9)

	AMOUNTS PER 100 KILOJOULES	
	MINIMUM	MAXIMUM
Vitamin A (mg/L)	0.263	0.541
Vitamin C (g/L)	0.0278	N.S
Vitamin E (mg/L)	0.327	N.S.
Calcium (mg/dL)	17.56	N.S
Magnesium (mg/dL)	20.49	N.S
Iron (mg/dL)	0.366	N.S
Zinc (μg/dL)	175.6	N.S
Protein (g/100ml)	0.629	1.405
Fat (g/100ml)	1.171	2.195
Carbohydrates (g/100ml)	2.30	5.36

N.S. = Not specified

Table 4.6.1.2 indicates the nutritional variables measured for all the bottle feeds containing coffee. In comparison with the CODEX standards the nutritional contents of the coffee samples for vitamin A and lactose were more than the standards set by CODEX. The remaining nutritional variables all met the standards set by CODEX. It seems unlikely that coffee can meet CODEX standards. However, as mentioned earlier, these coffee samples were a mixture of milk and coffee, with added sugar.

Table 4.6.1.2 The Nutritional content for the bottle feeds containing coffee (n=9)

VARIABLE	n	$\bar{x}$	MEDIAN	σ_{n-1}	% OF CODEX STD
Vitamin C (g/L)	9	0.0080	0.0003	0.001	28.78
Vitamin A (mg/L)	9	0.0531	0.0000	0.0740	191.0
Vitamin E (mg/L)	9	0.0000	0.0000	0.0000	0.00
Calcium (mg/dL)	9	0.8022	0.5800	0.5731	4.57
Magnesium (mg/dL)	9	5.6278	4.9400	2.6905	27.47
Zinc (µg/dL)	9	46.556	28.000	46.0112	26.51
Iron (mg/dL)	9	0.0000	0.0000	0.0000	0.00
Fat (g/100mL)	9	1.0633	0.9000	0.6620	90.80
Protein (g/100mL)	9	0.6200	0.4700	0.4646	99.53
Carbohydrates (g/100mL)	9	5.6111	6.1500	1.9667	243.96
Solids (g/100mL)	9	8.0767	8.5300	2.6783	-

n = number of samples analysed; $\bar{x}$ = Mean; σ_{n-1} = Standard deviation; STD = standard

4.6.2 THE MICROBIAL ANALYSES PERFORMED ON THE BOTTLE FEEDS CONTAINING COFFEE

Table 4.6.2.1 gives an indication of the standard plate counts present in the bottle feeds containing coffee. When comparing the results with the standards

set by the Foodstuffs, Cosmetics and Disinfectants Act 54, 1972 [188], 55.55% of the standard plate counts did not meet the standard and 44.44% did.

Table 4.6.2.1 The standard plate counts performed on the bottle feeds containing coffee (n=9)

STANDARD PLATE COUNT	FREQUENCY
0 – 50 000	4
50 001 – 100 000	0
100 001 – 500 000	0
500 001 – 1 000 000	0
1 000 001 – 1 500 000	0
1 500 001 – 2 000 000	2
TNTC	3

Standard plate count ≤ 50 000 organisms/ml = Fit for human consumption; > 50 000 organisms/ml = Unfit for human consumption. TNTC = Too Numerous To Count

Table 4.6.2.2 indicates the number of total coliforms present in the bottle feeds containing coffee. According to the standards set by the Foodstuffs, Cosmetics and Disinfectants Act 54, 1972 [188], only 11.11% of the coffee feeds did not meet the standards set. The majority, 88.89% did meet the standards of the Act mentioned above.

Table 4.6.2.2 The total coliform counts performed on the bottle feeds containing coffee (n=9)

TOTAL COLIFORM COUNT	FREQUENCY
0 – 10	8
11 – 20	0
21 – 30	0
31 – 40	0
41 – 50	0
> 50	1

Total coliform count ≤ 10 coliforms/ml = Fit for human consumption; > 10 coliforms/ml = Unfit for human consumption.

Table 4.6.2.3 summarises Tables 4.6.2.1 and Table 4.6.2.2 expressing the standard plate counts and total coliform counts as fit or unfit for human consumption. With the standard plate count and total coliform count in mind, 55.55% of the coffee feeds were unfit for human consumption leaving 45.45% fit for human consumption.

Table 4.6.2.3 The standard plate counts and total coliform counts for coffee samples expressed as fit or unfit for human consumption (n=9)

VARIABLE	FIT FOR HUMAN CONSUMPTION	UNFIT FOR HUMAN CONSUMPTION
Standard plate count	4	5
Total coliform count	8	1

Standard plate count ≤ 50 000 organisms/ml = Fit for human consumption; > 50 000 organisms/ml = Unfit for human consumption; Total coliform count ≤ 10 coliforms/ml = Fit for human consumption; > 10 coliforms/ml = Unfit for human consumption

Table 4.6.2.4 indicates the number of positive and negative *E. coli,* confirmed by the Indole test. In the case of the bottle feeds containing coffee there was no *E. coli* present making it fit for human consumption regarding *E. coli.*

Table 4.6.2.4 *E. coli* identified and confirmed by the Indole test for the bottle feeds containing Coffee (n=9)

VARIABLE	POSITIVE	NEGATIVE
E. coli	0	9
Indole	0	9

E. coli positive = unfit for human consumption; *E. coli* negative = fit for human consumption; Indole positive = confirms *E.coli*; Indole negative = confirms absence of *E. coli*

4.6.3 FRUIT JUICE

In order to compare the nutritional content of fruit juice with the CODEX standard of milk, the expected contents as determined by CODEX, were adapted according to the kilojoule content of the juice [248]. Table 4.6.3.1 shows the adapted CODEX standard for fruit juice.

Table 4.6.3.1 The adapted nutritional CODEX standards for the bottle feeds containing fruit juice per 100 kilojoules (n=2)

VARIABLES	AMOUNTS PER 100 KILOJOULES	
	MINIMUM	MAXIMUM
Vitamin A (mg/L)	0.327	0.672
Vitamin C (g/L)	0.0345	N.S.
Vitamin E (mg/L)	0.406	N.S.
Calcium (mg/dL)	21.80	N.S.
Magnesium (mg/dL)	25.44	N.S.
Iron (mg/dL)	0.4543	N.S.
Zinc (µg/dL)	218.04	N.S.
Protein (g/100ml)	0.781	1.744
Fat (g/100ml)	1.448	2.73
Carbohydrates (g/100ml)	2.85	6.66

N.S. = Not specified

The nutritional variables measured for the bottle feeds containing fruit juice is shown in Table 4.6.3.2. According to the CODEX standards only lactose met the CODEX standards for the fruit juice samples, leaving all the other nutritional variables for fruit juice short of the CODEX standards.

