FOLIC ACID NUTRITIONAL STATUS OF BRITISH COLUMBIA INDIAN POPULATIONS

by

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ABSTRACT .

Recent studies suggest that folic acid nutritional status may be poor among Canadian Indians, particularly among those living in isolated areas. However the prevalence and causes of folic acid deficiency have not been assessed. The present study was conducted in order to assess the magnitude of the problem among British Columbia Indians and to examine the possible relationship between low dietary intakes of folic acid and the occurrence of low blood folate values.

Using a 24-hour diet recall, dietary folate intakes were estimated at four relatively isolated Indian reserves (106 subjects) and at three reserves adjacent to urban centres (144 subjects).

A more detailed study, involving estimation of dietary folate intake, measurement of serum and red blood cell folate, and examination of related hematological parameters was undertaken at one isolated reserve (Fort Ware, 28 subjects) and two non-isolated reserves (Necoslie and Sechelt, 63 subjects) as well as at a school residence (70 children, age 6 to 16 years). Meal samples were collected and assayed for folic acid, in order to verify the recall calculations.

Results indicate that calculated and assayed folate values are similar and are significantly correlated (r=.9694). Total folate consumption is significantly higher at non-isolated reserves than at isolated reserves, and males consume significantly more folic acid than do females. Dietary folic acid intake is higher at the residence than at the reserves.

Serum folate values are significantly correlated with dietary folate intake. Serum values are lower at Fort Ware than at Necoslie and Sechelt. Children living on reserves have lower serum folate values than do children living in residence, and have a larger proportion of children classified as "at risk". On the basis of red cell folate values, 16 to 45% of the subjects at the three reserves are classified as "at risk", however, no evidence of megaloblastic anemia is indicated from the hematological examinations. It is concluded that many individuals are either bordering on or are deficient with respect to folic acid. This appears to be a more serious problem at isolated reserves than at those adjacent to urban centres and it is suggested that this is a consequence of the availability, variety and selection of foods.

TABLE OF CONTENTS

Page

Chapter

LIST OF TABLES

Table		Page

LIST OF FIGURES

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CHAPTER I

REVIEW OF THE LITERATURE

Folic Acid Deficiency and Megaloblastic Anemia

Folic acid (monopteroylglutamic acid) is a vitamin which serves as a cofactor in most one-carbon transfer systems. Folate is the collective term that comprises folic acid and its derivatives, i. e. coenzymes and their precursors which have a pteroylglutamate structure. Folic acid deficiency is widespread throughout the world, ranging from a mild deficiency to severe megaloblastic anemia (Herbert, 1962). The majority of cases of megaloblastic anemia result from folic acid and/or Vitamin B_{12} deficiency (Johns and Bertino, 1965 and Herbert, 1968).

The earliest indication of folate deficiency is a fall in serum folate activity, followed by nuclear hypersegmentation of the neutrophilic polymorphonuclear leucocytes. The normal nuclear lobe count of two to five increases to six or more. Red blood cell folate levels decrease along with decreases in liver folate stores. Macrocytes appear in the peripheral blood, as macroovalocytosis becomes defined and mean corpuscular volume increases. The eventual development of overt megaloblastic anemia is characterized by the presence of megaloblasts in the bone marrow, which relates directly to the degree of anemia present. A folate deficiency, per se, may be defined by any stage in the series of changes leading to the development of megaloblastic anemia. As the changes approach the stage of anemia they indicate more severe or intense depletion of folic acid.

Herbert (1962) summarized the sequence of events which occurs when the body is depleted of folate as a result of a daily intake of 5 μg. or less.

HEMATOLOGIC AND BIOCHEMICAL SEQUENCE OF CHANGES IN DIETARY FOLIC ACID DEPRIVATION IN MAN (HERBERT, 1962)

Sequence of Changes	Time of Change (Days)
Low serum folate (3ng/ml)	22
Hypersegmentation	49
High urine FIGLU	95
Low RBC folate (20ng/ml)	123
Macroovalocytosis	127
Megaloblastic marrow	134
Anemia	137

Assessment of Folic Acid Status

Many biochemical measurements are available which provide information concerning the folic acid status of individuals or population groups. For adequate interpretation of results it is important to understand that different parameters reflect different aspects of folate metabolism.

A. Indirect measures of folate status

1. Urinary FIGLU

Several indirect tests for identifying folate deficiency are based on the measurement of urinary formiminoglutamic acid (FIGLU) and its precursor, urocanic acid (Herbert, 1967). Under normal conditions formiminoglutamic acid is formed as a result of histidine degradation and is subsequently converted to glutamic acid by a tetrahydrofolate - dependent reaction. Folate deficiency inhibits this conversion, leading to increased levels of urinary FIGLU and urocanic acid, especially after the oral administration of an L-histidine load (Chanarin, 1964 and Herbert, 1967). The drawback of using a urinary FIGLU test as an index of folate status is its lack of specificity (Sauberlich et al, 1974), since folate deficiency is not distinguishable from numerous forms of metabolic blockage which also result in high FIGLU excretions. Abnormal FIGLU excretions are seen in subjects with liver damage, protein malnutrition, and congenital formiminotransferase deficiency.

2. Urinary folate

According to Cooperman et al (1970) urinary folate excretion averages approximately 1% of the dietary intake. This varies from 1 to 10 µg per day and is not a sensitive measure of dietary folate consumption. However, a comparison of urinary folate excretion after injected and oral administration of folic acid is valuable in the diagnosis of folate malabsorption states (Johns and Bertino, 1965).

3. Plasma folate clearance

The rate of plasma clearance of injected doses of folic acid is abnormally rapid in subjects with primary folic acid deficiency (Johns and Bertino, 1965). The use of this rate measure as an index of folate deficiency is impractical for survey application, since serial measurements of plasma folate must be taken to determine the clearance rate.

4. Leucocyte folate levels

Folate levels in the leucocytes correlate with red cell folate levels and are used as an index of folate deficiency (Hoffbrand and Newcombe, 1967). Leucocyte values will not distinguish between subjects with folate deficiency and those with pernicious anemia (Hoffbrand and Newcombe, 1967), and those with dietary vitamin B_{12} deficiency (Vitler et al, 1963). Measurement of leucocyte folate offers no advantage over measurement of serum and red cell folate, and is technically more difficult to perform (Hoffbrand and Newcombe, 1967).

B. Direct measure of folate status

Measurements of serum and red cell folate are widely used indices of folate status (Johns and Bertino, 1965).

Serum folate levels reflect transient changes in dietary folate intakes (Joint FAO/WHO Expert Group, 1970), therefore, distinctly low levels are not necessarily evidence of tissue depletion or protracted folate deficiency (Joint FAO/WHO Expert Group, 1970 and Hall et al, 1975). The folate status and incidence of deficiency in a population cannot be determined from serum folate values alone.

Red blood cell folate activity is a better indicator of deficiency than serum folate activity (Liu, 1974). In the absense of B_{12} deficiency red cell folate activity is a quantitative index of the severity of folate deficiency (Herbert, 1965 and Hoffbrand et al, 1966) and an indicator of the folate content of body tissues (Omer et al, 1970).

In vitamin B_{12} deficiency, serum folate levels increase while red cell folate decreases (Herbert and Zulusky, 1962; Cooper and Lowenstein 1964; and Hoffbrand et al, 1966). In subjects with this pattern suspected of folate deficiency further evaluation should therefore be made to eliminate the possibility of pernicious anemia or dietary vitamin B_{12} deficiency (Sauberlich et al, 1974).

The presence of megaloblastic anemia and low serum and red cell folate is strong evidence that folate deficiency exists (Cooper and Lowenstein, 1966 and Hoffbrand et al, 1966) but does not rule out the possibility that iron deficiency could be a coexisting cause of the anemia (Hall et al, 1975). Saraya et al (1971) reported that iron deficiency resulted in decreased serum folate values although this was not confirmed by others (Omer et al, 1970 and Saraya et al, 1973). Omer et al (1970), Roberts et al (1971) and Saraya et al (1973) reported significant increases in red cell folate in conditions of iron deficiency while Hershko et al, (1975) reported that red cell folate was unaffected by a coexistent iron deficiency. The effect of iron deficiency on folate metabolism is poorly understood (Hershko et al, 1975) and the data are contradictory.

