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PREVALENCE AND RISK FACTORS OF IRON DEFICIENCY IN INFANTS

by

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ABSTRACT

High rates of iron deficiency anemia (IDA) (> 24%) and iron deficiency without anemia (ID) (> 50%) have been previously reported among infants. In order to determine whether these rates pertain in a population with a high rate of breast-feeding and universal health care, given that the reformulation of infant cereals in 1976 in Canada increased the bioavailability of iron fourfold, a random sample of 320 6-18 month old infants of all social classes was assessed. Hemoglobin (Hgb), serum ferritin (SF), free erythrocyte protoporphyrin (FEP), height, weight, socioeconomic status (SES), dietary history and 24-hour dietary intake were studied. The prevalence of IDA (Hgb< 110 g/l) was 3.5% while ID (SF< 10 ug/l with Hgb $\geq$ 110g/l) was 10.5%. Nutritional risk factors for iron deficiency include lack of breast-feeding (or low degree or duration), estimated iron intake less than 125% of the RNI and the use of infant cereals for less than 3 months duration. Lack of knowledge of iron-rich foods and perceived lack of physician's counselling on infant nutrition were contributing factors. Weight for length percentiles <10 or >90 and low SES were risk factors. The low SES group demonstrated a higher degree of nutritional risk factors. As premature and low birth weight infants are routinely supplemented with iron, the effect of prematurity and low birth weight on iron status was small. FEP was not found to be a reliable

screening tool for ID. Because of its fairly low sensitivity for Hgb< 110 g/l (63%), its ability to screen for IDA is also limited. Normal and abnormal values for SF and FEP are markedly overlapped and thus, the three stage model for the development of IDA is not generally applicable to infants. As the rates of IDA and ID are low, and especially when it is considered that the importance of the ID state is controversial, it is felt that screening for ID and/or IDA should be limited to those infants most at risk, the level of risk being proportionate to the number of risk factors demonstrated.

DEDICATION

This thesis is dedicated to my husband, Hillel, in appreciation of his ongoing encouragement and support.

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PREFACE

The great questions of time are not decided by speeches and majority...
... but by iron and blood.

Otto von Bismark .
Speech to the Prussian Diet
(1862)

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ABBREVIATIONS

ID.....iron deficiency without anemia

IDA.....iron deficiency anemia

SF.....serum ferritin

FEP.....free erythrocyte protoporphyrin

Hgb.....hemoglobin

rbcs.....red blood cells

CV.....coefficient of variation

r.....Pearson's correlation coefficient

df.....degrees of freedom

SEM.....standard error of the mean

RNI.....Canadian Recommended Nutrient Intake

Part I

INTRODUCTION

Iron deficiency is the most common of all nutritional disorders and infants between six to eighteen months of age are particularly vulnerable (Layrisse, Roche, and Baker, 1976). To date, few studies have examined the prevalence of the iron deficiency state without anemia (ID) and iron deficiency anemia (IDA), and the concurrent iron intakes of infants in Canada.

The prevalence of ID and IDA varies from study to study. The findings are dependent on the criteria employed for diagnosis, and the socioeconomic status and dietary habits of the population (Oski, 1980). The diagnosis of iron deficiency should begin with its definition. A practical definition of iron deficiency would be to consider it present when iron supply is inadequate for the normal synthesis of essential iron compounds (Finch, 1977). There are three definite, but overlapping stages of iron deficiency. Screening tests are available for the detection of each stage (Dallman, 1977).

In the first stage, sometimes termed iron depletion, iron stores are decreased. Serum ferritin (SF), a soluble

protein, is directly proportional to iron stores in normal individuals (Lipschitz, Cook and Finch, 1974). However, because it is an acute phase reactant and rises with inflammation, infection and liver disease, a normal value does not rule out ID (Reeves, Yip, Kiley and Dallman, 1984; Dallman, Siimes and Stekel, 1980). A low value (< 10 ug/l) however, is entirely specific for ID (Labbe and Finch, 1980). Saarinen and Siimes (1978) determined that in severe IDA, low SF values are found. In milder cases, other signs of ID may appear before body iron stores are exhausted and/or SF has reached a subnormal level. It was concluded that SF alone is not a useful screening test for mild ID in infancy. Iron depletion may correct itself without treatment, particularly in late infancy when the rate of growth slows, and if the contribution of iron-containing foods in the diet is increased (Dallman, 1977).

The measurement of bone marrow iron from reticulo-endothelial cells has been the time-honoured method for assessing storage iron. Limitations to this method include disparities between bone marrow aspirates and biopsies, and the discomfort associated with the invasiveness of this method (Cook, 1982).

The second stage is iron deficient erythropoiesis. Erythroid iron supply is decreased but circulating hemoglobin (Hgb) is not significantly diminished. This stage is characterized by a fall in serum iron and a rise in total

iron binding capacity. Almost all the iron in serum is bound to the iron binding protein transferrrin and the ratio of serum iron to total iron binding capacity is termed transferrin saturation. Transferrin saturation is often used as a confirmatory test for ID, but this test has certain limitations. It can fall with a relatively mild infection or with inflammmatory disease. Therefore a low transferrin saturation is not specific for ID (Cook and Finch, 1979).

Another detector of iron deficient erythropoiesis is free erythrocyte protoporphyrin (FEP), or its zinc chelate, zinc erythrocyte protoporphyrin. Since protoporphyrin is the complex that combines with iron to form Hgb, any insufficiency in iron supply is reflected by an accumulation of unbound protoporphyrin in circulating red blood cells (rbcs). FEP levels greater than 100 ug/dl rbcs indicate overt iron deficient erythropoiesis (Yip, Johnson and Dallman, 1984). Limitations of this test include its elevation with inflammatory disease, exposure to lead and protoporphyria. Because of this, abnormal values are not specific to ID (Chisolm and Brown, 1975). FEP may be the more advantageous of the two tests for iron deficient erythropoiesis for the following reasons. FEP is useful in distinguishing ID from thalassemia minor as it rises with ID but not with thalassemia minor. As well, it requires only 0.1 ml of blood (Day, 1976), is more stable (Dallman et al., 1980), and there is uncertainty regarding the lower limit of

normal transferrin saturation in infants (Oski, 1980; Dallman et al, 1980). FEP values during infancy are somewhat higher than adult levels, but it has not been concluded if this is due to ID (Dallman et al., 1980). There is some evidence that the sensitivity of FEP in diagnosing IDA is increased when expressed in relation to Hgb (Thomas, Koenig, Lightsey, and Green, 1977), packed red cell volume or hematocrit (Cook, 1982), or heme concentration (Labbe, Finch, Smith, Doan, Sood and Madan, 1979).

FEP has been advocated as a screening tool for ID (Yip, Schwartz and Dienard, 1983). When assayed using a hematofluorometer, it is quickly and easily determined on a single drop of capillary blood (Blumberg, Eisinger and Lamola, 1977). If the goal of screening for IDA is to detect infants at risk of developing IDA, then FEP is a more direct indicator of inadequate iron nutrition than SF, which indicates inadequate iron stores.

Red blood cell indices such as mean corpuscular volume and mean corpuscular hemoglobin concentration measure microcytosis and hypochromia, respectively and are intermediate in terms of sensitivity between measurements of iron deficient erythropoiesis and frank IDA. A low mean corpuscular volume is also characteristic of thalassemia minor (Cook, 1982).

The third and final stage of iron deficiency is associated with a significant decrease in circulating Hgb,

in other words - overt IDA. The World Health Organization (WHO; 1972) has proposed that a Hgb less than 110 g/l or a hematocrit (packed red cell volume) less than 0.33 l/l be used to identify anemia in children between 6 months to 6 years who are living at sea level. Using frequency distributions of Hgb levels, Dallman and Siimes (1979) have confirmed the WHO lower limit of normality of Hgb. Blacks normally have a Hgb that is 5 g/l lower than that of whites at all ages after the perinatal period but there is some evidence (Reeves, Driggers, Lo and Dallman, 1981) that screening for IDA using identical criteria for blacks and whites is effective. While iron deficiency is the most common cause of anemia, other causes such as inflammatory disease and thalassemia minor are not uncommon among infants and children. Where possible, IDA should be confirmed with other laboratory tests or by a therapeutic trial of iron (Dallman et al., 1980).

The therapeutic iron trial measuring Hgb response is considered to be the "gold standard" against which all other measurements of IDA should be measured. Its limitations include possible compliance problems and high cost (Cook, 1982).

Combinations of hematologic lab tests have been proposed (Saarinen and Siimes, 1978; Thomas et al, 1977). Cook, Finch and Smith (1976) assessed the iron status of a population and indicated that the probability that IDA is

present increases with the number of abnormal blood values discovered. Two abnormal values is arbitrarily recommended for diagnosis . However, a study designed to determine lab test predictors for a significant Hgb response to iron therapy in infants with Hgb less than 115 g/l found that while over half the infants meeting predetermined criteria for iron deficiency had a therapeutic response to oral iron, a large percentage of responders would have been missed if two or more confirmatory tests had had to be abnormal (Dallman, Reeves Driggers and Lo, 1981).

The relationship between socioeconomic status and child health was examined and it was found that the prevalence of ID and IDA, and notably the severe degrees of anemia were more common among the poor. The study was unable to draw inferences about causality (Egbuonu and Starfield, 1982). Selected physical, social and nutritional correlates of IDA in infants of low socioeconomic groups were studied. When compared to non-anemic controls the child with IDA drank more milk, consumed less iron, took less iron supplements, and was introduced to strained food at a later age (Czajka-Nairns, Haddy and Kalbn, 1978).

Myers (1979) retrospectively studied infant feeding patterns in Canada between 1970 and 1972 and found that among 250 infants under one year of age, the lower the family income, the lower were the iron intakes, the use of infant cereals, and the practice of breastfeeding in the family.