Table 4.6.3.2 The nutritional quality for the bottle feeds containing fruit juice (n=2)

VARIABLE	N	$\bar{x}$	MEDIAN	σ_{n-1}	% OF CODEX STD
Vitamin C (g/L)	2	0.01	0.01	0.01	28.99
Vitamin A (mg/L)	2	0.02	0.02	0.03	6.11
Vitamin E (mg/L)	2	0.00	0.00	0.00	0.00
Calcium (mg/dL)	2	1.02	1.02	0.16	4.68
Magnesium (mg/dL)	2	2.08	2.08	0.22	8.177
Zinc (µg/dL)	2	3.50	3.50	0.71	1.61
Iron (mg/dL)	2	0.00	0.00	0.00	0.00
Fat (g/100mL)	2	0.63	0.63	0.04	43.51
Protein (g/100mL)	2	0.46	0.46	0.07	58.90
Carbohydrates (g/100mL)	2	8.82	8.82	1.48	309.47
Solids (g/100mL)	2	4.44	4.44	0.89	-

n = number of samples analysed; $\bar{x}$ = Mean; σ_{n-1} = Standard deviation

4.6.4 THE MICROBIAL ANALYSES PERFORMED ON THE BOTTLE FEEDS CONTAINING FRUIT JUICE

Table 4.6.4.1 gives an indication of the standard plate counts present in the bottle feeds containing fruit juice. When comparing the results with the standards set by the Foodstuffs, Cosmetics and Disinfectants Act 54, 1972 [188], 100% of the standard plate counts (only 2 of the 188 samples analysed), did not meet the standards.

Table 4.6.4.1 The standard plate counts performed on the bottle feeds containing fruit juice (n=2)

STANDARD PLATE COUNT	FRUIT JUICE
0 – 50 000	0
50 001 – 100 000	0
100 001 – 500 000	1
500 001 – 1 000 000	0
1 000 001 – 1 500 000	0
1 500 001 – 2 000 000	0
TNTC	1

Standard plate count ≤ 50 000 organisms/ml = Fit for human consumption; > 50 000 organisms/ml = Unfit for human consumption. TNTC = Too Numerous To Count

Table 4.6.4.2 indicates the total number of coliform organisms present in the bottle feeds containing fruit juice. According to the standards set by the Foodstuffs, Cosmetics and Disinfectants Act 54, 1972 [188] all of the fruit juice feeds met the standards.

Table 4.6.4.2 The total coliform counts performed on the bottle feeds containing fruit juice (n=2)

TOTAL COLIFORM COUNT	FREQUENCY
0 – 10	2
11 – 20	0
21 – 30	0
31 – 40	0
41 – 50	0
> 50	0

Total coliform count ≤ 10 coliforms/ml = Fit for human consumption; > 10 coliforms/ml = Unfit for human consumption.

Table 4.6.4.3 summarises Tables 4.6.4.1 and Table 4.6.4.2 expressing the standard plate counts and total coliform counts as fit or unfit for human consumption. With the standard plate count and total coliform count in mind, 100% of the fruit juice samples were unfit for human consumption due to the fact that all the standard plate counts exceeded the standards set by the Foodstuffs, Cosmetics and Disinfectants Act 54, 1972 [188].

Table 4.6.4.3 The standard plate counts and total coliform counts for fruit juice samples expressed as fit or unfit for human consumption (n=2)

VARIABLE	FIT FOR HUMAN CONSUMPTION	UNFIT FOR HUMAN CONSUMPTION
Standard plate count	0	2
Total coliform count	2	0

Standard plate count ≤ 50 000 organisms/ml = Fit for human consumption; > 50 000 organisms/ml = Unfit for human consumption; Total coliform count ≤ 10 coliforms/ml = Fit for human consumption ; > 10 coliforms/ml = Unfit for human consumption

Table 4.6.4.4 indicates the number of *E. coli* identified, confirmed by the Indole test. In the case of the bottle feeds containing fruit juice there was no *E. coli* present making it fit for human consumption regarding *E. coli*.

Table 4.6.4.4 *E. coli* identified and confirmed by the Indole test for the bottle feeds containing fruit juice (n=2)

VARIABLE	POSITIVE	NEGATIVE
E. coli	0	2
Indole	0	2

E. coli positive = unfit for human consumption; *E. coli* negative = fit for human consumption; Indole positive = confirms *E.coli*; Indole negative = confirms absence of *E. coli*

4.6.5 MILK

The CODEX standard as set for milk in Table 4.6.5.1 was used to compare the nutritional content of milk samples analysed with.

Table 4.6.5.1 The adapted nutritional CODEX standards for the bottle feeds containing milk per 100 kilojoules (n=148)

VARIABLE	AMOUNTS PER 100 KILOJOULES	
	MINIMUM	MAXIMUM
Vitamin A (mg/L)	0.351	0.722
Vitamin C (g/L)	0.037	N.S.
Vitamin E (mg/L)	0.436	N.S.
Calcium (mg/dL)	23.43	N.S.
Magnesium (mg/dL)	27.33	N.S.
Iron (mg/dL)	0.488	N.S.
Zinc (µg/dL)	234.25	N.S.
Protein (g/100ml)	0.839	1.874
Fat (g/100ml)	1.562	2.928
Carbohydrates (g/100ml)	3.064	7.153

N.S. = Not specified

The nutritional variables measured for the bottle feeds containing milk is shown in Table 4.6.5.2. When comparing the nutritional content of the milk samples with the CODEX standards, vitamins A, E, fat, protein, and lactose met the standards set by CODEX, with all the other nutritional variables below the required CODEX standard.