The proof of symptomatic folate deficiency usually depends on a combination of evidence rather than a single test, although the prompt response of megaloblastic anemia to physiologic amounts of folic acid would be the most conclusive evidence (Hall et al, 1975).

C. Iron indices related to folate status

An examination of folate status should include measurements indicative of iron status, since the existence of iron deficiency could have a significant effect on folate assessment. Hemoglobin values are used as an index of the severity of iron deficiency anemia (WHO Scientific Group on Nutritional Anemias, 1968). Hemoglobin concentrations will decrease when folate is deficient since folic acid is utilized in hemoglobin synthesis. The mean corpuscular hemoglobin concentration (MCHC) * reflects blood cell morphology and is a strong indicator of iron deficiency when values are low. The mean corpuscular volume (MCV)** increases in conditions of folate deficiency following the development of macroovalocytosis (Herbert, 1967; Armstrong et al, 1974; and Colman et al, 1975).

When comparing index values to reference standards it is important that the standards being used are applicable to the population under study. Significant racial and geographic differences in hemoglobin and hematocrit norms have been reported (Garn et al, 1974 and Owen et al, 1975).

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* MCHC (%) $= \frac{\text{HEMOGLOBIN (g/100 ml)}}{\text{HEMATOCRIT (\%)}} \times 100$

** MCV (u^3) $= \frac{10 \times \text{HEMATOCRIT (\%)}}{\text{RED CELL COUNT (MILLIONS/MM}^3\text{)}}$

In summary, iron indices which are important for assessment of folic acid nutritional status include measures of hemoglobin concentration, hematrocrit, mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV).

D. The folate assay

Radioactive methods of folate analysis have been developed and are being used increasingly. At present, folate levels in serum, red cells and other biological samples are most commonly measured by microbiological assay procedures, utilizing Lactobacillus casei, Streptococcus faecalis, or Pediococcus cerevesiae. Lactobacillus casei is the choice organism for evaluating human folacin nutritional status since it responds to the folate forms present in serum and red blood cells (Sauberlich et al, 1974).

There is good agreement within and between laboratories for L. casei folate assay results (WHO Group, Nutritional Anemias, 1972), yet due to the assay's complexity, constant monitoring and the regular use of reference preparations are necessary.

Guidelines have been set for the interpretation of serum folate and red cell folate values. They must be considered in terms of the microbiological assay procedure and the test organism employed (Refer to Appendix D for guidelines).

Dietary Folates

Folic acid deficiency commonly results from an inadequate diet, usually associated with alcoholism, increased physiological requirements, food faddism, poverty, or ignorance (Sullivan, 1967). It is important that dietary folate levels are evaluated when diagnosing folic acid deficiencies. Santini and Corcino (1974) stressed that dietary studies are essential for determining both the etiology and the proper treatment of nutritional anemias.

A. The nature and assay of dietary folates

Folates are present in a wide variety of foods, but folic acid (pteroylglutamic acid, PGA) constitutes only 5% of the total folate (Hurdle, 1973). Folates consist mainly of folic acid bound to a peptide chain of one to six L-gluatamic acid residues in γ-carboxy linkage (Bernstein et al, 1970)

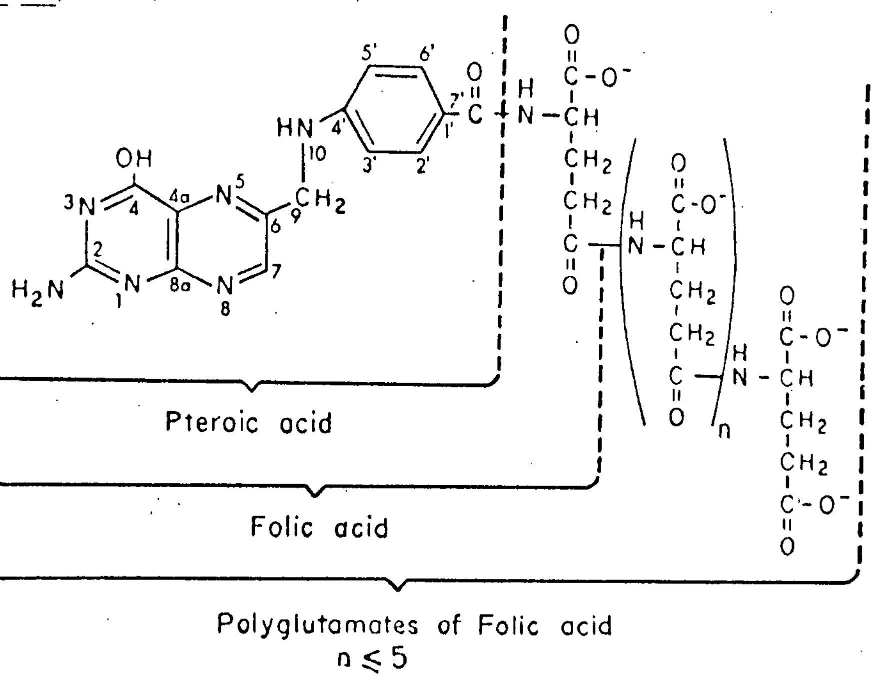


Figure 1: Folic Acid and its Polyglutamates*

Microbiological assay is the most common method of analysis of

* Bernstein et al (1970)

food folate activity. The different forms of folate vary with respect to their availability for assay organisms. According to Hoppner et al (1972) and O'Broin et al (1975), L. casei responds to most of the known pteroylglutamates with up to three L-glutamate residues.

Food folate can be classified into "free", "conjugated" and "total" fractions (Hoppner et al, 1972). The folate available prior to enzymatic hydrolysis with conjugase is referred to as "free" folate, while that which is active only after enzymatic hydrolysis is referred to as "conjugated". "Total" folate consists of both the "free" and "conjugated" forms.

There is uncertainty concerning the availability of different polyglutamate forms for absorption, and once absorbed, for utilization (Tamara and Stokstad, 1973). During digestion intestinal conjugase cleaves polyglutamyl folates to monoglutamyl forms in preparation for absorption (Butterworth et al, 1969), while similar cleavage also occurs in the food itself due to endogenous conjugase activity (O'Broin et al, 1975). Baugh et al (1971) and Baugh et al (1975) confirmed the biological availability of monoglutamyl forms of folate in a mammalian system, and showed that polyglutamyl forms are also readily absorbed as such from the lumen in the proximal jejunum of the dog. Butterworth et al (1969) demonstrated that ingested polyglutamates of folic acid are cleaved to the monoglutamate form in man in the process of absorption, although the site of cleavage cannot be stated with certainty. There can be little doubt that man is capable of deriving his nutritional folate requirement from conjugated forms present in the diet (Butterworth et al, 1969). The "total" folate value of food cannot be regarded as the amount available for absorption, since *in vivo* and *in vitro* conjugase activity are not necessarily the same. Pietarinen (1975) suggested that possibly the amount of available folate lies somewhere between the values representing the "free" and "total" fractions.

B. The estimation of dietary folate consumption

The determination of folate levels in food may be affected by many factors other than the assay itself such as the reliability of food collections, the interest, intelligence, and education levels of subjects involved in doing the collection, the storage facilities for the food samples and the storage time (Moscovitch and Cooper, 1973).

Heat labile, water soluble folates are lost during cooking and processing of food, with losses increasing when large amounts of water are used (Herbert, 1962).

There are few reliable food composition tables for estimating dietary folate consumption (Thenen, 1975), and values are available for a limited amount of foods. Food folate values reported prior to the use of ascorbate in the assay procedure are exceptionally low (Chanarin *et al*, 1972), since ascorbic acid prevents the oxidation of labile folates, keeping them available for the test organism. Hurdle (1973) reported folate values up to 40 times higher in foods assayed with ascorbate as compared to without.