Iron appears to be utilized more efficiently in breast-fed infants than in formula-fed infants (Woodruff, Latham and McDavid, 1977). A study by Saarinen (1978) suggests that breast-feeding alone may be insufficient to maintain iron status after six months and that iron supplementation be started after this time. Recently, infants exclusively breast-fed for nine months were studied and it was found that in the great majority of them, iron status was well maintained independent of whether or not the mothers received iron supplements (Siimes, Salmenpera, and Perheentopa, 1984).

In Canada, the practice of breast-feeding has almost tripled over the past 15 to 20 years. According to data collected during the Nutrition Canada Survey, covering the period between 1965-1971, only 26% of mothers initiated breast-feeding (Myers, 1979). In 1982, the national average for the incidence of breast-feeding was 69.4% (Myers, 1982). A recent study of infants in Montreal and Toronto revealed that 71% of mothers breast-fed their infants during the first week postpartum (Yeung, Pennell, Leung, and Hall, 1981a).

With the exception of a study of the iron status of native Canadians (Valberg, Birkett, Hiast and Zamecnik, 1979), there is a paucity of current data on the prevalence of ID and IDA among Canadians. A 1971 survey of child health centres in Toronto, demonstrated Hgb levels less than

100 g/l in 29% of 252 infants screened. The mean estimated dietary iron intake was 9 mg and no significant correlation between iron intake and Hgb levels was observed (Milne, Beaton, Latchford, Vaughn and Moss, 1971).

A major source of data is the Nutrition Canada Survey conducted between 1970 and 1972. Nutritional status was assessed according to region, population type, income and season. The response rate was 46%. An interpretive standard was developed for data analysis (Health and Welfare Canada, 1973). When the WHO (1972) criteria for identification of IDA are applied to the data (Health and Welfare Canada, 1975a), 13.2 to 18.8% of Canadian infants less than one year can be classified as anemic. In metropolitan areas of Ontario, iron status, as judged by transferrin saturation values less than 16%, was at a high risk level for 9.3% of the small sample of 23 infants less than 4 years of age. The concurrent mean dietary iron intake was 40 mg/day among infants less than one year in Ontario. The high mean intake of iron was due to the consumption of infant cereals which were particularly popular in Ontario. Nationally, 84% of the iron intake of infants less than one year was provided by infant cereals (Health and Welfare Canada, 1977).

Valberg, Sorbie, Ludwig and Pelletier (1976) measured iron stores of Canadians using SF assays on serum samples collected during the Nutrition Canada Survey. According to

the interpretive standard for SF, 29 and 48% of children ages 1 to 4 years had high (SF less than 10 ug/l) and moderate (SF between 10 to 20 ug/l) probabilities, respectively, that iron stores were diminished.

In the above three Canadian studies, mean iron intakes were well above daily requirements (Health and Welfare Canada, 1963, 1975b and 1983). The discrepancy between the hematologic values and the high iron intakes may be attributed to the low bioavailability of the iron contained in infant cereals at the time. Prior to 1976, the dietary iron in infant cereals was mainly in the form of sodium iron pyrophosphate with an absorbability of less than 1%. In 1976 in Canada (and in 1972 in the U.S.) this was replaced by reduced iron of small particle size which is absorbed at a level of about 4% (Rios, Hunter, Cook, Smith and Finch, 1975). This is an important change as infant cereals supply children under one year with an average of 62% (Yeung, Pennell, Leung, Hall and Anderson, 1981b) to 84% (Health and Welfare Canada, 1977) of their dietary iron.

Yeung et al. (1981b), have assessed the iron intakes of infants in Montreal and Toronto in a longitudinal study over the first 18 months of life. Food records indicated that among infants 3 to 10 months of age, infant cereals contributed about 70% of the dietary iron. Before and after this time the percentage contribution of cereal iron decreases. Although the mean dietary iron intake levels

were adequate except in the first and eighteenth months, between 18 to 39% of infants 6 to 18 months of age consumed less than the recommended iron intake. These authors, and others (Fomon, Filer, Anderson, and Ziegler, 1979) recommend that cereal feeding be continued until 18 to 24 months of age. Based on iron intake, iron deficiency was estimated by probability analysis and ranged from 21 to 39% at 6 and 18 months, respectively. No hematologic tests were done to confirm these estimates. Because of the limitations of the probability analysis technique these authors and the Canadian Paediatric Society (1979) support the use of biochemical data to determine the current prevalence of iron deficiency in Canadian infants.

Brault-Dubuc, Nadeau and Dickie (1983) longitudinally studied the dietary iron intake and the iron status of French-Canadian children from birth to 3 years. Between 6 and 18 months the percentage of infants with Hgb <11 g/l dropped from 7 to 1%. At 18 months, 29.2% of infants exhibited SF values <10 ug/l. Between 6 and 18 months of age, 11.2 to 55.3% of infants received less than the recommended levels of iron intake. The major limitations of this study are that the population had not been randomly sampled from French-Canadian families and that the children came from middle to upper class families. As iron deficiency is strongly related to socioeconomic status, the results cannot be extrapolated to the French-Canadian population as a whole.

Owen and Lippman (1977) reviewed studies conducted between 1967-1977 in the U.S.. Among the three national studies, the Ten State Survey, the Preschool Nutrition Study and the first Health and Nutrition Examination Survey, 20 to 30% of young children had iron intakes less than 5 mg/day; an unacceptable level. Nutrient intakes reflected the socioeconomic status of the family, when race, sex and age were held constant. A review of 25 regional and local studies indicated the prevalence of ID to be 23 to 50% and IDA to be 10 to 40% in infants.

Oski's review (1980), indicated the prevalence of IDA defined by Hgb less than 110 g/l to be 3 to 24% among infants 6 to 24 months old in the U.S.. Using transferrin saturation less than 16% as the criterion for iron deficiency, 29 to 68% of this population was found to be iron deficient. This summarizes studies published between 1970 to 1980.

It is possible that the type of health care delivered may play a role in the prevalence of ID and IDA. In studies of infants in private practices, the rate of ID was 7.4% (FEP >100 ug/dl rbcs) (Dine, 1980) and the rate of IDA was 14% (Hgb <110 g/l) (Fuerth, 1971). These rates are in the low to moderate ranges of the U.S. reviews described above (Owen and Lippman, 1977; Oski, 1980).

Because of the high risk of IDA, the American Academy of Pediatrics (1981) has recommended general screening for

anemia in infants at 6-7 months. Others have suggested general screenings between 9 to 24 months (Schmidt, 1984; Gershel, 1984). The screening of only high risk infants has also been advocated (Eggertsen, Schneeweiss and Bergman, 1980).

The Canadian Recommended Nutrient Intake (RNI) for iron was established to estimate the amount of dietary iron necessary to maintain reasonable degrees of iron storage, not upon amounts required to prevent IDA or other clinical symptoms. The regulation of iron levels in the body is governed by the absorption of iron from the intestinal tract. As deficiency develops, the body adapts by increasing the efficiency of iron absorption. When iron intake exceeds requirements, the efficiency of iron absorption decreases. Estimation of iron requirements is difficult in that it is necessary to establish the desired nutritional status and the efficiency of absorption associated with that target nutritional status (Health and Welfare Canada, 1983).

The bioavailablilty of iron is also affected by the nature of the diet (Layrisse, Martinez-Torres and Roche, 1968). Food iron is found in heme and non-heme forms. Non-heme iron is the primary form and it exists as inorganic iron III (ferric) complexes. Heme iron is found in the heme proteins, hemoglobin and myoglobin, which are present in meat. The absorption of heme iron is generally high and is

not as affected by the nature of the diet as non-heme iron (Dallman et al., 1980).

Over the past 16 years numerous publications (Canadian Paediatric Society, 1979, 1980; American Academy of Pediatrics, 1980, 1969) have reviewed iron requirements and have recommended several ways of meeting them. The RNI of iron for infants 6 to 12 months and 12 to 18 months is 7 and 6 mg/day respectively (Health and Welfare Canada, 1983). The U.S. Recommeded Dietary Allowance for iron is 15 mg/day for infants 0.5 to 3 years (National Research Council, 1980). It is suggested that iron supplementation start no later than 4 months (or 6 months in the exclusively breast-fed infant) in term infants, and no later than 2 months in pre-term infants and that it continue at least through the remainder of the first year (Canadian Paediatric Society, 1979; American Academy of Pediatrics, 1976) or to 18 months (Fomon et al., 1979). Recommended iron sources include iron fortified formulae and infant cereals and meats. If sufficient iron is not provided in the diet, medicinal iron preparations may be used (Canadian Paediatric Society, 1979, 1980; American Academy of Pediatrics, 1969; Fomon et al, 1979; Woodruff, 1978).

This study was undertaken, given that all patients under the Canadian health care system are private patients, that there has been a considerable increase in breastfeeding over the past 15 to 20 years and that since 1976 infant cereal,

as the single most important source of iron in infants' diets, has been improved. Its goals were to determine the prevalence of ID and IDA and to identify risk factors so that only those infants at risk need be screened. Additionally, improved nutrition education and compliance enhancing techniques could be targeted more specifically to high risk groups.

Part 2

METHOD

Ethics

The research protocol was found ethically acceptable by the research and ethics committee of the Children's Hospital of Eastern Ontario (CHEO) and review committees of the other hospitals concerned. Free, informed consent from parents was obtained and all information was held confidential.

Sample Population

Infants born in four Ottawa general hospitals with maternity services were randomly selected from case-room records. Those born and living in the Ottawa-Carleton area who were between six and eighteen months of age and living with their natural mothers were included in the study.

Sample Size

Based on an estimate of the prevalence of ID at 29% (Valberg et al., 1976) with 95% confidence limits at + 5% (e.g. 24 - 34%), the sample size required was determined to be 317 (Kahn, 1983). The number of infants enrolled in the study was 320.

Home Interviews

Infants' families were contacted by telephone and the nature of the study was explained to them. If permission was granted, the researcher visited the home for the structured interview and the drawing of the blood sample. Interviews were conducted in English or French. A publication containing information on dietary sources of iron and the role of iron in the body was reviewed with every family and left with them for reference (Ontario Ministry of Health, 1983).

