

VII

CONTENTS

| | Page No |
|--|---------|
| ACKNOWLEDGEMENTS | I-III |
| ABSTRACT | IV-VI |
| CONTENTS | VII-XIV |
| LIST OF TABLES | XV-XIX |
| LIST OF FIGURES | XX-XXI |
| CHAPTER 1 - INTRODUCTION AND REVIEW OF LITERATURE | 1-93 |
| 1.1 Classification of anaemias | 3 |
| 1.2 Iron deficiency anaemia | 5 |
| 1.2.1 Iron deficiency anaemia in different physiological stages of life | 7 |
| 1.2.1.1 Men | 7 |
| 1.2.1.2 Post-menopausal women | 8 |
| 1.2.1.3 Women of child bearing age | 9 |
| 1.2.1.4 Infants and children | 10 |
| 1.2.1.4.1 Epidemiology of iron deficiency anaemia | 12 |
| 1.2.1.4.2 Prevalence among children. | 13 |
| 1.2.1.4.3 The vulnerability of the weanling | 15 |
| 1.2.1.5 Adolescents | 17 |
| 1.2.2 Other Haematological effects of iron deficiency | 19 |
| 1.2.3 Non-haematological effects of iron deficiency. | 20 |

VIII

| | | |
|-------------|---|----|
| 1.2.3.1 | Iron deficiency and the central nervous system | 21 |
| 1.2.3.2 | Iron deficiency and infection | 22 |
| 1.2.3.3 | Effect of iron deficiency on epithelial structures | 23 |
| 1.2.4 | Pathogenesis of iron deficiency | 24 |
| 1.2.5 | Stages of iron deficiency | 26 |
| 1.2.6 | Diagnosis of iron deficiency | 29 |
| 1.2.6.1 | Histological diagnosis of iron deficiency | 30 |
| 1.2.6.2 | Haematological indices of iron deficiency | 30 |
| 1.2.6.3 | Biochemical tests for iron deficiency | 32 |
| 1.2.7 | Dietary sources of iron | 32 |
| 1.2.8 | Bioavailability of iron | 33 |
| 1.2.9 | Factors affecting Iron absorption from the diet | 35 |
| 1.2.9.1 | Heme-iron absorption. | 35 |
| 1.2.9.2 | Non-heme iron absorption | 36 |
| 1.2.9.3 | Dietary factors affecting iron absorption | 36 |
| 1.2.9.3.1 | Dietary factors inhibiting iron absorption | 37 |
| 1.2.9.3.1.1 | Phytate | 37 |
| 1.2.9.3.1.2 | Phenolic Compounds | 38 |
| 1.2.9.3.2 | Dietary factors enhancing iron absorption | 40 |
| 1.2.9.3.2.1 | Ascorbic acid (AA) | 40 |

IX

| | | |
|-------------|--|----|
| 1.2.9.3.2.2 | Other organic acids | 41 |
| 1.2.9.3.2.3 | Meat factor | 41 |
| 1.2.9.4 | Physiological factors affecting iron absorption | 42 |
| 1.2.10 | Mechanism of iron absorption and regulation | 44 |
| 1.2.11 | Transport of iron | 46 |
| 1.2.12 | Utilization of body iron | 48 |
| 1.2.13 | Storage of iron | 48 |
| 1.2.14 | Excretion | 50 |
| 1.2.15 | Iron balance considerations | 51 |
| 1.1.15.1 | A positive iron balance | 53 |
| 1.1.15.2 | A negative iron balance | 53 |
| 1.2.16 | Iron overload | 54 |
| 1.2.17 | Ways of combating iron deficiency | 55 |
| 1.2.18 | Iron requirements and nutritional allowances | 57 |
| 1.2.18.1 | Iron requirements in infancy and childhood | 58 |
| 1.2.19 | Studies on iron deficiency and anaemia in Sri Lanka, and the aetiological/risk factors | 59 |
| 1.3 | Weaning foods | 62 |
| 1.3.1 | Need for weaning foods | 62 |
| 1.3.2 | Characteristics of weaning foods | 63 |
| 1.3.3 | When should weaning foods be first introduced to infants? | 64 |
| 1.3.4 | Home made weaning foods | 66 |