The nutrient values for food items derived from direct analysis of the food do not necessarily agree exactly with values calculated from food tables since they provide somewhat different information (Whiting and Leverton, 1970). Chemical analysis provides a precise measure of the nutrients in the foods as eaten, whereas calculation provides an average nutritive value of a diet made up of similar foods.

The 24-hour recall is considered one of the most suitable methods for collecting dietary data in large surveys since it is readily applied to wide population groups, irrespective of age, education and intelligence (Mongeau, 1973). Significant limitations of the 24-hour recall are its heavy dependence on the sharpness of the subjects memory and its questionable representation of the habitual diet. Applied to Indian populations, Sabry (1975) questioned the value of 24-hour recalls, as Indians are accustomed to famine or feast eating patterns, where the availability of food is highly variable. According to Fidanza (1974) 24-hour recalls have limited use when evaluating the nutritional status of individuals. It is more valid to estimate group intakes using mean values from 24-hour recalls.

Studies Assessing Folate Status

Laboratory folate analyses suitable for use in community surveys have only recently become perfected, so that few surveys of folate nutritional status have been undertaken (WHO Group, Nutritional Anemias, 1972). There is general agreement that measurement of serum and red cell folate is the most practical and meaningful procedure for evaluating folate status in population groups. Folate results should be interpreted in terms other than mean values alone, since this could obscure the existence of a substantial number of low folate values indicative of widespread deficiency (Herbert, et al, 1975). Folate results could be expressed in terms of percent subnormal or deficient values, or by risk categories with percent distribution in each risk category.

A. Population surveys

A Barbados Nutrition Survey (1972) assessed the folate status of

415 male and female subjects of all age groups. Serum folate values <2 ng/ml and/or red cell folate <80 ng/ml were classed as indicating deficiency. Thirty-three percent of the preschool children were anemic, due almost equally to iron and folate deficiency. Twenty percent of the adult women were anemic, and while most of the anemias were due to iron deficiency, there was also a significant incidence of folate deficiency anemia. Very few older children and adult men had anemia, suggesting that hookworm infestations or other non-dietary factors were not likely important cases of anemia, since they would have affected the different age and sex groups more equally. Food consumption and socioeconomic findings indicated that anemia was largely due to dietary insufficiency of food folate and iron.

The vitamin B_{12} and folate status of 52 adults in a Himalayan village of Nepal was studied in early fall (Adams and Man Shresta, 1974). Despite the restricted food availability there was no evidence of folate or vitamin B_{12} deficiency based on serum and red cell measurements. In a study conducted the previous spring in a similar high altitude village 25% of the 67 subjects had definite polymorphonuclear hypersegmentation, suggesting B_{12} or folate deficiency. The seasonal availability of folate and vitamin B_{12} in these high altitude areas was suggested as having a significant influence on dietary intakes and deserved further evaluation. The varying incidence of megaloblastosis found in other studies has been attributed to dietary folate deficiency in off-season periods when the supply of fresh fruits and vegetables is low (Gatenby, 1956; Thompson, 1957; Coyle and Geoghegan, 1962; Pereira and Baker, 1966 and Chanarin et al, 1968).

Dietary and plasma folates were evaluated in healthy adolescents in Birmingham, Alabama (Daniel et al, 1975). The data were examined in relation to age and sex maturity ratings (SMR) according to classifications set by Tanner (1966). Females had higher plasma folate than males across all sex maturity ratings, yet their dietary folate intakes were lower. In both sexes, increased maturity was associated with a decrease in plasma folate values, yet dietary folate intake increased, possibly indicating greater tissue and cellular folate demands associated with adolescent growth.

Vitamin B_{12} and folate status was assessed in a group of 562 Seventh-Day Adventists in Australia (Armstrong et al, 1974). Seventy-seven percent of the subjects were vegetarian. Vegetarians had a significantly higher mean serum folate than the non-vegetarians. From a study reporting similar findings (Ellis and Montegriffo, 1970), it was postulated that elevated serum folate in vegetarians was a consequence of impaired folate metabolism, resulting from B_{12} deficiency. In the study by Armstrong et al (1974) serum vitamin B_{12} levels were significantly lower in vegetarians than subjects who consumed meat and eggs, while those with subnormal serum B_{12} values (i.e. 26% of the vegetarians) had significantly higher MCV and MCHC and lower red cell folate than the rest of the group. Similar findings were also reported by Ellis and Montegriffo (1970).

Colman et al (1975) reported widespread folate deficiency from a study of 469 Negro adults in a district of Nqutu, Kwa Zulu, South Africa. The subjects chosen were healthy, excluding pregnant women with hemoglobins less than 11g/100 ml from the study and excluding non-pregnant subjects receiving medical attention. The incidence of subnormal red cell folates

(160ng/ml) in men, non-pregnant women, and pregnant women was 18.6%, 32.1% and 43.8% respectively. Folic acid deficiency anemia was not reported, nor were any unequivocal cases of vitamin B_{12} deficiency. From these results it was recommended that maize meal be fortified at such a level that an average of 200 µg folic acid would be consumed per day. This would safeguard against folate deficiency in all segments of the population.

A nutrition survey conducted in Kiryat Shmoneh, an Upper Galilee community in Isreal, reported a high incidence of folate-and iron-deficiency anemia in women of childbearing age and in children (Levy et al 1975). From 26 fathers, 196 mothers and 160 children studied, the prevalence of sub-normal red cell folates was 0%, 47% and 53% respectively. Mean daily intakes for the same groups were 265 µg, 160 µg, and 158 µg total folate, the intake for adult men being significantly greater than for women or children. Similar trends were evident for iron, where low intakes in women and children correlated with subnormal serum iron values. This survey substantiated previous assumptions that folate and iron-deficiency anemia was related, at least in part, to low intakes of iron and folic acid.

The nutritional status of 41 families was assessed in Macon County, Alabama (Prothro et al, 1976). The sample of 102 individuals was 76% black and 24% nonblack. Mean serum folates for all age groups were low ($<$5ng/ml), with 20% of the subjects showing serum values $<$3ng/ml. Based on serum values, folate deficiency was the most extensive nutrient deficiency. The lengthy cooking processes involved in food preparation were thought to be responsible for extensive destruction and leaching of food folates, significantly lowering the vitamin's availability.

Kaufman et al (1975) studied the nutritional status of Florida seasonal farm workers. The sample population included 973 households, about one-third Spanish-Americans and two-thirds blacks. Iron deficiency was the most serious nutritional problem, yet folate deficiency was also significant. Approximately 40% of the black and 35% of the Spanish-Americans had subnormal red cell folate values.

Folate status was studied in healthy white hospital personnel, black clientele of an urban health centre, and black migrant Florida farm workers (Hall et al, 1975). In search of a reference group it was realized that plasma folate levels can vary significantly from one healthy population to another, and direct comparisons are not necessarily meaningful. If inappropriate reference values are used, a test could readily mislead more than inform (Bech, 1974), such that folate levels of a particular population could appear high or low.

Certain segments of a population show indications of being particularly prone to folate deficiency: (i.e.) pregnant women in both developed and developing countries (Sauberlich et al, 1974; Baker et al, 1975, and Herbert et al, 1975), low birth weight infants (Mathoth et al, 1964 b), artificially fed, as opposed to breast fed, infants (Matoth et al, 1964 a), geriatric patients (Hurdle and Williams, 1966 and Hurdle, 1968), and virtually all alcoholics (Herbert and Zulusky, 1961 and Baker et al, 1975 b). Reports by Shojania et al (1971), Pietarinen (1975) and Prasad et al (1975) indicate that women taking oral contraceptives are prone to folate deficiency while Spray (1968), Pritchard et al (1971) and Paine et al (1975) report contradictory evidence indicating that this is not so.

B. The Nutrition Canada Survey

The Nutrition Canada Survey (1973) evaluated serum folate levels in a wide distribution of population groups in Canada. Serum values of <2.5 ng/ml were classified as high risk values, 2.5 to 5.0 ng/ml were moderate risk, and >5.0 ng/ml were low risk. According to the national report serum folate values were not significantly different between male and female, and children in general had higher values than adults. Approximately one-half of the population was classified at moderate risk and 10 to 20% of teenagers and adults at high risk.