Questionnaire

The questionnaire consisted of a health review, a socioeconomic profile, and a nutritional history (see Appendix A), and was modelled after the Nutrition Canada Survey questionnaire (Health and Welfare Canada, 1973).

Participants were divided into three broad socioeconomic categories using the Blishen scale of parental occupation (Blishen, 1976). When these groupings were compared with income statistics from the 1981 Canadian Census, it was found that there was a slight underrepresentation of the lowest socioeconomic group.

Iron intake

Iron intake was estimated from a 24 - hour food recall. The 24 - hour recall has been determined to be a reliable method with which to estimate the dietary intakes of children when parents provide the child's food (Klesges, Klesges, Brown, Weber, Manderfeld and Swenson, 1985). Utensils used by the parent were often examined by the interviewer to verify food quantities. If the recall was not considered to be representative of the infant's intake, for example during sickness, it was noted to be atypical. Atypical intakes were not retained for analysis. The number of discarded food diaries was 19, or 5.9% of the total sample.

The 24 - hour recall was used to determine the dietary intake of iron which was then expressed as a percentage of the RNI (Health and Welfare Canada, 1983) to reflect adequacy, as mg iron per 1,000 kcal (4.184 MJ) to reflect the iron density of the diet and as mg iron/kg body weight to reflect iron intake for size. The computerized NUTS - Nutritional Assessment System (Quilchena Consulting Ltd., Victoria, B.C.) was used to analyze the dietary data. The limitations of this program are the same as those inherent in manual nutritional assessment. Nutient values used in food composition tables, which form the data bank of the program, are extremely variable due to hereditary, environmental, processing and analytical factors. Iron

values of foods included in the program represent the mean values of two or more pooled samples and, as such, can be considered generally representative of the infants' actual intakes.

The iron content of breast milk was estimated in the following manner. The energy content of the diet was calculated from the 24 - hour recall and was subtracted from the infant's recommended energy intake based on the RNI. Any remainder was assumed to be filled by energy from breast milk. The iron concentration of breast milk was calculated assuming that it contains less than 1 mg iron/ 1,000 kcal (4.184 MJ; Dallman et al., 1980)

Physical Measurement

A metric infant scale (Continental Scale Corp, Model 380) was used for weight determination. The accuracy of the scale was verified using standardized weights. Infants were weighed in diapers and light clothing.

Length was measured by lying the infant in a supine position on examining paper on a hard surface and marking off the head and feet (while flexed). A metal tape measure was used to measure the length in centimeters.

Blood Procurement Technique

Capillary blood samples were collected using microtainer collector tubes. The CHEO (1983) micromethod (see Appendix B) was used with the exception that an automatic skin puncture instrument, or hemalet with sterile needles, (Canlab, Montreal, Que.) was used instead of sterile lancets. Microtainer tubes with potassium EDTA as anticoagulant (Benton Dickinson Vacutainer Systems, Rutherford, New Jersey) were used for sample collection and 300 ul of blood were collected in each of 2 tubes from each infant.

For practical reasons, the quantity of blood available for testing and hence, the number of tests that could be performed, was limited by the amount of blood that could be obtained by capillary sampling.

Complete Blood Count

Hgb, hematocrit and red cell indices (mean corpuscular volume and mean corpuscular hemoglobin concentration) were determined electronically using either the Ortho Elt-800 Hematalogy Analyzer (Ortho Diagnostic Systems, Westwood, Mass.) or the Coulter Counter Model M430 (Coulter Electronics, Hialiah, Fla.).

The Ortho Elt-800 and the Coulter M430 usually agree within 1 standard deviation of each other. The day to day coefficient of variation (CV) for Hgb is determined using

commercial controls; Paralaser control (Streck Labs, Inc., Omaha, Neb.) for the Ortho Elt-800 and M-cal Calibration (Coulter, Houston, Tex.) for the Coulter M430. On the Ortho Elt-800, the CV for Hgb, based on 27 days of testing was 1.1%. On the Coulter M430 the CV for Hgb, based on 31 days of testing was 1.0%.

Samples were analyzed within 4 hours of their procurement. Blood smears were prepared and examined by registered hematology technologists for ID and IDA characteristics such as irregularly shaped cells, hypochromia and microcytosis.

Free Erythrocyte Protoporphyrin

FEP was determined fluorometrically according to the acid extraction method of Nelson (Day, 1977). This technique was modified by the use of celite. One hundred microliters of each anticoagulated whole blood sample was added to 100 ul of 5% celite (weight/volume) in suspension in saline (0.9% NaCl) (Piomelli, 1973). The use of celite facilitated the purification of the porphyrin-containing ethyl acetate/glacial acetic acid solution by producing a celite-protein sediment that adhered firmly to the bottom of the test tube.

In states of iron deficiency and lead poisoning, most protoporphyrin is in the form of zinc protopophyrin IX. In the ethyl acetate/glacial acetic acid and the HCl

extractions, the zinc protoporphyrin is extracted from erythrocytes and dissociates. Upon dissociation it is known as free protoporphyrin di-cation, or FEP. The fluorescence of the extracted FEP in HCl is then measured (Chisolm and Brown, 1975).

The coproporphyrin-I standards (stock # COP-I-5, Sigma Chemical Co., St. Louis, Mo.) were prepared according to instructions and contained 0.5 ug coproporphyrin I/ml HCl. Coproporphyrin I at concentrations of 0.05, 0.10, 0.25 and 0.50 ug/ml were used as standards, and one blank and two identical whole blood pool samples were carried through the procedure. Samples were centrifuged in the IEC HN-S Centrifuge (Damon/IEC Division, Needham Hts, Mass.).

The final extract was measured in the Turner Fluorometer, Model 111 (G.K. Turner Assoc., Palo Alto, Ca.). This was equiped with a high sensitivity sample holder (Turner No. 110-865), a primary filter of 405 nm (Turner No. 110-812) for excitation and a secondary filter of 595 nm (Turner No. 110-820) for emission.

FEP ug/dl rbcs was calculated by dividing FEP/dl blood by Hct (l/l). All FEP tests were performed within one week of blood sample procurement.

Based on 12 assays, the day to day CV FEP of the pool was 6.5% with a mean of 32.1 ug/dl rbcs.

Serum Ferritin

SF was determined by a commercially available kit (Bio-Rad Laboratories (Canada) Ltd., Mississauga, Ont.). In this two-site immunoradiometric assay, the antigen (SF) is "sandwiched" between 125I-labelled antibody to ferritin and ferritin antibodies immobilized on polyacrylamide beads. The 125I-labelled antibody is the tracer and the immobilized antibodies provide the solid phase.

Samples were centrifuged in the IEC HN-S and IEC UV centrifuges (Damon/IEC Division, Needham Hts, Mass.). 125 I decay was measured in the Beckman Gamma 4000 (Beckman Instruments, Fullerton Ca.). Samples not being analyzed within a week of procurement were frozen until testing.

An extra control was used in the latter assays (Lyphochek Immunoassay control serum (human) - Level I, product No. c-370-5, Bio-Rad) to assess the accuracy and precision of the immunoassay procedure. Based on 5 assays, the day to day CV of the SF pool was 15.7% with a mean of 22.2 ug/l. Contributing to the high CV are the small number of samples and the larger variation of the first sample tested.

Chemicals

All chemicals used were reagent grade (A.C.S.; Fisher Scientific Ltd. Canada, Ottawa, Ont.) and were not further purified.

Statistical Analysis

Chi square, Fisher's exact test, test for linear trends, t-test and analysis of variance were used as appropriate. The distribution of iron intake, expressed as mg iron/RNI x 100, mg iron/1,000 kcal (4.184 MJ) and mg iron/kg body weight, were markedly skewed toward lower values; therefore, these data were analyzed after logarithmic transformation. The distribution of FEP and SF values were skewed toward higher values and were also transformed logarithmically. Duration of breastfeeding data was transformed by square root. Transformations resulted in more normal distributions and were used for analyses of variance and Pearson's correlations. When relationships between iron indices were studied using Pearson's correlation coefficient (r), scatterplots of the associated pairs of variables were examined to verify that linearity was present.

All data were computer analyzed using the Biomedical Computer Program (BMDP; Dixon, Brown, Engelman, Frane, Hill, Jennrich and Toporek, 1981) and Statistical Package for the Social Sciences (SPSS; Nie, Hull, Stienbrenner and Bent, 1975) programs. P values less than 0.05 are considered to be statistically significant.

Part 3

RESULTS

CHARACTERISTCS OF THE STUDY POPULATION

Parents were contacted by telephone and 90% agreed to have their infants participate. Parents of 35 infants declined to participate. There were no systematic attempts made to determine if there were significant differences between those who did and did not accept to be involved. However, the usual reasons given for declining e.g. the infant was afraid of needles, had recently had blood taken, etc. suggest that the sample was not biased in favour of better nourished infants or better educated parents.

All social classes were represented; the lowest grouping was slightly underrepresented presumably because entry into the study necessitated having a telephone.

The infants' ages ranged from 6 to 18 months, inclusively, with approximately 25 in each age group. The prematurity rate of the study infants (4.7%) was slightly less than the regional prematurity rate (6.6%).

PREVALENCE OF THE IRON DEFICIENCY STATE WITHOUT ANEMIA

The prevalence of ID was defined as serum ferritin < 10 ug/l (Dallman et al., 1980) with a Hgb of 110 g/l or more; 10.5% of infants fell into this category.

With a sample size of 320, the 95% confidence interval for the rate of ID in this population is 10.5 ± 3.3% (7.2 - 13.8%).