Table 4.6.5.2 The nutritional quality for the bottle feeds containing milk (n=148)

VARIABLE	n	$\bar{x}$	MEDIAN	σ_{n-1}	% OF CODEX STD
Vitamin C (g/L)	148	0.009	0.003	0.0185	24.26
Vitamin A (mg/L)	148	0.57	0.34	0.65	162.39
Vitamin E (mg/L)	148	0.81	0.00	1.69	185.78
Calcium (mg/dL)	148	3.15	3.28	0.87	13.44
Magnesium (mg/dL)	148	11.68	9.61	7.32	42.74
Zinc (µg/dL)	148	120.35	95	91.48	51.38
Iron (mg/dL)	148	0.34	0.25	0.33	69.67
Fat (g/100mL)	148	2.18	2.04	0.91	139.56
Protein (g/100mL)	148	1.57	1.3	0.95	187.13
Carbohydrates (g/100mL)	148	5.04	4.855	2.25	164.49
Solids (g/100mL)	148	9.30	9.21	3.64	-

n = number of samples analysed; $\bar{x}$ = Mean; σ_{n-1} = Standard deviation; STD = Standard

4.6.6 THE MICROBIAL ANALYSES PERFORMED ON THE BOTTLE FEEDS CONTAINING MILK

Table 4.6.6.1 gives an indication of the standard plate counts present in the bottle feeds containing milk. When comparing the results with the standards set by the Foodstuffs, Cosmetics and Disinfectants Act 54, 1972 [188], 81.08% of the standard plate counts did not meet the standards. Of the 148 samples analysed only 18.92% had counts of less than 50 000 organisms/ml.

Table 4.6.6.1 The standard plate counts performed on the bottle feeds containing milk (n=148)

STANDARD PLATE COUNT	FREQUENCY
0 – 50 000	29
50 001 – 100 000	4
100 001 – 500 000	22
500 001 – 1 000 000	11
1 000 001 – 1 500 000	14
1 500 001 – 2 000 000	18
TNTC	50

Standard plate count ≤ 50 000 organisms/ml = Fit for human consumption; > 50 000 organisms/ml = Unfit for human consumption. TNTC = Too Numerous To Count

Table 4.6.6.2 is an indication of the total number of coliform organisms present in the bottle feeds containing milk. According to the standards set by the Foodstuffs, Cosmetics and Disinfectants Act 54, 1972 [188], 74.32% of the total coliform counts did not meet the standards, leaving a percentage of 25.68% that did meet the standards.

Table 4.6.6.2 The total coliform counts performed on the bottle feeds containing milk (n=148)

TOTAL COLIFORM COUNT	FREQUENCY
0 – 10	38
11 – 20	8
21 – 30	4
31 – 40	3
41 – 50	6
> 50	89

Total coliform count ≤ 10 coliforms/ml = Fit for human consumption; > 10 coliforms/ml = Unfit for human consumption.

Table 4.6.6.3 summarises Tables 4.6.6.1 and Table 4.6.6.2 expressing the standard plate counts and total coliform counts as fit or unfit for human consumption. Regarding the standard plate count and total coliform count, 93.92% of the milk samples were unfit for human consumption with 6.08% fit for human consumption.

Table 4.6.6.3 The standard plate counts and total coliform counts for milk samples expressed as fit or unfit for human consumption (n=148)

VARIABLE	FIT FOR HUMAN CONSUMPTION	UNFIT FOR HUMAN CONSUMPTION
Standard plate count	28	120
Total coliform count	38	110

Standard plate count ≤ 50 000 organisms/ml = Fit for human consumption; > 50 000 organisms/ml = Unfit for human consumption; Total coliform count ≤ 10 coliforms/ml = Fit for human consumption; > 10 coliforms/ml = Unfit for human consumption

Table 4.6.6.4 indicates the number of positive and negative *E. coli*, confirmed by the Indole test. In the case of the bottle feeds containing milk 18.2% were positive for *E. coli*. Of the 18.2% only 14.2% was positive in the confirmatory Indole test. Therefore only the 14.2% was regarded as *E. coli* positive.

Table 4.6.6.4 *E. coli* identified and confirmed by the Indole test for the bottle feeds containing milk (n=148)

VARIABLE	POSITIVE	NEGATIVE
E. coli	27	121
Indole	21	127

E. coli positive = unfit for human consumption; *E. coli* negative = fit for human consumption; Indole positive = confirms *E.coli*; Indole negative = confirms absence of *E. coli*

4.6.7 TEA

In order to compare the nutritional content of tea with the CODEX standard of milk, the expected contents as determined by CODEX was adapted according to the kilojoule content of the tea [248]. Table 4.6.7.1 shows the adapted CODEX standard for tea.

Table 4.6.7.1 The adapted nutritional CODEX standard for bottle feeds containing tea per 100 kilojoules (n=29)

VARIABLE	AMOUNTS PER 100 KILOJOULES	
	MINIMUM	MAXIMUM
Vitamin A (mg/L)	0.1998	0.411
Vitamin C (g/L)	0.021	N.S.
Vitamin E (mg/L)	0.248	N.S.
Calcium (mg/dL)	13.32	N.S.
Magnesium (mg/dL)	15.54	N.S.
Iron (mg/dL)	0.278	N.S.
Zinc (μg/dL)	133.2	N.S.
Protein (g/100ml)	0.477	1.066
Fat (g/100ml)	0.888	1.665
Carbohydrates (g/100ml)	1.742	4.067

N.S. = Not specified

The nutritional variables measured for the bottle feeds containing tea are shown in table 4.6.7.2. When comparing the nutritional content of the tea with the CODEX standards, only lactose met the standards, with all the other nutritional variables below the CODEX standards.

Table 4.6.7.2 The nutritional quality for the bottles containing tea (n=29)

VARIABLE	n	$\bar{x}$	MEDIAN	σ_{n-1}	% OF CODEX STD
Vitamin C (g/L)	29	0.001	0.0003	0.002	4.76
Vitamin A (mg/L)	29	0.01	0.00	0.01	5.01
Vitamin E (mg/L)	29	0.00	0.00	0.00	0.00
Calcium (mg/dL)	29	0.18	0.12	0.17	1.35
Magnesium (mg/dL)	29	2.61	2.49	0.84	16.80
Zinc (µg/dL)	29	6.69	4.00	7.11	50.23
Iron (mg/dL)	29	0.00	0.00	0.00	0.00
Fat (g/100mL)	29	0.38	0.37	0.07	42.79
Protein (g/100mL)	29	0.27	0.23	0.20	56.60
Carbohydrates (g/100mL)	29	5.41	5.07	3.28	310.56
Solids (g/100mL)	29	7.02	6.38	3.31	-

n = number of samples analysed; $\bar{x}$ = Mean; σ_{n-1} = Standard deviation; STD = Standard

4.13 THE MICROBIAL ANALYSES PERFORMED ON THE BOTTLE FEEDS CONTAINING TEA

Table 4.6.8.1 gives an indication of the standard plate counts present in the bottle feeds containing tea. When compared with the standards set by the Foodstuffs, Cosmetics and Disinfectants Act 54, 1972 [188], 62.07% of the standard plate counts did not meet the standards and 37.93% did.