The percentage of Indians classified at both moderate and high risk was greater than for the general population in most age categories (Nutrition Canada Indian Report, 1975). Indians in remote centres showed a significantly higher proportion of risk values than Indians closer to urban centres.

A higher percentage of Eskimos were at risk with respect to folic acid than either the Indian or General Population groups (Nutrition Canada Report, 1975).

TABLE I

Classification of serum folate values for the general, Indian and Eskimo populations: Percentage high risk values in different age categories (Nut. Can. Report, 1973)

POPULATION GROUP	AGE AND SEX CATEGORIES									
	0-4 MF	5-9 MF	10-19 M	20-39 M	40-64[a] M	65+[a] M	10-19 F	20-39 F	40-64[a] F	65+[a] F
General Population	9.6	4.8	10.3	11.1	14.0	17.7	13.5	21.2	12.5	13.7
Indian Population	3.1	7.7	14.3	14.8	24.4	19.5	12.1	23.1	19.5	26.8
Eskimo Population	38.5	27.2	51.5	69.6	58.1	77.2	40.9	84.4	65.5	77.8

a. Indian and Eskimo population (40-54 M&F, 54+ M&F).

The survey report indicated that the low serum folate values of the Canadian people should be viewed with concern, although low values are not necessarily indicative of a protracted folate deficiency (Nutrition Canada Report, 1973). Low serum values were not associated with the clinical manifestation of folate - vitamin B_{12} deficiency anemia. According to Sabry (1975) the high incidence of low serum values was unexpected and therefore additional measurements for interpreting serum folate values were not emphasized. Red cell folate values are important as indicators of body stores and the prevalence of folate deficiency (Omer et al, 1970 and Liu, 1974), blood smears give evidence of changes in cell morphology (Sauberlich et al, 1974), and an estimate of dietary folate intake from an accurate set of food tables is important for determining the cause of low serum values.

(Herbert, 1962 and Hoppner et al, 1972).

There is general agreement that the folate status of Canadian people is still in question and is an area of concern that deserves vigorous follow-up studies (Sabry, 1975).

C. Folic acid status of native Indians

White men have had both positive and negative effects on the lives of our native people (Haworth, 1975), while many changes in Indian lifestyle have a direct influence on their nutritional status (Lee et al, 1971).

Indian children across Canada are raised in such diverse social settings that it is difficult to generalize with respect to their nutritional status and speak of a "typical Indian child" (Smith, 1975). The variability in the nutritional habits depends on the relative importance of certain influences such as the basic traditional economy, the settlement patterns, the availability of white man's food, and the impact of their teaching (Smith, 1975). The nutritional status of different North West Coast Indian populations varies enough that it would be misleading to generalize from one group to another (Desai and Lee, 1974).

Foods known to be high in folic acid such as fresh fruits and vegetables are lacking in many Indian and Eskimo diets. A survey of Bush Indians in Northern Manitoba in 1942 found their diets deficient in almost all essential nutrients, with intakes of fruits and vegetables being extremely low (Moore, 1946). Indian children from Alert Bay, British Columbia consumed very few green and yellow vegetables (Dong and Feeney, 1968) and diets of Indians from isolated reserves in British Columbia were particularly low in fresh vegetables (Lee et al, 1971). According to

Smith (1975) the selection of fresh produce is often restricted in isolated places, canned vegetables are often not available, and native vegetables are seldom used.

The recent Nutrition Canada Survey has served as an important focus on potential and real problems in the nutrition of Indian people (Fraser, 1975). According to the Indian Survey Report (1975) the folate status, as indicated by the high incidence of low serum folate values, was generally poor for the total Indian population but tended to be lower for Indians in isolated areas as compared to those closer to urban centres. Beaton (1975) proposed that extremely low serum folate values in the Eskimo people could be attributed to the lack of fresh fruits and vegetables in their diet. The Nutrition Canada Survey did not analyze or calculate dietary folate intakes (Nutrition Canada Report, 1973) so one can only speculate that low serum folate values were a consequence of low dietary folate intakes.

CHAPTER II

INTRODUCTION

The native population of British Columbia consists of about 52,000 people, living primarily on reserves. Their way of life is based on a culture and tradition which tends to maintain them as a separate people. On the other hand, internal and external pressures are causing radical changes in the Indian lifestyle and are creating new problems and tensions for them. Interaction between Indian and non-Indian communities is increasing with the continuous development of highways and transportation facilities throughout the province. Indians are being forced to adjust from 'living off the land' to living the life of a wage-earner.

As Bryans (1967) stated: "The native Canadian is in a state of turmoil, trying to grasp the future with one hand and hold on to the old values with the other, moving from an ancient culture to the 20th century without the opportunity to evolve slowly over several generations."

Changes which affect the Indians' lifestyle will also have direct influence on their nutritional status. If nutritional problems of Indians living on reserves were identified and characterized, nutrition services and education programs could be more readily established to tend with the problems effectively. Although statistics are available concerning the morbidity and mortality of Canadian Indians in general, less is known about their health as related to nutritional practices.

The recent Nutritional Canada Survey (1973) has served to point out some major problems in the nutrition of Canadian people. Results indicate that the nutritional status of native Indians in Canada is poorer than is that of the general population. Folic acid in particular was suggested as being a nutrient of major concern. There was a high incidence of low serum folate values in the general population, but an even higher incidence in the Indian population. Folate values were lower for Indians on isolated as compared to non-isolated reserves. Since the prevalence of folate deficiency cannot be measured from serum values alone, it was recommended that further studies be conducted to more fully assess the problem.

Few studies have been published which assess the nutritional status of British Columbia Indians,and none, other than the Nutrition Canada Survey, have directly studied their folate status. Therefore, a need is indicated for further research in this area.

This present study was conducted to assess the folate status of native Indians in British Columbia. Two hundred and fifty subjects from seven reserves throughout the province and 70 subjects from an Indian Residential School in Sechelt, B. C. participated. Not all subjects participated in all parts of the study. This study primarily set out to determine if there really was a problem with respect to folate status among these people. If poor folate status was indicated by a high prevalence of subnormal serum folate values (i.e. less than 5 ng/ml) then the data reported by Nutrition Canada would be confirmed.

Additional hematological factors, including red cell folate determinations, thin blood smear preparations for microscopic examination, hemoglobin and hematocrit determinations and white blood cell and red blood cell counts were included as essential for the adequate assessment of folate status. Twenty-four hour dietary recalls were conducted and the data were used to calculate total folate, calorie, and iron intakes from food composition tables. In addition, food collections representing a 24-hour consumption period were obtained and total folate values were derived by direct folate analysis and calculation from food tables.