PREVALENCE OF IRON DEFICENCY ANEMIA

Mild anemia was defined as Hgb levels between 100 and 110 g/l; only 2.2% of infants had Hgb levels in this range. Moderate to severe anemia was defined as Hgb levels less than or equal to 100 g/l; only 1.3% of infants had Hgb levels in this category. Thus, 3.5% of infants were found to be anemic. All infants determined to be anemic had other evidence of iron deficiency (hematocrit < 0.33 1/1, mean corpuscular volume < 70 fl (Dallman and Siimes, 1979), SF < 10 ug/l , FEP > 100 ug/dl rbcs and/or a blood smear compatible with IDA).

With a sample size of 320, the 95% confidence interval for the rate of IDA in this population is 3.5 ± 2% (1.5 - 5.5%).

FREE ERYTHROCYTE PROTOPORPHYRIN IN SCREENING FOR IRON DEFICIENCY

As a screening tool it is necessary to select an appropriate FEP cutoff value so that infants with ID or IDA are detected (high sensitivity) whereas most infants who are free of these states are excluded (high specificity). Various FEP values from 40 ug/dl rbcs (50th percentile) to 100 ug/dl rbcs (98th percentile) were compared to determine the best cutoff point (see Table 1). The 98th percentile value of 100 ug/dl rbcs corresponds to the uppermost limit of FEP values among healthy 1 to 2 year olds who are free of ID, IDA and lead poisoning (Yip et al., 1984).

Of the infants with a low level of SF, only 5.9% were detected at the FEP cutoff point of 100 ug/ml rbc. At this point, the specificity (percent of infants with normal SF correctly identified as such) is very high at 98.2%.

At the lowermost cutoff point (40 ug/dl rbcs) 76.5% of infants with a low SF are detected. This however, is at the expense of a large decrease in specificy and only 52.5% of infants with normal SF levels are accurately identified by FEP. There is no cutoff point between these two values that can be effective for the screening of low SF values in

Table 1: Free Erythrocyte Protoporphyrin at Different Cutoff levels in the Detection of Infants with Low Levels of Serum Ferritin (<10 ug/l)

FEP (ug/dlrbcs) screening cutoff point (percentile rank)	* % of subjects with low SF correctly identified as low by FEP (SENSITIVITY)	** % of subjects with normal SF correctly identified as normal by FEP (SPECIFICITY)
40.2 (50.0%)	76.5	52.5
49.9 (75.0%)	47.1	77.5
63.5 (90.0%)	17.6	90.7
73.8 (95.0%)	11.8	95.7
86.2 (97.5%)	5.9	97.9
100.0 (98.0%)	5.9	98.2

* screen-positive subjects (SF< 10 ug/l and FEP> cutoff limit) divided by the total number of subjects with SF< 10 ug/l.
**screen-negative subjects (SF>= 10 ug/l and FEP< cutoff limit) divided by the total number of subjects with SF > 10 ug/l.

infants. In this study, the ability of FEP to screen for low SF with a satisfactory degree of sensitivity and specificity is poor at any cutoff level.

Table 2 compares different FEP values in the detection of infants with a low Hgb concentration (sensitivity) and the identification of those who are properly categorized as screen-negative (specificity). At the cutoff point of 40 ug/dl rbcs, sensitivity is fairly high at 81.8% but the specificity is low at 50.8%. A high specificity level of 97.0% can be attained at 73.8 ug/ml rbc (95th percentile)

and this is accompanied by a low to moderate rate of sensitivity (63.6%). The ability of FEP to be used as a screening test for low Hgb (IDA) is fair at the 73.8 ug/dl rbcs level.

Table 2: Free Erythrocyte Protoporphyrin at Different Cutoff Levels in the Detection of Infants with Low Levels of Hemoglobin (< 110g/l).

FEP (ug/dlrbcs) screening cutoff point (percentile rank)	* % of subjects with low Hgb correctly identified as low by FEP (SENSITIVITY)	** % of subjects with normal Hgb correctly identified as normal by FEP (SPECIFICITY)
40.2 (50.0%)	81.8	50.8
49.9 (75.0%)	72.7	76.7
63.5 (90.0%)	63.6	91.8
73.8 (95.0%)	63.6	97.0
86.2 (97.5%)	54.5	99.3
100.0 (98.0%)	45.5	99.3

* screen-positive subjects (Hgb<10 ug/l and FEP> cutoff limit) divided by the total number of subjects with Hgb< 10 ug/l.
**screen-negative subjects (Hgb>= 10 ug/l and FEP< cutoff limit) divided by the total number of subjects with Hgb > 110 g/l.

RELATIONSHIPS WITHIN AND BETWEEN IRON INDICES

A description of iron indices within the iron status groups, and in the total sample, is found below (see Table 3). The iron status groups are defined by Hgb and/or SF concentrations.

Table 3: Relationship Between Iron Status and Iron Indices

Iron Status (n)	Hgb (g/l)	FEP (ug/dl rbcs)	SF (ug/l)
Normal(271)	125 ± 0.4 (110-144)	41.1 ± 0.82 (12.3-104.7)	28.4 ± 1.2 (10.5-188.0)
ID (33)	122 ± 1.5 (110-148)	50.8 ± 3.58 (11.8-133.4)	7.1 ± 0.3 (2.5-9.9)
mild IDA(7)	107 ± 1.1 (102-109)	71.5 ± 15.30 (33.7-131.0)	35.6 ± 17.2 (10.5-137.0)
mod.-severe IDA (4)	87 ± 8.9 (61-100)	157.4 ± 48.83 (96.9-261.1)	10.9 ± 3.8 (2.0-20.5)
total sample(315)	124 ± 0.5 (61-148)	44.3 ± 1.22 (11.8-261.1)	26.0 ± 1.13 (2.0-188.0)

The results are expressed as means ± 1 standard error of the mean (SEM). Bracketed values denote ranges.

The greater the severity of iron deficiency the more abnormal the measurements of FEP become (Table 3). In milder cases, FEP shows a broader distribution extending from normal to abnormal values. Only one of the 271 infants with normal iron status (0.4%) and 4 of the 33 infants with ID (3%) had FEP levels above 100 ug/dl rbcs. This is in contrast to 3 of 7 mildly IDA (43%) and 3 of 4 (75%) moderate to severely IDA infants having FEP levels greater than 100 ug/dl rbcs.

FEP is inversely related to the Hgb level for the entire sample ($r= -0.36$, $p< 0.0001$). As the severity of iron deficiency increases, the relationship between FEP and Hgb becomes more closely correlated and r is significant in the groups with ID or IDA (see Table 4).

The positive correlation between Hgb and SF levels for the entire sample is weak ($r= 0.11$, $p= 0.02$). The relationship between SF and Hgb also becomes more closely correlated with increasing severity of iron deficiency, however none of the correlations are significant(Table 4).

The inverse correlation (r) between SF and FEP is -0.22 ($p= 0.0004$) for the entire sample. SF and FEP demonstrate a moderately strong and significant correlation ($r= -0.64$, $p= 0.02$) at the point of IDA (Hgb< 110 g/l) but there is little correlation between values among infants with normal iron status and ID (Table 4).

Table 4: Association Between Iron Status and Iron Indices Relationships

Iron Status	Correlations		
	Hgb x FEP	Hgb x SF	FEP x SF
Normal	r= -0.09 p= 0.07 (n=270)	r= 0.08 p= 0.11 (n=271)	r= -0.07 p= 0.12 (n=270)
ID	r= -0.37 p= 0.01 (n=35)	r= 0.28 p= 0.06 (n=33)	r= -0.16 p= 0.19 (n=33)
IDA	r= -0.69 p< 0.01 (n=11)	r= 0.31 p= 0.18 (n=11)	r= -0.64 p= 0.02 (n=11)

Pearson correlations were performed after FEP and SF values were transformed logarithmically to produce more normal distributions.

NUTRITIONAL FACTORS AND IRON STATUS

Breast-Feeding

There was a significantly higher degree of moderate to severe IDA among infants not breast-fed (4.0%) than among infants breastfed (0.4%) when they were compared to infants of normal iron status (Table 5).

Table 5: Association Between Breast-Feeding and Iron Status

Iron Status (n)	No Breast-Feeding (%)	Breast-Feeding (%)
normal (271)	81.2 a	87.5 b
iron deficient (33)	13.3	9.6
mild IDA (7)	1.3	2.5
moderate - severe IDA (4)	4.0 a	0.4 b

Values in a horizontal line followed by different letters are significantly different from each other. Fisher's exact test, p= 0.04.

There was a tendency for the degree of breast-feeding to influence iron status. When ID and IDA were pooled, it was

found that they varied inversely with increasing degrees of exclusivity of breast-feeding (Table 6).

Table 6: Association between Degree of Breast-Feeding and Iron Status

Iron Status (n)	No Breast-Feeding (%)	Breast-feeding & Formula (%)	Exclusive Breast-feeding (%)
normal (271)	81.3	82.9	88.3
ID/IDA (44)	18.7	17.1	11.7

Chi-square = 2.5 with 2 degrees of freedom (df)
p = 0.28.

Duration of breast-feeding and its effect on iron status was studied. The prevalence of ID and/or IDA tended to increase with decreasing duration of breast-feeding (see Table 7).

The mean breast-feeding duration ± SEM for infants of normal iron status was 4.5 ± 0.25 while that for infants with ID and/or IDA was 3.7 ± 0.59. After logarithmic transformation, a t-test was performed and no significant differences were found (t = 1.46, 313 df, p= 0.14).

Table 7: Association Between Duration of Breast-Feeding and Iron Status

Iron Status (n)	Duration of Breast-Feeding 0	< 6 months	>6 months
normal (271)	81.3	85.6	89.3
ID/IDA (44)	18.7	14.4	10.7

Chi-square = 2.5 with 2 df, p = 0.29;
Test for linear trends, p = 0.11.

Overall, 76% of mothers breast-fed their infants upon leaving the hospital. This compares favorably with the national rate of 69.4% (Myers, 1982).