Table 4.6.8.1 The standard plate counts performed on the bottle feeds containing tea (n=29)

STANDARD PLATE COUNT	FREQUENCY
0 – 50 000	11
50 001 – 100 000	1
100 001 – 500 000	5
500 001 – 1 000 000	1
1 000 001 – 1 500 000	3
1 500 001 – 2 000 000	4
TNTC	4

Standard plate count ≤ 50 000 organisms/ml = Fit for human consumption; > 50 000 organisms/ml = Unfit for human consumption. TNTC = Too Numerous To Count

Table 4.6.8.2 indicates the total number of coliform organisms present in the bottle feeds containing tea. According to the standards set by the Foodstuffs, Cosmetics and disinfectants Act 54, 1972 [188], 24.14%% of the total coliform counts did not meet the standards and 75.86% did.

Table 4.6.8.2 The total coliform counts performed on the bottle feeds containing tea (n=29)

TOTAL COLIFORM COUNT	FREQUENCY
0 – 10	22
11 – 20	1
21 – 30	1
31 – 40	1
41 – 50	0
> 50	4

Total coliform count ≤ 10 coliforms/ml = Fit for human consumption; > 10 coliforms/ml = Unfit for human consumption.

Table 4.6.8.3 summarises Tables 4.6.8.1 and Table 4.6.8.2 expressing the standard plate counts and total coliform counts as fit or unfit for human consumption. Regarding the standard plate counts and total coliform counts, 65.52% of the tea samples were unfit for human consumption with 34.48% fit for human consumption.

Table 4.6.8.3 The standard plate counts and total coliform counts for tea samples expressed as fit or unfit for human consumption (n=29)

VARIABLE	FIT FOR HUMAN CONSUMPTION	UNFIT FOR HUMAN CONSUMPTION
Standard plate count	11	18
Total coliform count	22	7

Standard plate count ≤ 50 000 organisms/ml = Fit for human consumption; > 50 000 organisms/ml = Unfit for human consumption; Total coliform count ≤ 10 coliforms/ml = Fit for human consumption; > 10 coliforms = Unfit for human consumption

The number of samples positive for *E. coli* is indicated in Table 4.6.8.4. Of the 29 tea samples only 6.90% were positive for *E. coli*. The indole test was positive for all the *E. coli* identified in the tea samples.

Table 4.6.8.4 *E. coli* identified and confirmed by the Indole test for the bottle feeds containing tea (n=29)

VARIABLE	POSITIVE	NEGATIVE
E. coli	2	27
Indole	2	27

E. coli positive = unfit for human consumption; *E. coli* negative = fit for human consumption; Indole positive = confirms *E.coli*; Indole negative = confirms absence of *E. coli*

CHAPTER 5

DISCUSSION

5.1 Introduction

A total of one hundred and eighty-eight bottle-feed samples given to weaning infants were randomly collected from the Mangaung region, in Bloemfontein. The nutritional content and level of contamination in these bottle feeds were tabled in chapter 4.

An acceptable guideline had to be chosen in order to make reasonable assumptions regarding the quality of weaning feeds given to children in the Mangaung area. Only two guidelines are available, the CODEX and the RDA. The RDA is used on international scale as a guideline for required food intake over a period of 24 hours [51]. The aim of this study was not to evaluate the daily nutritional intake of weaning children (in order to address malnutrition). This study aimed at measuring the quality of specific bottle feeds given to children at the specific time of sampling. CODEX standards do not necessarily address the nutritional requirements of infants, but rather the standards for milk preparations themselves [248]. These guidelines are in line with the purpose of this study. The microbial contamination levels in the milk were determined by using the standard plate counts, the total coliform counts and the total *E. coli* counts. The contamination levels were then determined by comparing the results with the standards set by the Foodstuffs, Cosmetics and Disinfectant Act 54, 1972 [188].

This chapter will discuss the data for the group as a whole and then as divided into the 4 categories, namely: coffee, fruit juice, milk and tea.

5.2 Discussion of group data

The CODEX standards provide prescribed standards that every commercial company must meet regarding the nutritional quality of its product, before marketing [248]. Through evaluation of the mean values of the nutritional variables (Table 4.3.2) in comparison with the CODEX standards (Table 4.3.1) we could assess whether the bottle feeds were adequate in nutritional content. For the group data, vitamin A, vitamin E, fat, protein and lactose were above the recommendations set by CODEX. The nutritional variables that did not meet the prescribed CODEX standards included vitamin C, calcium, magnesium, zinc, and iron.

The vitamin C content of the bottle feeds was only 23.5% of the given CODEX standard (Table 4.3.2). The low vitamin C content arouses concern. Ascorbic acid is an antioxidant and protects vitamins A and E, as well as polyunsaturated fatty acids, from excessive oxidation [113]. It is suspected that the lack of vitamin C can be attributed mainly to incorrect preparation of the bottle feeds by caregivers. During the study it was observed that it is a common practice among caregivers to add the formula powder directly to boiling water. After preparation, bottles are stored unprotected from light on the kitchen shelf. Vitamin C is both light and heat sensitive. Therefore, this malpractice leads to denaturation of the vitamin C molecule [113]. Insufficient vitamin C intake for prolonged periods of time causes growth retardation, anaemia, poor wound healing and increased susceptibility to infection in the growing infant [113].

Calcium (11.9% of CODEX), magnesium (39.1% of CODEX), zinc (45.5% of CODEX) and iron (60.1% of CODEX) content of the bottle feeds were all below

the minimum requirements. These minerals are all physiologically important minerals [50]. A deficiency of these minerals will enhance the infants' susceptibility to disease [50, 53, 70, 84, 85]. In practice, an astounding addition of 88.1% calcium is needed in order to meet the minimum requirement as determined by CODEX [248].