CHAPTER III

MATERIALS AND METHODS

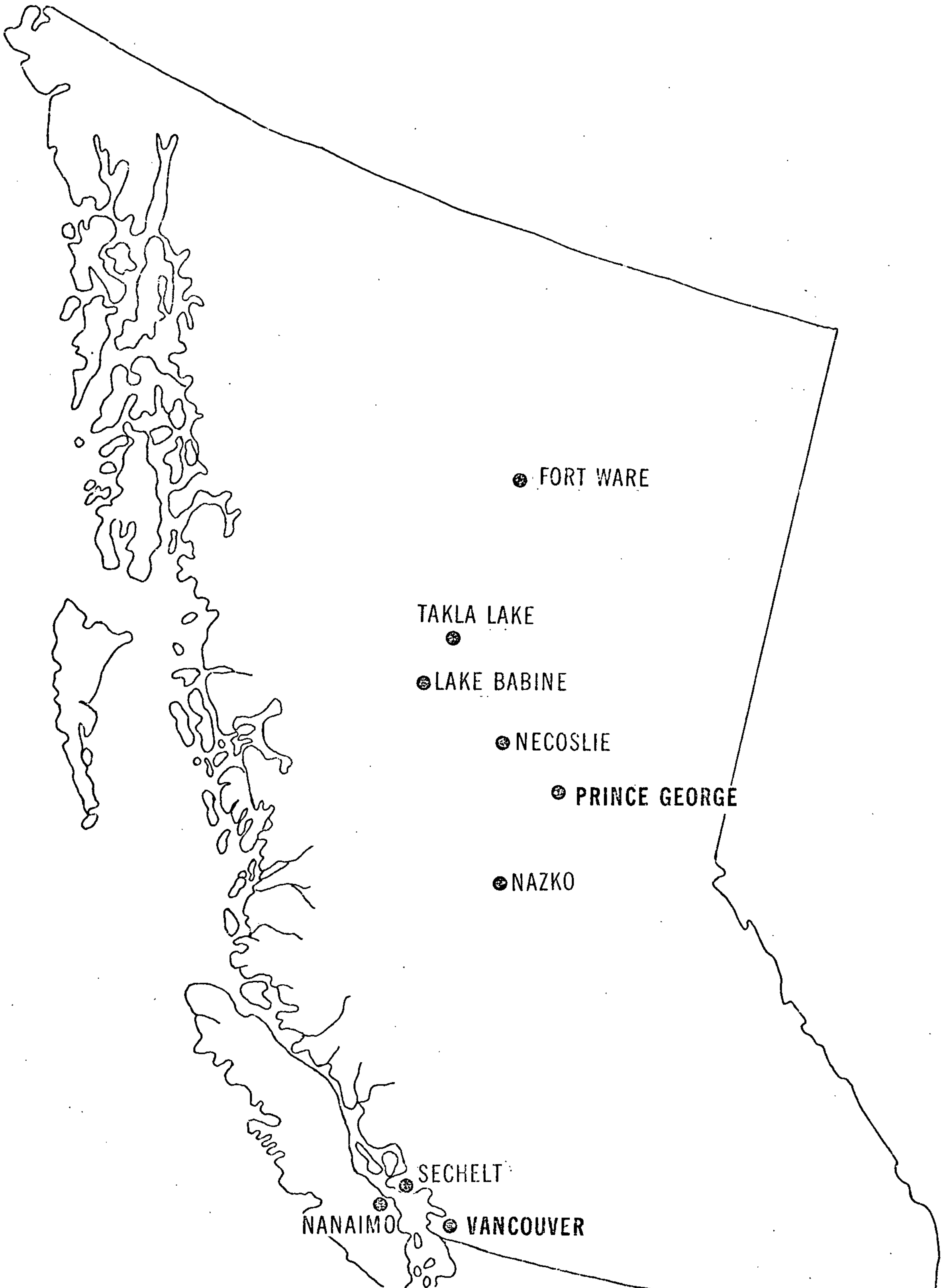
Sample Population

The study of folate status in British Columbia Indians was divided into two parts. In the first part of the study, a 24-hour recall was used to assess dietary folate status at seven reserves throughout British Columbia i.e. Sechelt Indian Reserve, Sechelt; Fort Ware Indian Reserve, Ware; Necoslie Indian Reserve, Fort Saint James; Nanaimo Indian Reserve, Nanaimo; Nazko Indian Reserve, Quesnel; and Lake Babine and Takla Lake Reserves. (See Figure 2 for locations).

Reserves were classified with respect to their state of 'isolation' where 'isolation' was assessed in terms of the distance from urban centres. Reserves located in or adjacent to urban centres of less than 5,000 people were classed as isolated and those located in or adjacent to urban centres of greater than 5,000 people were classed as non-isolated. Isolated reserves had, at most, one general store with a limited availability and selection of food stuffs. At least two food stores or supermarkets were located on or adjacent to the non-isolated reserves, offering a wider selection of food stuffs. Permanent health personnel or hospital facilities were available for the non-isolated but not the isolated reserves.

The second part of the study was a more comprehensive assessment of folate status, involving the use of dietary recalls, 24-hour food sample collections, and serum and whole blood folate measurements. This was

Figure 2: British Columbia Indian Reserves
Involved in the Study of Folic Acid Nutritional Status.


FORT WARE
TAKLA LAKE
LAKE BABINE
NECOSLIE
PRINCE GEORGE
NAZKO
SECHELT
NANAIMO
VANCOUVER

conducted among a subsample of the subjects at Sechelt Indian Reserve, Fort Ware Reserve, and Necoslie Reserve, as well as among a group of children living at the Sechelt Indian Residential School, Sechelt, B. C. Seventy children ranging from 5 to 16 years (34 male and 36 female), representing 90% of the total enrollment, participated from the Residential School.

A summary of reserves is presented in Table II, indicating the population size of each reserve, the sample size for both parts of the folate study, and the state of 'isolation', linguistic group, and cultural area of each reserve.

Subjects and reserves were not selected randomly, and were therefore not considered to be representative of all British Columbia reserve Indians. The Sechelt Indian residence and the seven reserves were selected in consultation with Medical Services, Department of National Health and Welfare, in order to assure accessability and local cooperation. Approval for this research was obtained from the Health Sciences Screening Committee, University of British Columbia, from the reserve Band Councils, and from each subject who participated. Consent forms were signed by an adult or responsible family member of each household which participated in the more comprehensive part of the study, which required both a dietary assessment and blood samples from each subject. (See Appendix A for a copy of the consent form).

TABLE II

Indian Reserves in British Columbia involved in the assessment of folate status

Reserve	Linguistic Group	Cultural Area	State of Isolation	Population Size*	Sample Size: 1. Dietary folate study: 24-hr. Recalls	Sample Size: 2. Comprehensive Folate Study: Dietary and Hematological Measures
Sechelt	Salishan	Pacific Coast	Non-isolated	380	53	30
Fort Ware	Athapaskan	MacKenzie R.	Isolated	165	32	28
Necoslie	Athapaskan	Plateau	Non-isolated	534	35	33
Nanaimo	Salishan	Pacific Coast	Non-isolated	415	57	
Nazko	Athapaskan	Plateau	Isolated	100	23	
Lake Babine	Athapaskan	Plateau	Isolated	115	21	
Takla Lake	Athapaskan	Plateau	Isolated	265	30	

* Represents the number of status Indians presently living on the reserve

Dietary Recalls, Food Collections and Analysis

A. Dietary recalls and analysis

Twenty-four hour dietary recalls were conducted on the seven reserves. The matriarchial head of the household was interviewed and asked to recall all food items consumed by herself and other members in the preceding 24 hours. Other adults and older children present at the time of the interview were also interviewed.*

The 24-hour recall data were coded into Dietary Analysis Coding Booklets, together with the subject's sex, age and identification number. Total folate, iron and caloric intakes were calculated for each subject.

Dietary folate was calculated using values from the published tables of Butterfield and Calloway (1972), Hoppner et al (1972), and Hurdle et al (1968). Food items not listed in the composition tables were individually analyzed for total folate as discussed in the following section. Food values for iron and calories were calculated from U.S. Department of Agriculture Nutrient Composition values. The 24-hour recall data were computed for the comprehensive folate status study as well, since the subjects involved were a subgroup from the larger dietary study sample.

B. Collection of food samples and analysis

For the more comprehensive part of the study, in addition to 24-hour dietary recalls, food samples representing a 24-hour consumption period were collected from Sechelt Residence and from Sechelt and Necoslie reserves.

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*All 24-hour dietary recalls were conducted by Yolanda Stepien, Masters student in Human Nutrition, School of Home Economics, U. B. C.

There was little variety in the foods consumed at Fort Ware and consistent eating patterns were seldom followed. Therefore, instead of collecting 24-hour food samples at Fort Ware, samples were collected of the major food items consumed in the diet, as determined from the 24-hour recalls.

Plastic buckets containing 500 ml phosphate buffer (pH6.1) and 150 mg% ascorbic acid were used to collect the 24-hour food samples. Samples from the residential school were prepared by observing the meals and snacks eaten by the children and collecting duplicate food samples representative of a child's total consumption over the 24-hour period. Records were kept of all food items added to the bucket. The 24-hour food samples from the two reserves were collected and recorded by women from each household. They were instructed to add to the bucket duplicate samples of all foods they had eaten for one 24-hour period, and to keep a written record of the amount of every item that was added. A 24-hour recall for the collection period was used to cross-check the written record. Food buckets were kept refrigerated during the sample collection.