Introduction of Solids

Delayed introduction of solids did not contribute to an increase in ID and/or IDA. Conversely, earlier introduction of solids was associated with an increased risk and infants receiving solids prior to 2 months of age experienced almost twice the rate of ID and/or IDA than those receiving solids after this time (Table 8).

Table 8: Relationship Between Age Introduction of Solids and the Prevalence of ID/IDA

Age of Introduction of Solids (months)	ID/IDA (%)
0 < 2 (n=26)	23.1
2 < 4 (n=79)	13.9
4 < 6 (n=121)	12.4
6 + (n=89)	13.5

Iron Intake

Only 3.8% of infants were receiving iron supplements at the time of the interview. Iron intake was expressed in a variety of ways as described in the Method section. Results of iron intake for the sample population are found below (see Table 9).

When the effect of iron intake on iron status (normal, ID, mild IDA, moderate to severe IDA) was analyzed by analysis of variance, no significant relationships were found.

Infants were grouped according to their consumption of iron as a percentage of the RNI, and the percentage of infants within each group with ID and/or IDA was determined (Table 10). Although there were no statistically

Table 9: Iron Intake of the Sample Population

Iron Intake	Mean ± SEM	Median	Range
mg/24 hours	9.5 ± 0.28	8.7	2.1 - 30.3
% RNI	148.3 ± 4.20	135.0	31.4 - 432.9
mg/1,000 kcal (4.184 MJ)	9.7 ± 0.32	7.8	2.4 - 35.3
mg/kg body weight	1.0 ± 0.03	0.8	0.2 - 3.2

significant differences between the expected and the observed distributions in the chi-square analysis, a significant linear trend was observed.

Table 10: Relationship Between Iron Intake and Iron Status

% RNI FOR IRON (n)	ID/IDA (%)
< 75 (37)	21.6
75 - 125 (90)	17.8
>= 125 (169)	10.7

Chi-square = 4.4 with 2 df, p = 0.11;
Test for linear trends, p = 0.04.

The median value (50th percentile) for iron density of the diet was 7 75 mg iron/1,000 kcal (4.184 MJ). Among infants receiving less than the median value, the percentage of infants with ID and/or IDA was 17.8. Among those receiving at least the median value the percentage of ID and/or IDA was 10.7 (Chi-square = 3.1 with 1 df, p = 0.08).

It has been suggested that term infants consume 1 mg iron/kg body weight per day to a maximum of 15 mg, starting at 4 months of age (National Research Council, 1980; American Academy of Pediatrics, 1976; Canadian Paediatric Society, 1972). Among infants receiving less than 1 mg iron/kg body weight the rate of ID and/or IDA was 15.8% while among those receiving at least 1 mg iron/kg body weight the rate of ID and/or IDA was 11.6% (Chi-square = 0.9 with 1 df, p = 0.32).

Infant Cereals

One of the most important factors to influence the iron content of the infant's diet is iron-fortified infant cereal. Only 7 infants in the sample (2.2%) never consumed infant cereals. Iron intake was significantly higher among infants who were still receiving infant cereals than among those who had discontinued infant cereals. This observation held true when iron intake was expressed as mg iron per 24 hours, per 1,000 kcal (4.184 MJ), per kg body weight or as a percentage of RNI. Using t-tests, significance was found at < 0.0001 level (see Table 11).

Table 11: Relationship Between Cereal Consumption and Iron Intake

Measures of Iron Intake	No Cereals (n= 157)	Cereals (n= 158)
mg Fe/24 hours	7.7 ± 0.29a	11.5 ± 0.44b
% RNI for Fe	125.3 ± 4.70a	171.8 ± 6.60b
mg Fe/1,000 kcal (4.184 MJ)	6.8 ± 0.25a	12.4 ± 0.49b
mg Fe/kg body weight	0.8 ± 0.03a	1.2 ± 0.05b

The results are expressed as means ± SEM of raw values. After these values were logarithmically transformed to produce a more normal distribution, t-tests were performed. Values in horizontal lines followed by different letters are significantly different from each other ($p < 0.0001$). A separate t-test was performed for each measure of iron intake.

The prevalence of IDA in infants who had been on infant cereals a minimum of 3 months was decreased by at least 47% when compared with infants who had consumed infant cereal for less than 3 months. None of the 12 infants who received cereals for longer than 12 months had IDA (see Table 12). These differences were not accounted for by age as there were no significant differences between the mean ages of infants in the normal, ID and IDA groups when they were

analyzed by analysis of variance. No trends were evident when ID and duration of cereal consumption were studied:

Table 12: Association Between Duration of Infant Cereal Consumption and Iron Deficiency Anemia

Months Duration	(n)	% IDA
0 < 3	(88)	5.7
3 < 6	(100)	3.0
6 < 9	(78)	2.6
9 < 12	(37)	2.7
12 +	(12)	0

Identification of Iron-Containing Foods

Parents' knowledge of dietary sources of iron was examined. Thirty-three percent of parents could not identify a single source of iron. Of the 4 infants with moderate to severe IDA, 3 had parents who could not identify a single iron source. The rate of ID and/or IDA among infants whose parents could not identify an iron source was 17.1%, while the rate among infants whose parents identified at least one iron source was 12.4% (Chi-square = 1.3, 1 df, $p = 0.25$).

Parents' Perception of Physicians' Counselling on Infant Nutrition

Parents were asked how often their infants' primary care physicians discussed nutrition during well-baby visits. Forty-nine percent responded "always", 45% "sometimes" and 5% "never". There was a clear inverse relationship between the perceived frequency of nutritional counselling and the rate of ID and/or IDA. The prevalence of ID and/or IDA among infants whose parents perceived the physician always to discuss nutrition was almost 3 times lower than among those whose parents stated that the physician never discussed nutrition (Table 13).

Table 13: Relationship Between Nutritional Counselling by Physicians and Iron Status

Nutrition Discussed (n)	ID and/or IDA (%)
always (155)	11.0a
sometimes (144)	15.3
never (16)	31.3b

Values in a column followed by different letters are significantly different from each other (Fisher's exact test, p = 0.04).

MEDICAL FACTORS AND IRON STATUS

Prematurity

The relationship between prematurity for date and/or weight and iron status was studied. Since it is the policy of physicians in the Ottawa-Carlton area to prescribe supplemental iron for premature or low birth weight infants, only 1 infant of 7 less than 36 weeks and weighing less than 2,500g at birth, had a Hgb < 110 g/L.

Percentile Weight for Length

The rate of ID and/or IDA among infants below the 10th and above the 90th pecentile weight for length was approximately twice that among infants between the 10th and 90th pecentiles (Table 14).

Various factors were examined to explore why a higher rate of ID and/or IDA was observed among infants in the high and low percentile groups. Nutritional factors included energy and iron intakes expressed as percentages of RNI to reflect adequacy and iron intake per kg body weight to reflect iron need for size. Non-nutritional factors included whether the infant was sick at the time of the interview and the number of times the infant was seen by a physician in the previous 3 months for other than well-baby

Table 14: Relationship between Percentile Weight for Length and ID/IDA

Percentile Weight for Length (n)	ID/IDA (%)
<10 (31)	22.6
10 - 90 (268)	12.3
>= 90 (16)	25.0

visits. All of these factors may affect iron status. Results are summarized below (see Table 15).

A lower percentage of infants in the <10th percentile category consumed less than the RNI for energy and iron and less than 1 mg iron/kg body weight. However, more infants in this category were sick and a higher percentage had seen a physician in the previous 3 months for other than a well-baby visit.

The profile is reversed for infants in the > 90th percentile group. By chi-square analysis, distributions in the 3 percentile categories were significantly different in the energy as a percentage of RNI and mg iron per kg body weight categories with higher percentages of infants in the >90 percentile group receiving less than 100% of the RNI for energy and less than 1 mg iron per kg body weight.

Table 15: Association Between Various Factors and Percentile Weight for Length

Factors	Percentiles <10 (%)	10 - 90 (%)	>= 90 (%)	chi-square analysis
< 100 % RNI for energy	32	42	71	chi-square =6.1; 2 df, p<0.05
< 100% RNI for iron	25	31	36	*
< 1 mg iron/kg body weight	46	62	93	chi-square =8.5; 2 df, p=0.01
sick	67	58	43	*
> 1 visit to MD in previous 3 months	55	44	25	*

* Chi-square analysis could not be done as there were too few observations per cell.

SOCIOECONOMIC FACTORS AND IRON STATUS

Differences in nutritional and hematologic characteristics of the social groups appear to be most striking between the high and low, and the middle and low groups. For the purpose of identifying statistically significant differences more easily, the high and middle groups were pooled and their results were compared with those of the low social group. Table 16 summarizes the results. The prevalence of moderate-severe IDA, total IDA and pooled ID and IDA are 2 to 5 times higher among those infants in the low social group than those among those in the middle-high group.

Nutritional factors varied between social groups as follows. The proportion of infants breast-feeding was 30% lower in the low social group than in the middle to high group and these differences were significant ($p <= 0.0001$). Of the infants who had discontinued infant cereals, a higher proportion of those in the low social group discontinued after less than 3 months duration of cereal feeding and a lower proportion continued cereals for longer than 6 months duration ($p=0.15$). Iron intake expressed as total iron/24 hours and percent iron per RNI was lower ($p=0.05$) among

infants in the low social class and when expressed as mg iron/1,000 kcal (4.184 MJ) and mg iron/kg body weight, iron intake was significantly lower ($p < 0.05$) for infants in this group.

When parents' knowledge of iron sources was evaluated, it was found that a significantly higher (P=0.03) percentage of parents in the low social group could not identify a single iron source.

Socioeconomic status was significantly related to the educational level of both the mother (Chi-square = 73.8, 4 df, $p<0.0001$) and the father (Chi-square = 96.2, 4 df, $p<0.0001$).