Vitamin C and certain amino acids may also increase calcium absorption. The lack of vitamin D seriously impairs calcium absorption [85]. The bottle feeds contained inadequate amounts of vitamin C. Therefore, it can also be speculated that the bio-availability of the available calcium is low. This presents even further complications. A lack of calcium for prolonged periods of time during infancy contributes to impaired bone and teeth development and increased susceptibility of the infant to hypocalcemia and tetany [85, 88].

The magnesium content of bottle feeds also falls well below the CODEX standards. The characteristic symptoms manifested by infant magnesium deficiency are muscle tremor, paresthesias, convulsive seizures and delirium [84]. Decreased blood magnesium levels contribute to hypocalcemic tetany. Magnesium deficiency can mainly be ascribed to the curtailment of food intake or lowered absorption, or both, resulting in a great deal of infant mortality due to malabsorption syndromes such as sprue, kwashiorkor, vomiting and diabetic acidosis [84, 18].

Table 4.3.2 indicates that the zinc content of bottle feeds was only 45.5% of the CODEX standard. Zinc deficiency is responsible mainly for growth retardation, poor wound healing, mental lethargy, increased susceptibility to infections, etc., thus overlapping most of the other conditions caused by calcium and magnesium deficiency [70]. Therefore, a deficiency of all these minerals may increase the susceptibility of the infant to develop associated abnormalities.

The content of iron in the bottle feeds was 60.1% of the CODEX standard, the highest of all the minerals analysed. Iron is the mineral element requiring the most emphasis during infant feeding, especially for infants of lower socio-economic groups [52]. The prevalence of iron deficiency is the single most common nutrient deficiency known. [249]. It has recently been estimated that at least 1 billion people are iron deficient, with the most vulnerable groups being infants, children and women [249, 250]. Depleted iron stores lead to infant weakness and fatigue [53]. It was common that mothers within our subject population complained about fatigued infants. According to the caregivers, "milk does not provide energy". Consequently, infants are given gruels that, according to them, provide more "strength" to the infant. It is a common myth within our population group that milk is inadequate for infant nutrition, due to the belief that milk is only passed as urine and has no ability to provide "strength".

Observed fatigue symptoms may possibly indicate the onset of iron-deficiency-anaemia. At first the symptoms are non-specific, and can only be diagnosed using expensive laboratory tests [53, 249]. Most of the mothers do not have access to appropriate medical facilities, nor the financial backup of a medical aid. Apart from this, most mothers are unaware of the fate of their child if this is not treated.

Although the blood iron levels are low at birth, breast-milk contains sufficient amounts of iron for the first four to six months, and thereafter iron supplementation is needed [251]. Iron deficiency can be precipitated by several factors other than poor iron intake and/or absorption [249]. It is well known that subjects with thalassaemia are often anaemic [252]. Increased blood loss caused by parasites such as hookworm [253], cow's milk intolerance or feeding fresh cow's milk to infants [254] can also lead to anaemia. Gastrointestinal diseases like celiac disease or Crohn's disease are also associated with iron deficiency. A recent study in Italy showed that children with these diseases had a very high

prevalence of iron deficiency: 84% and 72%, respectively [255]. It was shown that iron malabsorption was very common among these subjects, most likely due to the mucosal damage that had occurred. Children with Phenylketonuria (PKU) have also been reported to have a high incidence of iron deficiency, even though their daily intake appeared adequate, perhaps suggesting higher requirements for iron [256]. Low birth-weight infants are at higher risk due to lower iron stores at birth; therefore, it is recommended that these infants be given supplemental iron earlier [257]. Especially infants receiving tea (as refreshment) during the day are at risk of developing iron deficiency. This was a common practice within our study group. Tea is known to inhibit iron absorption. This practice is also prevalent within the Russian community, where iron deficiency is common. It is believed that their tea drinking habits can be a causative factor [258].

The lack of sufficient vitamin C, calcium, magnesium, zinc and iron content within the analysed bottle feeds can contribute towards the development of underweight, malnourished infants. According to the anthropometric data (Table 4.5.1) the mean age of the population group used in this study was 9 months, ranging form 0.6 months to 23.8 months, with a mean weight of 8548.9g. It could not be established whether the children were undernourished because of the lack of a 24hr recall or food frequency questionnaire. However, it can be assumed that if the analysed bottle samples represent a fair percentage of every feeding preparation given to the child, malnutrition will definitely be encountered later in life.

Evaluation of the contamination level of the infant feeds is cause for concern. The contamination level was determined by evaluating the standard plate counts and total coliform counts. From the total of 188 feeds analysed 145 samples (Table 4.4.1) had standard plate counts above 50 000 organisms/ml (Foodstuffs, Cosmetics and Disinfectants Act 54, 1972 standards for milk for human

consumption) [188]. Depending on the immune status of the infant, feeds with counts as high as this will definitely lead to diarrhoea and disease. When evaluating the coliform counts, the Foodstuffs, Cosmetics and Disinfectants Act 54, 1972, states that counts higher than 10 coliforms/ml are not fit for human consumption [188]. According to this 118 of the analysed infant feeds did not meet this standard. A percentage of 12.2 were tested positive for *E. coli* confirmed by the Indole test. The presence of *E. coli* in the bottle feeds accompanied by the high total and coliform counts often results in severe diarrhoea. This is the major cause of child morbidity and mortality [18]. With all this in mind only 11.7% of the infants were suffering from diarrhoea, with 3.7% of the mothers unsure as to whether the babies had diarrhoea or a loose stool. These values are contradictory. According to the data 84.6% of the infant feeds were not fit for human consumption. It is suspected that the prevalence of diarrhoea should be much higher than indicated. The low incidence of diarrhoea can be attributed to the fact that most of these children have a continuous intake of contaminated food from birth. Another important factor may also include the mother's unwillingness to admit that her baby has diarrhoea. Most parents are adamant about their children's health and persist that they receive healthy nutrition.