| | | |
|-----------------------------------|---|---------|
| 1.3.5 | Weaning foods used in developing countries | 68 |
| 1.3.6 | Village level weaning foods | 70 |
| 1.3.7 | Weaning foods in industrialized countries | 72 |
| 1.3.8 | Weaning foods used in Sri Lanka | 73 |
| 1.3.9 | Nutritional evaluation of the weaning foods | 74 |
| 1.3.9.1 | Nutritional quality | 76 |
| 1.3.9.1.1 | Protein quality | 77 |
| 1.3.9.1.2 | Biological value as a measure of protein quality | 79 |
| 1.3.9.1.3 | True digestibility and net protein utilization (NPU) | 81 |
| 1.3.9.1.4 | Studies on bioavailability of iron | 84 |
| 1.3.9.1.4.1 | Measurement of the Bio-available iron content | 85 |
| 1.4 | Objectives of the study | 89 |
| CHAPTER 2 - MATERIALS AND METHODS | | 102-158 |
| 2.1 | Selection of Study Sample | 103 |
| 2.2 | Dietary survey | 103 |
| 2.3 | Assessment of iron status of the preschool children | 104 |
| 2.3.1 | Haemoglobin concentration | 104 |
| 2.3.2 | Packed Cell Volume (PCV) | 106 |
| 2.3.3 | Serum Iron (SI) | 107 |
| 2.3.4 | Total Iron Binding Capacity (TIBC) | 110 |
| 2.3.5 | Serum Ferritin | 112 |
| 2.4 | Nutritional evaluation of the weaning foods | 118 |
| 2.4.1 | Chemical evaluation | 118 |
| 2.4.1.2 | Total nitrogen | 119 |

| | | |
|---------------------------------------|---|---------|
| 2.4.1.3 | Fat | 119 |
| 2.4.1.4 | Vitamins | 119 |
| 2.4.1.4.1 | Vitamine A (Beta carotene) | 119 |
| 2.4.1.4.2 | Vitamine B ₁ and B ₂ | 124 |
| 2.4.1.4.3 | Vitamine C | 125 |
| 2.4.1.5 | Minerals (Fe and Zn) | 129 |
| 2.4.1.5.1 | Total iron | 129 |
| 2.4.1.5.2 | Determination of the Zinc content | 132 |
| 2.4.1.6 | Analysis of individual amino acids | 133 |
| 2.4.2 | Biological evaluation of the protein quality | 133 |
| 2.4.3 | Measurement of iron absorption in humans and the effect of ascorbic on iron absorption using the extrinsic tag method | 139 |
| 2.4.3.1 | Experimental design and iron isotope labelling of the gruel | 140 |
| 2.4.3.2 | Iron absorption measurements | 140 |
| 2.4.4 | Measurement of available iron (exchangeable iron) content (in-vitro) | 141 |
| 2.4.5 | Determination of inhibitory factors of iron in the weaning foods. | 145 |
| 2.4.5.1 | Iron binding phenolic groups | 145 |
| 2.4.5.2 | Phytate | 149 |
| CHAPTER 3 - ASSESSMENT OF IRON STATUS | | 159-187 |
| 3.1 | Introduction | 160 |
| 3.1.1 | Assessment of iron status in infants and children | 160 |
| 3.1.2 | Criteria for defining iron deficiency | 161 |

| | | |
|--|---|---------|
| 3.1.3 | Laboratory assessment of iron status | 163 |
| 3.1.3.1 | Haemoglobin | 163 |
| 3.1.3.2 | Packed cell volume | 164 |
| 3.1.3.3 | Serum iron | 165 |
| 3.1.3.4 | Total iron binding capacity and transferrin saturation | 166 |
| 3.1.3.5 | Serum ferritin | 168 |
| 3.1.3.6 | Free erythrocyte protoporphyrin | 172 |
| 3.1.4 | Comparison of biochemical parameters for assessing iron deficiency. | 172 |
| 3.2 | Methodology | 173 |
| 3.3 | Results | 174 |
| 3.3.1 | Haemoglobin concentration | 174 |
| 3.3.2 | Packed cell volume | 174 |
| 3.3.3 | Serum iron and transferrin saturation | 174 |
| 3.3.4 | Serum ferritin level | 175 |
| 3.3.5 | Anaemia and iron deficiency in the study population | 175 |
| 3.3.6 | Iron intake from diet | 176 |
| 3.4. | Conclusion | 177 |
| CHAPTER 4 - NUTRITIONAL EVALUATION OF TRADITIONAL SRI LANKAN WEANING FOODS | | 188-207 |
| 4.1 | Introduction | 189 |
| 4.2 | Methodology | 192 |
| 4.3 | Results | 193 |
| 4.4 | Conclusion | 197 |
| CHAPTER 5 - IRON ABSORPTION FROM TRADITIONAL AND OTHER WEANING FOODS | | 208-249 |