Table 16: Hematologic and Nutritional Correlates of Social Groups

Correlates	Social Grouping Low	Middle-High	Statistical Analysis
Hematologic			
Moderate-severe IDA (%)	4.1	0.8	Fisher's exact test (1 tail) p=0.10
IDA (%)	8.2	2.6	Fisher's exact test (1 tail) p=0.07
ID/IDA (%)	22.4	12.4	Chi-square =2.7, 1 df p=0.10
Nutritional			
Breastfeeding (%)	51.0	81.0	Chi-square =21.4, 1 df p<0.0001
Duration of infant cereals			Chi-square
< 3 mo (%)	32.4	20.1	=2.0, 1 df
> 6 mo (%)	29.4	42.0	p= 0.15
Inability to identify an Fe -rich food (%)	47.1	31.2	Chi-square =4.6, 1 df p=0.03
*Total Fe/ 24 hrs (x + SEM)	8.6 ± 0.78	9.9 ± 0.31	t= 1.93 298 df p= 0.05
* % RNI (x + SEM)	132.6 ± 11.30	152.8 ± 4.70	t= 1.95 298 df p= 0.05
* mg Fe/ 1,000kcal (x + SEM)	8.1 ± 0.75	10.0 ± 0.36	t= 2.38 298 df p= 0.02
* mg Fe/kg body weight (x + SEM)	0.9 ± 0.08	1.0 ± 0.04	t= 2.10 298 df p= 0.04

* The results are expressed as means ± SEM of raw values. After these values were logarithmically transformed to produce a more normal distribution, a t-test was performed on each measure of iron intake.

Part 4

DISCUSSION

STATISTICS

The power of an experiment is the probability that a given experiment will detect an effect of a given size, if that effect is present (Berwick, 1980; Maisels, 1977).

The sample size for this study was chosen primarily to provide a sufficient n in order that 95% confidence limits be no larger than + 5%. However, this did not provide an adequate sample size to furnish the statistical power to determine the effects of certain risk factors.

Because of the low frequency of ID (10.5%) and IDA (3.5%) and the regrouping of these variables by independent variables, larger sample sizes are necessary if the study is to have the statistical power to detect relatively small differences between these groupings. The hazard of this situation is missing an effect that is really present, which would be committing a type II or beta error.

Although many of the crosstabulations in this study have indicated trends in relationships, in some cases they may lack statistical significance in their association due to the low freguency of ID and IDA and the relatively small

sample size. Many of the trends that have emerged from this study are important in that they appear to have clinical significance and they point to directions for further research. Future studies may wish to elaborate, in a statistically rigorous manner, on the degree of influence of these trends as risk factors.

Follow-up statistical analysis for this study should include a discriminant analysis. This analysis can determine whether or not and to what extent independent variables such as nutritional, medical and socioeconomic factors can predict iron deficiency in the sample studied. If certain variables are found to be predictive, then they can be used to predict iron deficiency in other samples, e.g.patients, as well. The discriminant analysis weighs and linearly combines the discriminating variables so that the iron deficient and normal iron status groups are as statistically distinct as possible. The variables which contribute the most to discrimination are identified with weighted coefficients. This method can select the variables that improve discriminating ability and only the variables that contribute to discrimination are retained (Nie et al., 1975).

IRON INDICES

This study examined the subset of infants with abnormal SF (< 10 ug/l) and Hgb (< 110 g/l) levels to determine the prevalence of ID and IDA and risk factors associated with these states. The value of these iron indices can be determined by their measurement against standards. SF can be measured against the standard of bone marrow iron, whose magnitude it reflects. As such, SF is the most sensitive indicator of early iron deficiency. The therapeutic response to iron is the standard for IDA, as it is the most sensitive indicator of advanced iron deficiency (Cook, 1982).

It is recognized from the poor correlations between SF, and FEP and Hgb that factors other than low iron stores affect the development of iron deficiency anemia and that some of these factors reduce the sensitivity of SF in the detection of ID. While the accuracy of using SF to predict iron stores has not been studied in infants, it has been found to reflect iron stores in adults (Lipschitz et al., 1974). Furthermore, SF changes in infants and children tend to parallel known changes in iron stores in normal development (Deinard, Schwartz and Yip, 1983) as well as iron deficiency (Siimes, Addiego and Dallman, 1974; Smith,

Rosello, Say and Yeya, 1955). The exceptions to this are during certain conditions such as infection and inflammation when SF levels are increased.

Low SF values are almost invariably diagnostic for ID and no condition has been found to give "false" low values when SF levels of infants and children in states of health and disease have been studied (Siimes et al., 1974). It has been noted that SF levels < 10 ug/l are entirely specific for ID (Labbe and Finch, 1980). Considering these factors, infants with SF levels in the abnormal range are a valid group to study. While not all infants with ID may have been recognized as such by SF testing, all of those who have been characterized as having ID by SF testing, likely do have depleted iron stores (Siimes et al., 1974; Lipschitz et al., 1974). Not all of these infants would be expected to respond to a therapeutic trial of iron for some of the reasons which will be described below.

For this population of 6 to 18 month old infants IDA was diagnosed by Hgb < 110g/l (Dallman and Siimes, 1979). IDA was confirmed in each infant by at least one other indicator (SF, FEP, mean corpuscular volume, hematocrit or blood smear) being characteristic of iron deficiency. The IDA group is thereby also a valid group to study.

SF, FEP and Hgb determinations are valuable tools in identifying iron status on a population basis. It is their ability to determine iron status on an individual level that

is limited and this is demonstrated by the generally low level of correlation between the iron indices (especially among non-anemic infants) and the limited ability of each index to predict the values of the remaining indices for ID and IDA.

Similar results have been found in other studies designed to test laboratory predictors of iron deficiency in infants. Very recent evidence (Jiminez, Lozoff and Jiminez, 1985) supports the finding that SF as well as FEP are not particularly sensitive or specific indicators of ID in infants. Response to iron occurred in over half the non-anemic infants whether their SF and/or FEP levels were abnormal or not. These results suggest that Hgb production may be limited by a lack of iron even when SF or FEP are within the normal range. Among those infants with SF< 12 ug/l and/or FEP> 100 ug/dl rbcs (Jiminez et al., 1985) and among those with SF< 10ug/l (Dallman et al., 1981) between 31 to approximately 40% did not achieve a Hgb response. These studies illustrate the unpredictable relationships between iron indices in infants and how the development of IDA does not necessarily follow the three stage model decribed previously. Possible reasons for these observations are described below.

In this study, the overall correlation coefficient of SF and FEP was low (r= -0.22, p=0.0004). A study by Yip, Schwartz and Dienard (1983) found a stronger correlation (r=

-0.66, $p<0.001$) among 4,160 children but it is not readily comparable to the correlation in this study as they excluded subjects with acute illness at the time of testing. This is an important consideration as there is evidence that even mild antecedent infection can significantly increase SF and FEP levels (Reeves et al., 1984). Twenty-six percent of infants in this study experienced a cold or infection within a month of testing.

The reliability of SF in predicting ID and IDA is an area of uncertainty. There is evidence that while some subjects with high FEP or low Hgb levels have low SF values, a large percentage do not (Yip et al, 1983; Saarinen and Siimes, 1978). In this study SF levels within normal limits were found in 71% of infants with abnormal FEP levels and and 91% of infants with abnormal Hgb levels. This suggests that either conventional SF cutoff limits (10-15 ug/l; Dallman et al., 1980; Yip et al., 1983) are too low, or far more likely, that there are factors other than intactness of iron stores that affect heme synthesis.

This study has demonstrated that FEP has limited use as the sole criterion of iron deficiency in infants between 6 and 18 months of age. At any cutoff level tested, FEP was a poor predictor of low SF when sensitivity and specificity were taken into account. At the 73.8 ug/dl rbcs cutoff point, FEP displayed excellent specificity (97.0%) and fair to moderately good sensitivity (63.0%). This limited

sensitivity would make FEP an ineffective tool for the diagnosis of IDA in individual infants. It may have some value in the screening of populations for epidemiological purposes or as one of a number of confirmatory tests for ID and/or IDA.

There are several possible explanations of why the correlations between iron indices and the reliability of the measurements used to predict iron deficiency are not greater. Not all individuals with abnormal values are expected to have ID or IDA. The normal ranges used for laboratory tests have 95% confidence limits (Henry and Reed, 1974); approximately 1 in 20 normal individuals will have a value outside the normal range. As described earlier, abnormal values are not always specific to iron deficiency. Infection, inflammation, liver disease, thalassemia minor are some of the factors that can affect SF, FEP and/or Hgb levels. As well, there may be errors due to biologic variability in the individual (Dallman et al., 1980).

Abnormal SF may be a poor predictor of low FEP and both SF and FEP may be poor predictors of IDA (Hgb< 110g/l) because of the chronological sequence in which they change. SF, which reflects the size of iron stores, should theoretically drop before FEP becomes abnormal. FEP increases only after several weeks of erythroid iron depletion and because of this it is not possible to identify subjects with iron deficient erythropoiesis at the onset

(Cook, 1980). FEP concentrations should, in turn, rise to a certain point before heme production is affected. It is possible that a proportion of infants with abnormal SF or FEP levels have not yet reached a stage where heme production is significantly affected (Dallman et al., 1981).

The possibility exists that the cutoff values selected were not ideal for separating ID and IDA infants from normal iron status infants. However, the use of normative data (Yip et al., 1983; Dallman and Siimes, 1979; Saarinen and Siimes, 1978) should have helped to improve their reliability.

Among middle to upper class French-Canadian 18 month old infants (Brault-Dubuc et al., 1983) the level of ID was far higher (29.2%) than that found among the infants in this study (10.5 ± 3.3%). This finding is surprising as iron deficiency is inversely related to socioeconomic status (Egbuonu and Starfield, 1982). Brault-Dubuc et al. concede that this is a rather high figure as only 11.5% of these infants were consuming less than 100% of the RNI for iron. Perhaps the higher rate of ID is related to the lower rate of breast-feeding among French-Canadians as compared to English-Canadians (Yeung et al., 1981a) although by 18 months of age, the importance of breast-milk as a source of iron would be expected to diminish as the intake of iron from other rich sources increases. The level of IDA among infants in the present study (3.5 ± 2%) was comparable to

that of the French-Canadian infants between 6 to 18 months of age in the study by Brault-Dubuc et al. (1 to 7%).