Ignorance on the mother's side regarding the purchase and preparation of bottle feeds is believed to be an additional factor contributing towards increased contamination levels. This may be ascribed mainly to the educational level of the parents. When purchasing a specific infant formula it is very important that the caregiver has adequate knowledge regarding the best formula for the infant's needs. Because of these communities' financial implications the caregivers often choose a formula which he/she can afford, but which is not necessarily of good quality. For instance unpasteurised milk should not be used as a weaning food for infants because of the pathogen risks involved. The quality of the purchased infant formula is very important for it will reduce the susceptibility of the infant to

nutritional deficiencies and the risk of contamination present at the time of purchasing. Another factor contributing to contaminated bottle feeds is the preparation methods applied by the caregivers. Most caregivers admitted to washing their hands prior to preparing the bottle feeds. However, when asked to show the interviewer how bottles were prepared, only a small minority of caregivers washed their hands prior to handling the bottle. This is a direct indication that the caregivers have the necessary knowledge, but do not apply their knowledge in practice. It is evident that the implication of their deeds is not fully realised. The lack of education further contributes to high levels of contamination. It was observed that only a limited number of caregivers used prescribed methods for sterilisation of bottles, others only washed the bottles in water. Bottles were then left on the shelf to dry without any special attention to the bottle teats.

In conclusion, if a summary of all the above-mentioned data could be made, it is understandable why the level of contamination within bottle feed samples is so high.

5.3 Discussion of individual data

5.3.1 Coffee

Coffee, not a natural weaning food, was present in nine of the hundred and eighty-eight samples analysed. When comparing the coffee's nutritional results with the CODEX standard it was evident that only vitamin C and lactose had percentages above 100%, thus above the CODEX standard. All coffee samples were milk-based. Infants weaned on coffee will definitely lack adequate nutritional intake. Besides the fact that all the other nutrient markers were below the CODEX standard, iron and vitamin E were not present at all. Therefore, if the

mother persists in weaning the infant on coffee it can lead to devastating long-term effects like malnutrition, iron deficiency, osteoporosis, and growth and mental retardation [53, 121]. A percentage of 55.6 of the coffee samples were unfit for human consumption based on the standard plate and total coliform counts.

5.3.2 Fruit Juice

The nutritional quality of fruit juice was even poorer than that of coffee. Only the carbohydrate content met the CODEX standards for milk. All the other nutritional markers were deficient according to the CODEX standard, making the infant very susceptible to malnutrition. These children are especially susceptible to kwashiorkor. The protein content of fruit juice is very low [131]. Fortunately, only two of the hundred and eighty-eight samples contained fruit juice. The microbiology, based on the standard plate counts and total coliform counts, indicates that both fruit juice samples were unfit for human consumption when evaluated according to the Foodstuffs, Cosmetics and Disinfectants Act 54, 1972 [188]. From all this it is evident that fruit juice as a weaning alternative must be eliminated because it can lead to even more severe and long-term abnormalities when compared with milk. However, it is suspected that fruit juice is only given as a supplement to daily nutrition. Overall, fruit juice is more expensive than most milk preparations. Therefore, only parents with a high socio-economic background will give fruit juice. It is most likely that these children will also receive milk in their diet.

5.3.3 Milk

Milk, the most natural weaning food, either breast milk or formula milk, was present in one hundred and forty-eight of the hundred and eighty-eight bottle samples. When comparing the nutritional quality of the milk with the CODEX

standards it was evident that vitamins A, E, fat, protein and lactose met the CODEX standards, leaving just the minerals and vitamin C below the CODEX standards. The microbiology results indicate that 93.9% of the milk samples were unfit for human consumption in comparison with the Foodstuffs, Cosmetics and Disinfectant Act 54, 1972 [188]. The *E.coli* identified and confirmed with the Indole test, indicates that 14.2% of the milk samples tested positive for *E. coli*. According to these results the contamination levels were very high and could be ascribed to the quality of milk used and the preparation methods applied. Caregivers did not follow prescribed preparation instructions and the quality of milk purchased arouses suspicion for some caregivers used unpasteurised milk or milk of poor quality. Somewhere, from the original container to the babies' mouths, samples are exposed to a high level of contamination. In spite of all this, milk still remains the most appropriate weaning food because of its nutritional quality. This is probably the most important reason for us to ensure that these children receive adequate nutrients in the form of high quality milk supplemented in a safe and healthy manner as well as promotion of breastfeeding practices.

5.3.4 Tea

Tea as a weaning food was encountered in twenty-nine of the hundred and eighty-eight samples. When compared with the CODEX standards for milk it is evident that only the carbohydrates in tea met the standards set by CODEX. This is mainly due to the fact that most caregivers add sugar (sucrose) to the tea. Some of the Paediatricians interviewed during the study stated that they never prescribe tea before the age of six months. Tea contains tannin which interferes with iron absorption, leaving infants more susceptible to iron deficiency [258]. Also, the tea samples themselves contained no iron. Of the tea samples analysed 65.5% were unfit for human consumption, based on the standard plate counts. A percentage of 24.1 exceeded the Foodstuffs, Cosmetics and Disinfectants

Act 54, 1972, standards for coliform counts [188]. Of the 29 tea samples only 6.9% were *E. coli* positive. The low percentages of *E.coli* in the tea could be attributed to the fact that tea is prepared using boiling water, which can reduce *E.coli* counts.

5.4 General Discussion

After comparison of all the results with either the CODEX standards [248] or the Foodstuffs, Cosmetics and Disinfectants Act 54, 1972, based on their nutritional content or their contamination levels, a total of 84.6% of the feeds were not adequate for human consumption. A study conducted by the University of Cape Town (UCT) within the Cape Metropolitan Area determined how socio-demographic and economic factors influence hygiene practices, resulting in the microbial contamination of infant formulas and the occurrence of the infantile diarrhoea [259]. Results from this study indicated that only 2% of the infant formulas were contaminated and concluded that this was not the leading cause of infantile diarrhoea in that population. Thirteen percent of the infants had diarrhoea at the time and 56% had past episodes of diarrhoea. The results from our study indicate that 84.6% of the bottle feeds were contaminated. Only 11.7% of the infants contracted diarrhoea within the previous two weeks prior to sampling, with 3.7% of the mothers unsure as to whether the infant had only a loose stool or dairrhoea. Therefore, the two studies seem to contradict each other. The study performed in the Cape Metropolitan Area [259] indicated low contamination levels with a high diarrhoeal incidence. Results from this study indicated a high frequency of contamination (84.6% contamination level) but only a 11.7% diarrhoeal incidence. These differences may also be ascribed to differences in sample handling and quality control measurements. Another study conducted in an urban slum with a high prevalence of infantile diarrhoea in

Northern Brazil, investigated possible sources of contamination of infant food. It was found that i) the low prevalence of hand washing, ii) not using boiled water for the reconstitution of infant formulas, iii) increased prevalence of bottle feeding and lastly, iv) the use of leftover formula, were the most likely sources of contamination [260]. However, by contrast, the diarrhoeal incidence remained low within our subject population. With all this in mind it can be assumed that there must be other factors protecting the infants from contracting diarrhoea from their contaminated infant feeds. These may include both genetic and/or environmental factors and which future investigations could possibly pursue.

incidence, 12.2% of the samples tested positive for *E. coli* and 11.7% of the infants receiving these feeds reported to have diarrhoea. Thus, there exists a good correlation between the *E.coli* and diarrhoeal incidence. The standards set by the Foodstuffs, Cosmetics and Disinfectants Act 54, 1972, stipulate that the standard plate counts must not exceed 50 000 organisms/ml and the total coliform count must not exceed 10 coliforms/ml. In our results, 84.6% exceeded one or both of these counts. Therefore, this emphasises the existence of definite factors protecting the infants from infantile diarrhoea. Further investigation regarding these protective factors is recommended.