After collection the food samples were homogenized for one minute in a Waring Blender. Aliquots of homogenate were immediately refrigerated in air tight containers to prevent oxidation and frozen at -20°C within 24 hours. These samples were used for food folate analysis. Individual food samples from Fort Ware were homogenized and stored in a similar manner.

Total folate values for the 24-hour food collections and individual food samples were determined by the Lactobacillus casei microbiological method of Herbert (1963), utilizing chicken pancreas conjugase.* Bacto-Lactobacilli Broth AOAC** was used as the maintenance

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* Difco Chicken pancreas, from Difco Laboratories, Detroit, Michigan
** Difco certified, from Difco Laboratories, Detroit, Michigan.

culture and Bacto Folic Acid Casei Medium* was the assay medium.

Two groups of pooled sera were run as controls with each assay, a 'low folate' pool (1.2 to 2.0ng/ml) and a 'high folate' pool (10.5 to 12.0ng/ml). Folic acid (pteroyl-glutamic acid) "Baker Grade"** was used to prepare standard folate solutions.

Blood Collection, Treatment, and Analysis

A. Collection and treatment of blood samples

The comprehensive part of the folate study involved collections of blood samples from each subject. Blood was collected after a minimum fast of 3 hours. Three venous blood samples were obtained from each subject; one into a 10ml vacutainer tube*** with no additive, the second into a 4.5ml vacutainer containing 0.5ml of 3.8% sodium citrate, and the third into a 7ml vacutainer containing 10.5 mg. disodium ethylenediamine tetraacetic acid (EDTA).

The whole blood collected without anticoagulant was kept at room temperature for approximately 1 hour to allow firm clot formation. It was then centrifuged at 2000 rpm for 15 minutes and the serum was transferred to plastic tubes **** with a pasteur pipette. The sera were frozen at -20°C within 8 hours of collection and stored for serum folate analysis.

A microhematocrit was performed on citrated blood samples and the remaining whole blood was frozen at -20°C within 8 hours of collection and stored for red cell folate analysis.

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* Difco certified, from Difco Laboratories, Detroit, Michigan.
** J. T. Baker Chemical Co., New Jersey Lot No. 1-755
*** Vacutainer tubes, Becton, Dickenson and Co., Canada, Limited
**** Falcon polystyrene tubes.

A thin blood smear was prepared from samples collected into EDTA - containing vacutainers and was used for microscopic examination. Hemoglobin, hematocrit and white blood cell counts, red blood cell counts, and red cell indices were determined on the EDTA-treated blood samples, with a Model S Coulter Counter.

Blood samples were refrigerated immediately after collection and were packed in ice during transport.

B. Blood Folate analysis

Serum and red cell folates were assayed using *Lactobacillus Casei* microbiological methods. Serum folate was determined by the method of Baker *et al* (1959) and red cell folate by the modified methods of Hoffbrand *et al* (1966) and Spray (1969). Serum and whole blood samples were thawed for analysis and immediately diluted with sodium phosphate buffer (pH 6.1) containing 150 mg% ascorbic acid. The materials used in preparation of the maintenance culture, assay medium, and standard curve were those described for the assay of food folate.

The pooled control sera were run with each assay. As a further control measure, selected serum and whole blood samples were also assayed in an independent laboratory.*

Statistical Analysis

All statistical analyses were performed with an IBM 360/370 computer at the computing centre of the University of British Columbia.

A linear correlation analysis was used to measure the relationship between the assayed and calculated folate values from the 24-hour food collections. The Pearson Product-Moment Correlation Coefficient was calculated.

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* Hematology Department, Saint Paul's Hospital, Vancouver, B. C.

The Trip /360 program was employed to compute the mean, standard deviation and correlation matrix of the biochemical and dietary parameters, which were grouped according to sex, location, and specific age categories.

Regression analysis was conducted for blood and food folate data from three reserves involved in the comprehensive part of the study. Regression equations were tested for equality between sexes and the different locations by means of an S:SL Test (Equality of Slope Test). The TRIP regression program tested the goodness of fit for the regression lines, testing the relationship between serum and red cell folate levels and dietary folate intake.

The Student's t-test with $p=.05$ was used to test, for a given variable, statistical significance between two sample means. Comparisons were made for hematological and dietary variables, grouping the means in terms of sex, location, or degree of isolation.

A one-way analysis of variance with $p=0.05$ was chosen to test, for a given variable, statistical significance among three or more samples means. Again, comparisons were made for hematological and dietary variables, grouping the means by location, sex and age categories. A Duncan's Multiple Range Test was performed, when means were significantly different, to determine both homogeneous subsets within the group of means and the means which differed significantly from the rest of the group.

CHAPTER IV

RESULTS

Dietary Folate Data

A. Assayed vs. calculated values

Results of the Pearson Product - Moment Test of Correlation between assayed and calculated total folate values for the 24-hour food collections are presented in Table III. An r-value of .9694 suggests a highly significant linear correlation between the assayed and calculated values, suggesting that the computed folate values are a reliable estimate of the actual folate intakes.

The scatter diagram for assayed and calculated total folate values from the 24-hour food collections (Figure 3) illustrates the correlation between the variable pairs. The individual folate values are listed in Appendix B.

TABLE III

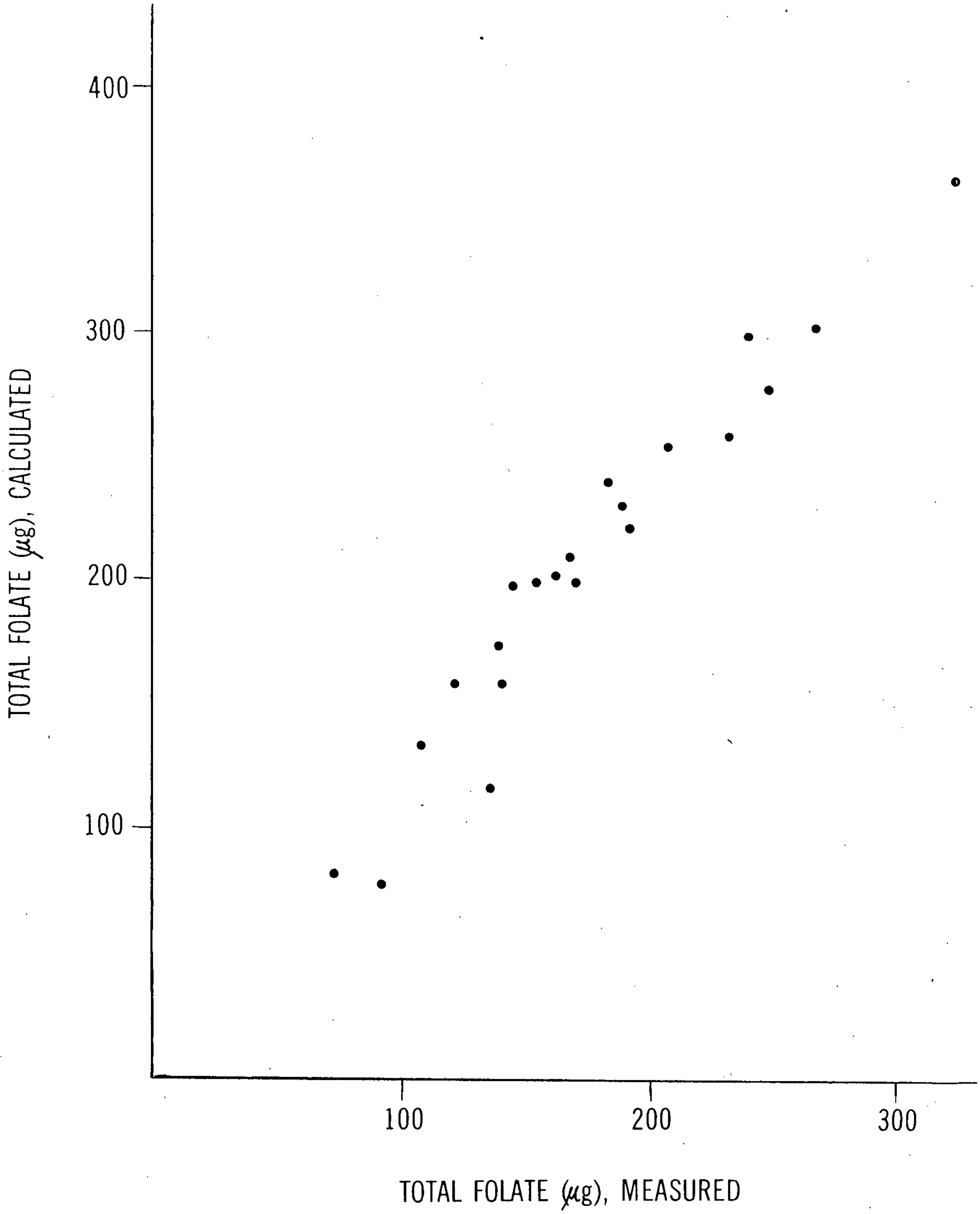
Pearson Product-Moment Correlation Coefficient: Test of correlation between assayed and calculated values for total folate from 24-hour food collections (21 paired values).