The importance of iron deficiency in the absence of anemia among infants is controversial as to whether it is a health risk (Deinard, Gilbert, Dodds and Egeland, 1981; Oski, Honig, Helu and Howanitz, 1983). It may increase the risk of the development of IDA but it seems clear that heme production may be limited by a lack of iron whether SF and FEP concentrations are abnormal or not (Jiminez et el., 1985; Dallman et al.,1981). The actual mental and physical hazards associated with the iron deficient state need to be clarified.

Those infants with evidence of low iron stores as determined by SF and those with anemia due to a lack of iron as determined by Hgb and confirmed by one other indicator formed the iron deficient group. The factors determined to be 'risk factors' for this study were associated with an increase in the prevalence of ID and/or IDA among this iron deficient group. These risk factors would be valid on group basis and not necessarily on an individual level.

NUTRITIONAL FACTORS AND IRON STATUS

Breast-fed infants experienced a significantly lower prevalence of moderate to severe anemia than infants who were not breast-fed. Additionally, the degree of breast-feeding appeared to have a substantial effect on the prevalence of ID and/or IDA; infants breast and bottle-fed and those never breast-fed respectively experienced 46 and 60% increase in ID and/or IDA over infants receiving at least some exclusive breast-feeding. Duration of breast-feeding also affected the prevalence of ID and/or IDA; infants who were breast-fed for over 6 months experienced a 35% lower prevalence of ID and/or IDA than those breast-fed less than 6 months.

The reason for the beneficial effect of breast-feeding protecting against ID and/or IDA seems to lie not in the amount of iron contained in breast milk, but rather in the amount of iron absorbed from breast milk. Breast milk has been reported to have an initial concentration of 0.6 mg iron/l. This falls to approximately 0.3 mg iron/l after 5 months (Siimes, Vuori and Kuitunen, 1979). Other reports have suggested concentrations of up to 1 mg iron/l breast milk or, expresssed in relation to energy, less than 1 mg

iron/1,000 kcal (4.184 MJ) (Dallman et al., 1980). During the first several months of life the iron contribution from breast milk likely does not exceed 1 mg/day. This amount would seem to be insufficient to meet the needs of rapidly growing infants. However, the low iron content of breast milk is compensated by the very high bioavailability of its iron. An average of 49% of the iron in breast milk is absorbed; this is almost a 5 fold increase over the 10 to 12% absorbed from unfortified cow's milk or cow's milk formulae (Saarinen and Siimes, 1977).

The basis for the excellent absorption of iron from breast milk is not known. The high lactose, high ascorbic acid, low phosphorus and low protein content of breast milk facilitate iron absorption but this may not explain the phenomenon entirely (McMillan, Landaw and Oski, 1976). There is some evidence that ingestion of breast milk may condition the intestinal mucosa in a way that facilitates the absorption of iron, even when it is not consumed with breast milk (Saarinen, Siimes and Dallman, 1977). These findings may help to explain the observation that prolonged breast feeding confers some protection against the development of ID and/or IDA.

Neonatal iron store depletion usually occurs between 4 to 6 months of age (Canadian Paediatric Society, 1979; American Academy of Pediatrics, 1976). There is evidence that breast-fed infants rarely develop ID and/or IDA prior to 6

months of age (Saarinen, 1978; Siimes et al, 1984; MacMillan et al., 1976) and retain substantial iron stores based on their serum ferritin at that age (Saarinen and Siimes, 1978) so that iron supplementation is not necessary for them until 6 months of age (Canadian Paediatric Society, 1979). It has been recommended that formula-fed infants receive some form of iron supplementation by 4 months of age (Canadian Paediatric Society, 1979; American Academy of Pediatrics, 1976). This study's finding that breast-feeding (degree and duration) was associated with a decrease in the prevalence of ID and/or IDA support these recommendations.

An infant's iron needs can be adequately met by the introduction of solid foods containing iron. Infant cereals are traditionally the first solid food introduced. An increased prevalence of ID and/or IDA was not associated with the late introduction of solids (beyond 4 to 6 months). Instead, ID and/or IDA was increased with the introduction of solid foods prior to 2 months of age.

Reexamination of the data revealed an inverse association of breast-feeding, which has a protective effect against iron deficiency, and the early introduction of solids. The age of introduction of solids appears to be influenced by the type of milk-feeding chosen (Table 17). The higher the degree of breast-feeding, the later was the introduction of solids. A trend in the introduction of solids and the degree of breast feeding is evident. The higher rate of ID

and/or IDA among infants receiving solids prior to 2 months may be secondary to the decreased incidence of breast feeding in this population; of infants receiving solids before 2 months of age only a third were breast fed.

Table 17: Relationship Between Degree of Breast-Feeding and Age of Introduction of Solids

Degree of Breast-Feeding (n)	Age of Introduction of Solids (months)
No breast-feeding (76)	3.3 ± 0.21 a
Breast and bottle-feeding (36)	4.5 ± 0.25 b
Exclusive Breast-feeding (208)	4.6 ± 0.11 b

Values are means ± 1 SEM. Values in the column followed by different letters are significantly different from each other ($p = 0.0003$).

This relationship was also evident in a study based on Nutrition Canada Survey data (Myers, 1979). It may reflect differences in maternal knowledge of infant nutrition.

The 24-hour dietary recall was used to assess iron intake. When parents provide their child's food this method has been determined to be a strong predictor of actual food intake (Klesges et al., 1985) and in the majority of cases in this study, parents interviewed did provide their child's

intake. However, since the infants of some participating parents were cared for in day-care centres or by babysitters, the 24-hour recall was likely less reliable for this group. Consequently, the 24-hour recall can only be considered to provide an estimate of the actual iron intake of this sample population.

Although the association of iron intake and iron status was not found to be significant by analysis of variance or chi-square analysis, a linear trend was evident. The prevalence of ID and/or IDA was twice as high among infants receiving less than 75% of the RNI for iron than among those receiving at least 125% of the RNI for iron. Similiarly, infants receiving less than the 50th percentile value for mg iron/1,000 kcal (4.184 MJ) and those receiving < 1 mg/kg body weight experienced a higher prevalence of ID and/or IDA. (Some error was likely introduced into the mg iron/ kg body weight measurement as infants were not weighed completely unclothed.) This evidence suggests that a higher RNI may be more appropriate for the maintenance of adequate iron status in this population. This hypothesis could be tested in a longitudinal dietary survey.

The RNI levels for iron are presumed to be the level of dietary intake needed to maintain health in the majority of healthy infants. At two standard deviations above what is thought to be the average level of requirement, this level should exceed the actual requirement of most individuals but

the lower the iron intake is in relation to the RNI, the greater the risk of the individual not meeting his/her requirement. These data suggest that children with the lowest intake of dietary iron are the most likely to experience ID or IDA.

Many factors influence individual iron requirements. Bioavailability of iron is affected by the chemical form of the iron as well as its interaction with other foods simultaneously ingested. In this study, intakes of dietary iron were calculated, but no attempt was made to estimate the availability of iron from mixed foods.

The consumption of infant cereals had an important impact on the iron intakes of infants in this study. Infants not consuming infant cereals received significantly less iron in their diets than those infants who were consuming infant cereals. As well, the prevalence of IDA varied inversely with the duration of infant cereals. The withdrawal of infant cereals prior to 3 months duration was associated with approximately a two-fold increase in the prevalence of IDA. Prolonged infant cereal feeding (over 12 months) by a small group of 12 infants was associated with a rate of IDA at 0%. No trend was evident with respect to the prevalence of ID and duration of infant cereals.

Given the popularity of infant cereals and their reliability as a vehicle for dietary iron, it would seem prudent to recommmend their feeding for at least 3 months

duration. This suggestion is based upon the correlation of the lowered prevalence of IDA with the increased duration of cereal feeding. Causation cannot be implied from the data available.

The ability of parents to correctly identify at least one iron source was related to a decreased rate of ID and/or IDA. As with breast feeding, this could be a function of parental education and interest in infant nutrition.

There is an inverse relationship between parents' perceptions of frequency of nutritional counselling by their infants' physician and the presence of ID and/or IDA. It may be that when primary care physicians are perceived by their patients' parents as concerned about nutritional issues, parents are more compliant with nutritional recommendations such as breast feeding and feeding infant cereals for appropriate durations. These infant feeding practices may decrease the risk of ID and/or IDA.

MEDICAL FACTORS AND IRON STATUS

In low birthweight infants, the total body iron at birth is less than that of the full-term infant, however, the iron content of the preterm infant is proportionate to body weight. Because of the preterm infants' rapid postnatal growth, iron stores are depleted between 2 and 3 months of age (Dallman et al., 1980; Canadian Paediatric Society, 1981). Because of this, their requirements for exogenous iron are greater than those of the full-term infant and there is some evidence that IDA will develop after this time unless supplements are given (Lundstrum, Siimes and Dallman, 1977). Because low birth weight and preterm infants in Ottawa-Carlton are routinely supplemented with iron, the effect of prematurity or low birthweight on iron status was small.

Infants in the upper (> 90th) and lower (< 10th) percentiles for weight for length exhibited twice the rate of ID and/or IDA compared to infants between the 10th and 90th percentiles (Table 10). Among infants with percentiles less than 10, there was little evidence of low energy and consequently low iron intake. There was, however, a higher percentage of infants in this category who were sick at the

time of the interview and who had been seen by their physician at least once in the previous 3 months for a non well baby visit. Infants in the > 90 percentile category experienced the lowest rates of illness.