6.2 Samples categorised according to the bottle content

The different bottle feeds obtained during the study varied from coffee, milk, fruit juice to tea. When comparing the nutritional value of all four different sample types, milk still remains the most nutrient-rich weaning supplement to infants. However, supplemented milk-like formula, cow's milk, etc., will never be as nutritious as the mother's breast-milk, for industry can only strive to market a formula approximately equivalent to mother's-milk. The contamination levels of the different types of bottle feeds indicated that fruit juice had the highest level of contamination (although just two samples were analysed), followed by milk, tea and coffee, for reasons discussed in Chapter 5.

6.3 Recommendations

From the results certain recommendations can be made in order to reduce the severity of the current situation:

- By informing the mother that breast-feeding still remains the best feeding practice, a high nutritional quality of the infant's diet as well as much

diminished contamination levels can be ensured. The infants' susceptibility to infection and, ultimately, to morbidity and mortality can be decreased.

- If supplemental feeding needs to be implemented it is important that the correct preparation methods, sterilisation, feeding procedures and product quality be brought to the attention of the caregiver.

- Teach mothers to use available resources responsibly (for example good hygiene practises).

- Address basic causes of malnutrition through a comprehensive, multidisciplinary, holistic approach including employment, housing, poverty, education (illiteracy) etc.

- To ensure maximum education we recommend that a Health Program with an accompanying pamphlet in different languages, by trained mothers and caregivers themselves, be implemented in clinics. The aim of this program must be to make caregivers aware of the dangers involved during inappropriate weaning, as well as education in the correct methods of food preparation and methods of feeding to raise healthy infants. Furthermore, rural and urban health clinics need to play a leading role in such a program, for they are the institutions that can evaluate appropriate weaning practices implemented by the caregivers. If clinic personnel can encourage the caregiver to apply appropriate weaning practices, a decrease in infant susceptibility to infection, diarrhoea and ultimately death should be encountered. From this study it is clear that the current Nutritional Health Program used at clinics regarding the education of mothers and caregivers is not adequate. It seems that the Nutrition Advisors working at clinics are not able to convey the importance of appropriate weaning to the caregiver or

mother. The main cause of this may be insufficient training of the Nutrition Advisors. Dieticians should be responsible for the proper training of Nutrition Advisors regarding infant weaning practises and more emphasis should be placed on the use of appropriate weaning practices.

Another area of concern is the supplement industry. Tins of infant formula were randomly selected and instructions on the tins/sachets investigated. Most products indicate no precautions regarding personal hygiene, like washing of hands, etc. There is no illustration that the bottle needs to be cooled down prior to the addition of the formula powder. This aspect only appears in writing. Most manufacturers use only one language. The method of preparation should be supplied in a more consumer-friendly manner, by the addition of more illustrations for the illiterate. Another recommendation should be the use of a variety of languages such as English, Afrikaans and Sotho, to explain instructions. When the industry and Educational Programs begin to join forces the problems involving lack of knowledge on the part of mothers/caregivers will definitely be decreased, leading to healthier and happier infants.

Because our children are the future of our land, we need to protect, nourish and love them to ensure a better future for their children. As it is said, a full tummy makes a growing baby happy.

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QUESTIONNAIRE

INFANT FOOD PREPARATION KNOWLEDGE AND PRACTICES OF CAREGIVERS

For office use only

Questionnaire Number_____ [ ][ ][ ] 1-3

House Number _____ [ ][ ][ ][ ][ ] 4-8

Interviewer Name _____ [ ] 9

Date _____ [ ][ ][ ][ ][ ][ ][ ][ ] 10-17

_____

Area _____ [ ] 18

I GENERAL BACKGROUND AND HOUSEHOLD DATA

1. Name of baby _____

2. Birth date(DDMMYY) _____ [ ][ ][ ][ ][ ][ ][ ][ ] 19-26

3. How old is the baby? [ ][ ] 27-28

		Months

4. Sex [ ] 29

Boy	1	Girl	2

5. How many people live in the house? (five days or more)_____ [ ][ ] 30-31

6. How many children (less than 13 years) are there living in the house?_____ [ ] 32

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7. How many rooms are in the house?
_____ ☐☐ 33

8. How many people contribute to the income of the family?_____ ☐ 34

9. What is the relation between the baby and the person being interviewed (caregiver)?

Mother	1
Grandmother	2
Aunt	3
Sibling	4
Father	5
Other(specify)_____	

☐ 35

10. What is the highest education level of the above person?

_____ ☐☐ 36-37

11. What is the age of the above person?

	Years

☐☐ 38-39

12. What facilities are available for cooking?

Facility	Yes	No
Primus	1	2
Electric stove/Electric plate	1	2
Open fire	1	2
Other methods of cooking food:		

☐☐ 40-41

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13. Do you have access to the following?