Variable Pair	Coefficients	
	r*	r^2**
Total Folate Value Assayed vs. Calculated	.9694	.9397

* Measure of linear Correlation between the two variables

** 100 x r^2% of the variation in the values of one variable may be accounted for by the linear relationship with the other variable.

Figure 3: Scatter diagram showing the degree of correlation between calculated and assayed values of total folate from 24-hour food collections.


400
300
200
100
TOTAL FOLATE (µg), CALCULATED
100
200
300
TOTAL FOLATE (µg), MEASURED

B. Comparison of mean folate intakes for the seven reserves

A Multiple Range Test was performed on mean folate scores calculated from the 24-hour dietary recalls conducted on the seven reserves. As shown in Table IV mean intakes range from 125.4 µg total folate for Fort Ware to 236.2µg for Nanaimo. The highest and lowest means are significantly different at $P<0.05$. Individual values for nutrient intakes are listed in Appendix E.

A Multiple Range Test was performed on mean folate scores for all seven reserves for different age and sex groupings (Table V). Results indicate that mean folate intakes do not differ significantly among the three age categories in the population as a whole. Significant differences are only seen for males, where 10 to 19 year olds have a significantly lower mean intake than males 20 years of age or older ($P<0.05$). In general, these results indicate that folate consumption does not significantly increase with age.

TABLE IV

Multiple Range Test of mean scores for total folate intake for the seven reserves under study.

Location	N	Total Folate Intake (µg/Day)
Fort Ware	26	125.4^{a*}
Takla Lake	30	153.5^{ab}
Sechelt	53	171.6^{ab}
Nazko	23	186.9^{ab}
Necoslie	35	189.9^{ab}
Lake Babine	21	200.9^{ab}
Nanaimo	56	236.2^{b}

*Means not sharing the same superscript are significantly different at $P<0.05$

TABLE V

Multiple Range Test of mean scores for food folate intake for sex and age groupings for the seven reserves studied.

Age (Years)	Sex	N	Total Folate Intake (µg/Day)
0-9	M&F	53	189.7[a]*
10-19		51	190.9[a]
20+		146	191.6[a]
0-9	Male	17	248.6[ab]
10-19		25	210.6[a]
20+		45	280.3[b]
0-9	Female	36	161.8[a]
10-19		26	171.9[a]
20+		101	152.1[a]

*Means not sharing the same superscript within each group of 3 means are significantly different at $P<0.05$.

A t-test was performed comparing mean scores for food folate intake for the seven reserves with respect to isolation and sex groupings. As Table VI shows, mean intake of total folate is significantly greater on the non-isolated reserves as compared to the isolated reserves, both for the subjects as a whole, and for male and female subjects separately. The mean folate intake for males is significantly greater than the mean for females ($P<0.05$). Generally the results indicate that diets are poorest with respect to dietary folate intakes for females on isolated reserves and are better for males, particularly on non-isolated reserves.

As Table VII shows, the mean total folate intake for subjects from all seven reserves is below the Recommended Daily Allowance for total folate (200 µg for persons 1 to 12 years old and 400 µg for those 13 years of age and older). The mean calorie intake is low as compared to recommendations, for the females as a whole and for males from the isolated reserves. Iron intakes are adequate except for females on non-isolated reserves, where the mean is slightly below recommendations. (Refer to Appendix C for recommendations.)

TABLE VI

T-test of mean scores for folate intake for the seven reserves with respect to isolation and sex.

Reserve Grouping	Sex	N	Total Folate Mean + S. D. (µg/Day)	T-Test Values	
				T-Prob*	F-Prob**
Non-isolated	M&F	144	211.7±117.1	.000	.000
Isolated	M&F	106	163.1± 76.2		
Non-isolated	M	58	273.1±132.8	.046	-
Isolated	M	29	216.2±106.6		
Non-isolated	F	86	170.3± 83.2	.011	.000
Isolated	F	77	143.1± 48.5		
All Reserves	M	87	254.1±126.9	.000	.000
All Reserves	F	163	157.4± 70.2		

* Probability values $P<0.05$ indicate significant difference between sample means. Dashes (-) indicate no significant difference.

** Probability values $P<0.05$ indicate significant difference between sample variances. Dashes (-) indicate no significant difference.

TABLE VII

Mean daily intake of folate, calories, and iron for subjects from the seven reserves.

Group Mean	N	Total Folate (µg/Day)	Calories*	Iron (mg/Day*
1-12 Years	70	194.1±103.2[1]	1919±687	14.1±9.4
13+ Years	180	190.2±106.1[2]	1979±788	15.0±8.2
Females:				
Non-isolated reserves	86	170.3± 83.2	1727±383	10.5±3.4
Males:				
Non-isolated reserves	58	273.1±132.8	2803±848	17.7±6.3
Females:				
Isolated Reserves	77	143.1± 48.5	1673±542	15.2±5.3
Males:				
Isolated Reserves	29	216.2±106.6	2229±549	19.1±7.7

*See Appendix C for Canadian Recommended Intake.

[1] WHO Recommended Daily Allowance = 200 µg Total Folate

[2] WHO Recommended Daily Allowance = 400 µg Total Folate

C. Comparisons of mean folate intakes for subjects involved in the comprehensive part of the survey

Table VIII shows the results of a Multiple Range Test of means for total folate intake for reserve subjects involved in the comprehensive part of the study (i.e.) subgroup selected from Sechelt, Necoslie and Fort Ware reserves. The mean intake for Fort Ware is significantly lower than for Sechelt or Necoslie reserves ($P<0.05$).

The mean total folate consumption for children at the Sechelt Residential School is considerably greater than at any of the three reserves. (Individual values for nutrient intakes are presented in Appendix F.)

TABLE VIII

Multiple Range Test of mean scores for food folate intake for subjects involved in the comprehensive folate study from Sechelt, Necoslie, and Fort Ware Reserves **

Location	N	Total Folate Intake (μg/Day)
Fort Ware	20	130.6[a]*
Sechelt	30	165.6[b]
Necoslie	33	173.6[b]
Residence	70	310.1

* Means not sharing the same superscript are significantly different at $P < 0.05$.

** Mean reserve values were calculated from 24-hour recalls. The residence mean was calculated from a 3-day food record representing the average intake of all subjects.

The results of a t-test of mean scores for dietary folate intake for subjects involved in the comprehensive part of the folate study are presented in Table IX. The values indicate that the mean folate intake for Fort Ware, an isolated reserve, is significantly less than the mean intake for the non-isolated reserves, Sechelt and Necoslie. The mean folate intake for females from the group as a whole is significantly less than the mean for males ($P<0.05$). The results indicate that in general, diets are poorest with respect to dietary folate intake for females from the isolated reserve.

TABLE IX

T-test of mean scores for dietary folate intake for subjects involved in the comprehensive folate study from the three reserves.