There is some evidence that hematologic parameters change with mild antecedent infection in infants. Significant decreases in Hgb and increases in SF and FEP have been observed among such infants (Reeves et al., 1984). So while the rate of ID, based on SF, may decrease consequent to illness, the rate of IDA would be expected to increase. Among infants in the < 10th percentile weight for length group, mild antecedant infection may contribute to an increase in IDA, but not in ID, so infection would not explain this observation entirely.

Among infants in the >= 90th percentile group, it was found that a significantly higher percentage consumed less than the RNI for energy (calculated on an age and weight basis) and less than 1 mg/kg body weight. More infants in this group also consumed less than 100% of the RNI for iron but the differences between groups were not as striking.

It is an interesting finding that percentile weight for length varied inversely with energy intake. The opposite situation would be expected. Although parental reporting of the 24-hour dietary recall has been found to be a strong predictor of children's actual intakes (Klesges et al., 1985), there is some evidence of the under-reporting of

large intakes and the over-reporting of small intakes that has been observed in validity studies of the 24-h dietary recall (Gersovitz, Madden and Smiklas-Wright, 1978; Madden, Goodman, and Guthrie, 1976). This "flat slope syndrome" may help to explain the phenomenon of the relatively low energy intake among the high weight for length infants. Other factors that may affect energy intake include differences in metabolic rate, activity level, and efficiency of energy utilization between groups but none of these factors were studied.

Iron intake below recommended levels, particularly when calculated on a per kg basis, seems to be an important contributor to the higher rate of ID and IDA observed among infants in the >= 90th percentile group. However, this iron intake data is subject to the potential limitations of the 24-h dietary recall as described above. Further analysis of more specific medical and nutritional factors could reveal other associations.

SOCIOECONOMIC FACTORS AND IRON STATUS

Relationships between socioeconomic status and hematologic and dietary factors have been observed (Table 16).

The percentage of ID and/or IDA among the infants in this study was twice as high in the lower social group than in the middle-high group. As well, the prevalence of total IDA and moderate-severe IDA was 3 and 5 times higher, respectively, among those in the lower social group. Similiarly, a review of studies conducted over the past 2 decades revealed the percentage of 1 to 5 year olds having IDA to be 3 times greater among the poor than the nonpoor, and the severe degrees of IDA to be twice as common among the poor (Egbuonu and Starfield, 1982).

It is unlikely that low socioeconomic status per se is responsible for ID and/or IDA, but rather that specific practices associated with poverty may be contributing factors. The economic status of the family and the cultural background are factors that determine the types of foods consumed and feeding practices (Czajka-Nairns et al., 1978). In this study there were important gradients in breastfeeding, duration of infant cereal consumption and

iron intake that varied with SE status. Among infants in the lowest socioeconomic group, the proportion of those breastfed and the duration of use of infant cereals were lower than among infants in the middle to high group. The average iron density of the diet was almost 2 mg lower in the low socioeconomic group than in the middle-high group. This may be a result of the decreased use of infant cereals in the low socioeconomic group.

Similiar results were found in data from the Nutrition Canada Survey (Myers, 1979). Education has been found to be an important factor in the choice of breastfeeding and its duration (Pursall, Jepson, Smith, and Emery, 1978; Yeung et al., 1981a). In this study as well, socioeconomic status was significantly related to to parental educational level ($p < 0.0001$) and is implicated in other specific nutritional practices that have been associated with ID and/or IDA.

The assumption of the effect of socioeconomic status on nutritional practice, is that parents who are better educated and hence, may be in a higher socioeconomic group, are more likely to seek information on infant nutrition. This study found that 15% more parents in this middle-high group could identify at least one source of iron in their infant's diet. Dietary knowledge and certain nutritional practices (e.g. breastfeeding, prolonged infant cereal feeding) may contribute towards decreasing the prevalence of ID and IDA.

Part 5

CONCLUSION

The following conclusions may be drawn from the results of this study.

Based on identical criteria used in the Nutrition Canada Survey (1970-1972), the prevalence of ID among infants has decreased from 29 to 10.5% and the prevalence of IDA has decreased from 18.8 to 3.5%. Possible reasons include a three-fold increase in the practice of breast-feeding and an increase in the bioavailability of the iron added to infant cereal.

FEP is a poor screening tool for ID in infancy. As a screening tool for IDA, FEP is fair at the 73.8 ug/dl rbcs cutoff point. While its sensitivity (63.6%) at this level is too poor for it to be used to predict IDA in individuals, it may have some value in population surveys or as one of a number of confirmatory tests for iron deficiency.

The three stage model for the development of IDA is not generally applicable to infants as there is a marked overlap of normal and abnormal values for SF and FEP. Despite their unpredictability on a population level, correlations between iron indices become stronger with increasing severity of IDA.

Risk factors are variables that are associated with an increased rate of ID and/or IDA. They do not imply causal relationships. Nutritional risk factors for iron deficiency include lack of breast-feeding (or low degree or duration), estimated iron intake less than 125% of the RNI and the use of infant cereals for less than 3 months duration. Lack of knowledge of iron-containing foods and perceived lack of physician's counselling on infant nutrition are also contributing factors.

With a protocol in which premature infants are routinely supplemented with iron, the effect of prematurity on iron status was small. Very low (< 10) and very high (>90) weight for length percentiles were risk factors for ID and IDA. Percentiles < 10 were associated with a higher rate of sickness and those > 90 were associated with a lower iron intake per kg body weight.

Low socioeconomic status is a risk factor. Lack of breast-feeding, decreased duration of infant cereal use; poor parental knowledge of iron-rich foods and lower iron content of the diet were more common among infants this group.

General screening for ID and/or IDA is not warranted and should be limited to those infants most at risk, the level of risk increasing with the number of risk factors demonstrated. Similarly, those families with infants at high risk should be the target of nutrition education programs.

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Appendix A

QUESTIONNAIRE

The prevalence of iron deficiency and iron deficiency anemia among 6 to 18 month old infants in Ottawa

A.1 Health Review

1. Name of child _____
2. Survey number _____
3. Sex: male _____ female _____
4. Age (months) _____
5. Date of interview _____
6. Length (cm) _____
7. Weight (kg) _____
8. Percentile weight for length _____
9. Weeks gestation _____ Don't know _____
10. Birth weight (g) _____
11. Does your child have any ongoing health problems for which he/she sees a doctor regularly?

    No _____

    Yes _____ for _____

12. Has your child seen a doctor or have you had to call a doctor in the past:

a) 1 to less than 4 weeks? No _____ Yes _____

if yes, for _____ How many times _____

and for _____ How many times _____

b) 4 to less than 12 weeks? No _____ Yes _____

if yes, for _____ How many times _____

and for _____ How many times _____

A. Check up _____ D. Contagious disease _____ G. Other _____

B. Fever _____ E. Colds _____

C. Infection _____ F. Diarrhea _____

13. Has your child ever eaten unusual things? No _____ Yes _____

a - soil?

b - starch?

c - paint?

d - other? _____

14. Has your child ever had an operation?

No _____

Yes _____ For? _____

When? _____

15. Does your doctor discuss infant nutrition with you at well-baby visits?

always sometimes never

A.2 Socioeconomic Profile

1. Usual language spoken at home

   English ____ French ____ Both ____ Other_____

2. Race

   Black ____ White ____ Oriental ____ Native Canadian___

3. Educational level of parents

   What was the last school year successfully completed?

   Mother _____

   Father _____

4. Occupation of parents (Blishen scale) -note type and place

   Mother _____

   Father _____

A.3 Nutritional History

1. Was/is the child breast fed?

   No _____ Yes _____

   If yes, a) exclusively breast fed?

   No _____ Yes _____

   b) breast fed with bottle supplementation?

   No _____ Yes _____

   Age at which breast feeding was discontinued

   (months) if applicable: _____

2. Was/is the child bottle fed? No _____ Yes _____

   if yes a) type of milk _____

   b) age at which bottle feeding was started

0 < 4 weeks _____

4 < 12 weeks _____

over 12 weeks _____

c) Has bottle feeding been discontinued?

No _____

Yes _____ 0 < 3 months _____

3 < 6 months _____

6 < 12 months _____

> 12 months _____

3. Age at which solid foods were started

months _____

4. Is child on a diet? No _____ Yes _____

For _____

5. Does the child take any vitamin/mineral supplements?

No _____ Sometimes _____ Regularly _____

Type: _____ Iron content _____

6. Does the child consume commercial infant cereals?

No _____ Yes _____

If yes, a) age at which they were started _____ months

b) Have infant cereal feeding been discontinued?

No _____

Yes _____ 0 < 6 months _____

6 < 9 months _____

9 < 12 months _____

12 < 15 months _____

15 > 18 months _____

c) Please name the product

i Gerber ii Heinz iii Milupa iv Pablum v Other

d) Does your baby eat other cereals?

(e.g. Corn Flakes, porridge) No _____ Yes _____

7. What foods containing iron does your child receive?

commercial infant cereals _____

iron fortified formula _____

meat, poultry, egg, liver _____

other _____

don't know _____

Appendix B

CHILDREN'S HOSPITAL OF EASTERN ONTARIO BLOOD

SPECIMEN COLLECTION

Micromethod with Microtainer Collector Tubes (1983)

Assemble all equipment necessary:

- alcohol swab
- dry swab
- sterile microlance
- Pre-assemble Microtainer Tube by removing lavender plug and replacing with flo top collector. Do not discard plug (note: make sure that little mixing balls are free of each other).

1) Select puncture site (finger or heel depending on age of child). Cleanse with alcohol.
2) Wipe alcohol off with dry swab.
3) Puncture skin with sterile lancet.
4) Wipe away first drop of blood.
5) Hold microtainer tube with flo top collector at an angle below horizontal with vent hole in upward position.
6) Touch tip of the flo top collector to underside

of blood drop. Do not scrape up blood sample.

7) When sufficient blood is collected, twist off flo top collector from tube and discard.

8) Seat lavender plug securely in tube opening, and gently invert tube about 8 times to ensure proper mixing of sample. Put label on tube and send to lab.