Refrigerator in the house	1	2	42
Refrigerator of a neighbour	1	2	43
Running water(tap)in the house	1	2	44
Running water outside house	1	2	45
Other source of water Specify.....			46-47
Electricity	1	2	48

14. Are pets allowed in the house?

Yes	1	No	2	49

15. What type of sanitary facilities is available?

None (open veld)	1
Put toilet	2
Bucket toilet	3
Bucket toilet	4
Other, specify.....	5

50

II ANTHROPOMETRIC DATA

1. What is the baby birth weight?

.....	Weight(g)

51-54

2. What is the baby's current weight?

.....	Weight(g)

55-58

3. What is the baby's current height?

.....	Height(cm

59-61

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III BOTTLE FEED AND COMPLEMENTARY FOOD PREPARATION PRACTICES

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CHECKLIST	YES 1	NO 2	Unknown 3		
Formal house					
Informal house (wood/metal sheets)					
Running tap on premises					
House looks clean overall					
Kitchen looks clean and hygienic					
Kitchen apparatus available					
Stove					
Kettle					
Pots and/or pans					
Cutlery					
Clean dishcloths					
Pets allowed in kitchen					
PLEASE INDICATE WHETHER THE FOLLOWING QUESTIONS WERE OBSERVED OR ASKED? Observed [3] Asked [4]					
PREPARATION CHECKLIST					
Clean hands with water					
Clean hands with soap & water					
Bottles sterilised in boiling water					
Teats sterilised in boiling water					
Bottle caps sterilised in boiling water					
Use of sterilising solution eg. Milton					
Water boiled before adding formula					
Formula added immediately to boiled water					
Addition of other food to formula feed					
Bottles prepared in morning and stored					
Bottles prepared on demand					
Prepared bottles stored in fridge					
Prepared bottles stored on shelf					
Prepared bottles stored uncovered					

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1. Is or was the baby breast-fed?

Yes, still breast feeding	1
Was breastfeeding	2
Never breast fed	3

☐ 7

2. If above answer is yes, how many times a day do you breastfeed the baby?

.....	Times

☐☐ 8-9

3. What is the main reason for breastfeeding your baby?

To feed your baby	1
To comfort your baby	2

☐ 10

4. When did the baby receive the first liquid other than breast milk (supplementary food, can be solid or liquid foods)?

.....	Weeks

☐☐ 11-12

5. Do you give the following food to your baby?

Food Type	**Yes**	**No**	
Meat	1	2	☐ 13
Eggs	1	2	☐ 14
Fruit and vegetables	1	2	☐ 15
Bread	1	2	☐ 16
Mieliepap	1	2	☐ 17
Commercial products	1	2	☐ 18

(Commercial products include Purity and other baby puree products)

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6. If yes, how old was the child when solid foods were included in the child's diet?

.....	Weeks

		19-20

7. Where did you learn to prepare a bottle-feed?

Where did you learn?	Yes	No
Read the label (yourself or someone else)	1	2
Family/friends showed you	1	2
Clinic	1	2
Work	1	2
Guess	1	2
Other, specify.....	1	2

	21
	22
	23
	24
	25
	26-27

8. Did the child have a loose tummy in the previous two weeks?
(More than three loose stools per day)

YES	1	NO	2	UNSURE	3

	28

9. What is the reason for a loose tummy?

.....

.....

	29-30

10. What is the best drink for your baby?

Tea	1
Cow's milk	2
Formula	3
Mothersmilk	4
Other, specify.....	5

	31-32

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11. At what age must a child start to eat solid foods?

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.....months	.....weeks	.....days

☐☐ 33-34

12. Do you agree/disagree or are you uncertain about the following statements?

Statement	Yes	No	Unsure
Leftover milk can be used for babies	1	2	3
It is best to keep prepared food for the baby on the shelf to ensure that it is at the right temperature.	1	2	3

☐ 35
☐ 36

13. What of the following does the baby consume in a bottle?

	Type	Yes	No
a	Formula	1	2
b	Cow's milk	1	2
c	Tea	1	2
d	Other,specify.....	1	2

☐ 37
☐ 38
☐ 39
☐ 40

Complete the relevant sections:

Part a: Formula

1. How old was the baby when you started giving him/her formula?

_____Weeks		41-42

1. Which brand of formula do you use?

	Brand		43-44

2. Why do you give formula to your child?

....		45-46

3. How do you measure the powder into the bottle?

	Heaped	Level	
A heaped spoon	1	2	
A level spoon	3	4	
The heaped scoop in tin	5	6	
The level scoop in tin	7	8	
Use tin lid	9	10	
Other, specify	11		47-48

4. How many of the above, question nr. 3 (scoops, etc.) do you use for one bottle feed?

		49-50

5. How much water do you use for one bottle feed?

	ml	51-53

6. How many of these bottles/feeds do you give to the baby per day (24 hours)?

54-55

End of part a _____

Part b: Cow's milk

1. How old was the baby when you first gave him/her cow's milk?

_____Weeks

56-57

1. Do you use fresh cow's milk or powdered cow's milk?

Fresh	1
Powdered	2

58

2. If it is powdered Cow's milk, which brand do you use?

	Brand

59-60

3. Why do you give Cow's milk to your baby?

.....

.....

61-62

4. How old was the baby when you first introduced Cow's milk into the diet?

63-64

		Weeks

End of part b _____

Part c: Tea

1. How old was your baby when you first gave him/her tea?

_____	Weeks

☐☐ 65-66

2. What type of tea do you give to the child?

Rooibos	1
Black tea	2
Other, specify.....	3

☐ 67

3. Is sugar added to the tea?

Yes	1	No	2

☐ 68

4. Is milk added to the tea?

Yes	1	No	2

☐ 69

5. How often and how much do you give tea to the baby?

.....	Times/day
.....	Ml

☐ 70

☐☐☐ 71-73

6. Why do you give tea to the baby?

.....

.....

☐☐ 74-75

End of part c _____

CHECKLIST	YES 1	NO 2	Unknown 3		
Formal house					62
Informal house (wood/metal sheets)					63
Running tap on premises					64
House looks clean overall					65
Kitchen looks clean and hygienic					66
Kitchen apparatus available					
Stove					67
Kettle					68
Pots and/or pans					69
Cutlery					70
Pets allowed in kitchen					71
PLEASE INDICATE WHETHER THE FOLLOWING QUESTIONS WERE OBSERVED OR ASKED? Observed [3] Asked [4]					72
PREPARATION CHECKLIST					
Clean hands with water					73
Clean hands with soap & water					74
Bottles sterilised in boiling water					75
Teats sterilised in boiling water					76
Bottle caps sterilised in boiling water					77
Use of sterilising solution eg. Milton					78
Water boiled before adding formula					79
Formula added immediately to boiled water					80
Addition of other food to formula feed					1
Bottles prepared in morning and stored					2
Bottles prepared on demand					3
Prepared bottles stored in fridge					4
Prepared bottles stored on shelf					5
Prepared bottles stored uncovered					6