| | | |
|---------|---|-----|
| 5.1 | Introduction | 209 |
| 5.2 | Methodology | 215 |
| 5.3 | Results | 215 |
| 5.3.1 | Bioavailability of iron from the <u>Centella gruel</u> (iron absorption study, (in vivo.)) | 215 |
| 5.3.2 | Iron availability (exchangeability) of the commonly used weaning foods in Sri Lanka (determined in-vitro) | 217 |
| 5.3.2.1 | Traditional gruels | 218 |
| 5.3.2.3 | Iron availability in leafy vegetables used in the preparation of weaning gruels | 219 |
| 5.3.2.4 | Marketed weaning foods | 220 |
| 5.3.3 | Supplementary effect of Thripasha on commonly used weaning foods | 220 |
| 5.3.4 | Supplementary effect of legumes or sprats on iron availability in traditional weaning foods. | 221 |
| 5.3.5 | Enhancing effect of ascorbic acid and/or ascorbic acid rich foods on iron absorption from traditional weaning foods | 222 |
| 5.3.6 | Inhibitory and enhancing factors in weaning preparations | 222 |

XIV

| | |
|--|---------|
| 5.3.6.1 Polyphenols and vitamin C (Ascorbic acid) in leafy vegetables used in the preparation of gruels | 223 |
| 5.3.6.2 The levels of inhibitors of iron absorption in (a) gruels prepared with extracts of leafy vegetables and (b) other weaning mixtures | 223 |
| 5.3.6.3 Phytate levels and iron availability in rice | 225 |
| 5.3.6.4 Phytate content and iron availability in traditional gruels supplemented with legumes | 226 |
| 5.4 Conclusion | 227 |
| CHAPTER 6 - DISCUSSION | 252-272 |
| RECOMMENDATIONS AND LIMITATIONS | 273-274 |
| REFERENCES | 275-319 |
| PUBLICATIONS AND COMMUNICATIONS | 321 |

XVI

List of tables

| | | Page No |
|----------|--|---------|
| Table 1 | Dietary factors increasing and decreasing iron bioavailability | 90 |
| Table 2 | Estimated iron requirement (mg/day) | 91 |
| Table 3 | Requirements for absorbed iron | 92 |
| Table 4 | Steps (1-8) of the radioimmunoassay in the determination of serum ferritin | 154 |
| Table 5 | Distribution of haematological variables in the population studied | 179 |
| Table 6 | Iron status of the study subjects | 179 |
| Table 7 | Prevalence of anaemia and iron deficiency in the population studied | 180 |
| Table 8 | Relation between haemoglobin and other laboratory values indicating iron status | 181 |
| Table 9 | Means and standard deviation of haemoglobin, packed cell volume, serum iron, transferrin saturation and serum ferritin in different age groups | 182 |
| Table 10 | Proportion of children in different age groups having below normal values of haemoglobin and other laboratory parameters | 183 |
| Table 11 | Estimated dietary requirements for iron (mg/day) for a low bioavailability diet | 184 |
| Table 12 | Daily iron intakes of children (9-24 months) | 184 |
| Table 13 | Serum ferritin level in relation to iron intake of children | 185 |
| Table 14 | Comparison of the nutrient content in gruels | |

XVII

| | | |
|----------|---|-----|
| | with commercially prepared weaning mixtures with respect to daily requirements, WHO/FAO/UNU | 198 |
| Table 15 | Amino acid composition (mg/g Nitrogen) in raw materials and leaf gruel | 200 |
| Table 16 | Essential amino acids in breast milk and leaf gruel compared WHO (1985) daily requirements for infants and young children of one to two years | 201 |
| Table 17 | Protein quality of some weaning foods | 202 |
| Table 18 | Nutrient content of raw materials of the leaf gruel and other analysed foods that could possibly be used to improve the nutrient content of the leaf gruel | 203 |
| Table 19 | Nutrient content of the leaf gruel supplemented with other foods | 205 |
| Table 20 | Methods used to determine iron availability | 229 |
| Table 21 | Hematological data of the subjects in in vivo study | 230 |
| Table 22 | Iron absorption measurements from the <u>Centella</u> gruel (with and without ascorbic acid) in the study sample | 231 |
| Table 23 | Bioavailable nutrient density (BND) in one feed of <u>Centella</u> gruel | 232 |
| Table 24 | Amount of absorbed iron and energy required daily by infants and young children | 233 |
| Table 25 | Required bioavailable nutrient density for absorbed iron in the <u>Centella</u> gruel if | |

XVIII

| | | |
|----------|---|-----|
| | it is to satisfy the physiological requirements of the children | 234 |
| Table 26 | Total iron and the available (exchangeable) iron in traditional weaning foods prepared with different types of leaves | 235 |
| Table 27 | Total iron and the available (exchangeable) iron in other common home made weaning mixtures | 236 |
| Table 28 | Iron availability (exchangeability) in common leafy vegetables that are used in the preparation of weaning foods | 237 |
| Table 29 | Total iron and the percentage of available (exchangeable) iron in commonly used marketed weaning foods | 238 |
| Table 30 | Supplementary effect of Thripasha on iron availability of some weaning foods | 239 |
| Table 31 | Supplementary effect of green gram or sprats on iron availability (exchangeability) of the traditional gruels | 240 |
| Table 32 | Enhancing effect of Ascorbic Acid (AA) and Lime juice on iron availability (exchangeability) from the weaning foods | 241 |
| Table 33 | Improvement of iron availability in the weaning foods when supplemented with tomatoes | 242 |
| Table 34 | Polyphenol content and vitamin C levels | |

XIX

| | | |
|----------|--|-----|
| | in the uncooked form of leafy vegetables used in preparation of gruels | 243 |
| Table 35 | Polyphenols, total phosphorous and phytates in the weaning foods | 244 |
| Table 36 | Phytate levels and iron availability in different rice varieties | 246 |
| Table 37 | Effect of supplementation of weaning foods with legumes on the phytate level and iron availability | 247 |

List of figures

| | | |
|--------|---|-----|
| Fig 1 | Changes in body iron during infancy | 94 |
| Fig 2 | The three stage for evaluating iron deficiency | 95 |
| Fig 3 | Schematic representation for the development of iron deficiency anaemia | 96 |
| Fig 4 | Iron absorption from vegetable and animal foods in normal and iron deficient subjects | 97 |
| Fig 5 | Iron in the diet | 98 |
| Fig 6 | Structure formulae of some phenolic compounds with different hydroxylation patterns | 99 |
| Fig 7 | Schematic representation of the transfer of iron from transferrin to an erythrocyte precursor | 100 |
| Fig 8 | Schematic outline of iron metabolism in adults | 101 |
| Fig 9 | Standard curve for the determination of haemoglobin | 155 |
| Fig 10 | Formation of anteferritin-ferritin-antiferritin- ¹²⁵ I complex | 156 |
| Fig 11 | Standard curve for the determination of ferritin | 157 |
| Fig 12 | Flow diagram for automated analysis of ascorbic acid, dehydroascorbic acid and | |

| | | |
|--------|--|-----|
| | total vitamin C in food products | 158 |
| Fig 13 | Distribution of haemoglobin levels of infants and children aged 9 to 24 months | 186 |
| Fig 14 | Serum ferritin levels in infants and children aged 9 to 24 months | 187 |
| Fig 15 | Correlation of serum ferritin levels with haemoglobin concentration of the study subjects | 188 |
| Fig 16 | Nutrient content of 500 ml breast milk and one feed of traditional leaf gruel expressed in % of recommended daily intake (WHO,1985) | 206 |
| Fig 17 | Nutrient content of 500 ml breast milk and one feed of (only the liquid part) traditional leaf gruel expressed in % of recommended daily intake (WHO,1985) | 207 |
| Fig 18 | Effect of ascorbic acid (AA) on iron absorption from the <u>Centella</u> gruel | 248 |
| Fig 19 | Correlation of serum ferritin with iron absorption in the study subject | 249 |
| Fig 20 | Effect of Thripasha on iron availability | 250 |
| Fig 21 | AA and lime juice on iron availability | 251 |