Means Compared	N	Total Folate Mean + S.D. (µg/Day)	T-test Values T-Prob*	T-test Values F-Prob**
Sechelt & Necoslie	63	169.8±79.8		
Fort Ware	20	130.6±53.2	.041	-
Reserve Males	34	185.1±94.4	.023	.001
Reserve Females	49	143.2±54.4		

* Probability value $P<0.05$ indicates significant difference between the sample means. Dashes (-) indicate no significant difference.

** Probability value $P<0.05$ indicates significant difference between the sample variances. Dashes (-) indicate no significant difference.

As shown in Table X, the mean total folate intake for reserve subjects involved in the comprehensive part of the study is well below the WHO Recommended Daily Allowance for total folate (200 µg for persons 1 to 12 years and 400 µg for those 13 years of age and older). The mean intake for residence subjects of 310.0 µg. total folate places the children from 1 to 12 years old in the recommended range for folate consumption. Mean calorie intakes are generally low for reserve subjects. In most cases the mean intake for the residence is more than 200 calories higher than the reserve means, and is closer to the recommended intake. Mean iron intakes for the reserve and residence subjects are adequate or slightly below Canadian recommendations. (Refer to Appendix C for Recommendations).

TABLE X

Mean daily intake of total folate, calories, and iron for location and age groupings for subjects involved in the comprehensive part of the study.

Location	Age (Years)	N	Total Folate (µg/Day)	Calories*	Iron (mg)*
Sechelt, Necoslie	1-12	18	163.8± 91.5[1]	1980±853	10.6±5.0
& Fort Ware	13+	65	159.4± 71.7[2]	1893±622	12.3±4.6
Sechelt & Necoslie	1-12	14	165.5± 93.7[1]	1846±888	9.7±5.2
	13+	49	171.1± 76.4[2]	1917±606	12.3±4.3
Fort Ware	1-12	4	158.1±101.0[1]	2450±905	13.9±6.1
	13+	16	123.8± 81.2[2]	1821±698	12.5±4.7
Residence	1-12	14	310.1	2227	13.4
	13+	56			

* See Appendix C for Canadian Recommended Intake

1 WHO Recommended Daily Allowance = 200 µg Total Folate
2 WHO Recommended Daily Allowance = 400 µg Total Folate

Hematological Data

All hematological data were obtained from the subgroup selected from Sechelt, Necoslie and Fort Ware reserves and from Sechelt Residence children. When data are presented in terms of specific reserve subjects they represent the values for the subgroup selected from the reserves.

A. Control sample values

Table XI shows the folate concentrations of the selected serum and whole blood samples, which were analyzed in duplicate (i.e. in this laboratory and in an independent laboratory).

TABLE XI

Comparison of serum and red cell folate values for duplicate samples run as a control measure.

Sample	Values Reported (ng/ml)	
	This Laboratory	Independent Laboratory*
Serum Folate		
1	2.0	2.3
2	12.0	13.0
3	3.9	4.6
4	5.7	5.0
5	14.9	14.0, 17.0
6	8.2	8.0, 9.0
Red Cell Folate		
1	210	199
2	117	103
3	94	109
4	198	181
5	64	54

* Hematology Department, Saint Paul's Hospital, Vancouver, B. C.

The results indicate that values for the paired samples for both serum and red cell folate are in reasonable agreement. The paired values are similar enough that one can be confident that very low values (i.e. 2.0 and 2.3 ng/ml) represent a sample with very low folate levels, and conversely, high values (i.e. 14.9, 14.0 and 17.0 ng/ml) truely represent a high folate containing sample.

B. Comparison of means for hematological variables

Table XII gives the results of a Multiple Range Test performed on mean scores for serum folate, red cell folate and hemoglobin measurements for subjects from the three reserves. (Individual values for hematological variables are listed in Appendix G.)

TABLE XII

Multiple range test of mean scores for serum folate, red cell folate and hemoglobin for the three reserves involved in the comprehensive part of the study.

Location	N	Serum Folate (ng/ml)	Red Cell Folate (ng/ml)	Hemoglobin g/100ml)
Sechelt	30	6.8^{b}*	251^{b}	13.3^{a}
Fort Ware	20	4.1^{a}	179^{a}	14.1^{a}
Necoslie	33	5.7^{b}	189^{a}	13.3^{a}
Residence	69	8.3	124	12.2

*Means not sharing the same superscript under each variable are significantly different at $P<0.05$.

The mean serum folate for Fort Ware is significantly lower than for Sechelt or Necoslie Reserves, whose means are not significantly different from one another ($P<0.05$). The mean red cell folate for Sechelt is significantly higher than for Necoslie or Fort Ware, whose means are not significantly different. Mean hemoglobin values are not significantly different among the three reserves ($P<0.05$).

The mean serum folate for Sechelt Residential School is considerably higher than the three reserve means, while the mean red cell folate is considerably lower.

Table XIII gives the results of a Multiple Range Test of mean scores for hemoglobin and folate variables grouped into age and sex categories for subjects from the three reserves. The serum folate mean for 0 to 9 year olds is significantly greater than for the older age categories ($P<0.05$), with values for females contributing most to the variability between the age groups. Mean red cell folate is significantly higher for 0 to 9 year olds than for older subjects ($P<0.05$) with values for male subjects contributing most to the variability. Hemoglobin means are not significantly different for the group as a whole, although adult males have a significantly higher mean than males from 0 to 9 years old and adult females have a significantly higher mean than females from 10 to 19 years old ($P<0.05$).

The results of a t-test performed on serum and red cell folate means are presented in Table XIV. Both the serum and red cell folate means for Fort Ware are significantly lower than the joint means for Sechelt and Necoslie reserves ($P<0.05$), indicating that folate status is significantly lower on the isolated reserve as compared to the non-isolated reserves. A comparison of means between reserve males and females indicates there is no significant difference for either serum or red cell folate variables.

TABLE XIII

Multiple range test of mean scores for serum folate, red cell folate, and hemoglobin for age and sex groupings for the three reserves involved in the comprehensive part of the study.

Age (Years)	Sex	N	Serum Folate (ng/ml)	Red Cell Folate (ng/ml)	Hemoglobin (g/100 ml)
0- 9	Male & Female	7	7.6^{b}*	243^{b}	12.7^{a}
10-19		26	5.6^{a}	212^{a}	13.3^{a}
20+		50	5.4^{a}	203^{a}	13.5^{a}
0- 9	Male	2	5.8^{a}	274^{c}	12.7^{a}
10-19		19	5.2^{a}	207^{b}	14.0^{ab}
20+		13	6.4^{a}	153^{a}	15.0^{b}
0- 9	Female	5	8.5^{b}	227^{a}	12.7^{ab}
10-19		7	6.5^{ab}	224^{a}	11.4^{a}
20+		37	5.0^{a}	221^{a}	13.0^{b}

* Means not sharing the same superscript under each variable, in each grouping of three means, are significantly different at $P<0.05$.

TABLE XIV

T-test of mean scores for serum and red cell folate variables for location and sex groupings for subjects involved in the comprehensive part of the survey.

Variable	Means Compared	N	Mean ± S.D.	T-test Values	
				T-Prob*	F-Prob**
Serum Folate (ng/ml)	Sechelt & Necoslie	63	6.2±2.4	.000	.031
	Fort Ware	20	4.1±1.5		
	Reserve Males	34	5.9±2.6	-	-
	Reserve Females	49	5.6±2.3		
Red Cell Folate (ng/ml)	Sechelt & Necoslie	63	218±82	.015	.047
	Fort Ware	20	179±54		
	Reserve Males	34	190±64	-	-
	Reserve Females	49	222±84		

* Probability value P<0.05 indicates significant difference between the sample means. Dashes (-) indicate no significant difference.

** Probability value P<0.05 indicates significant difference between the sample variances. Dashes (-) indicate no significant difference.

C. Risk distributions

Serum folate, red cell folate, and hemoglobin values are distributed into risk categories as illustrated in Figure 4. Standards used to designate the three risk grouping are presented in Appendix D. Subjects are classed "at risk" with respect to a variable if the value of the variable tested is in the range of either the high or moderate risk categories.

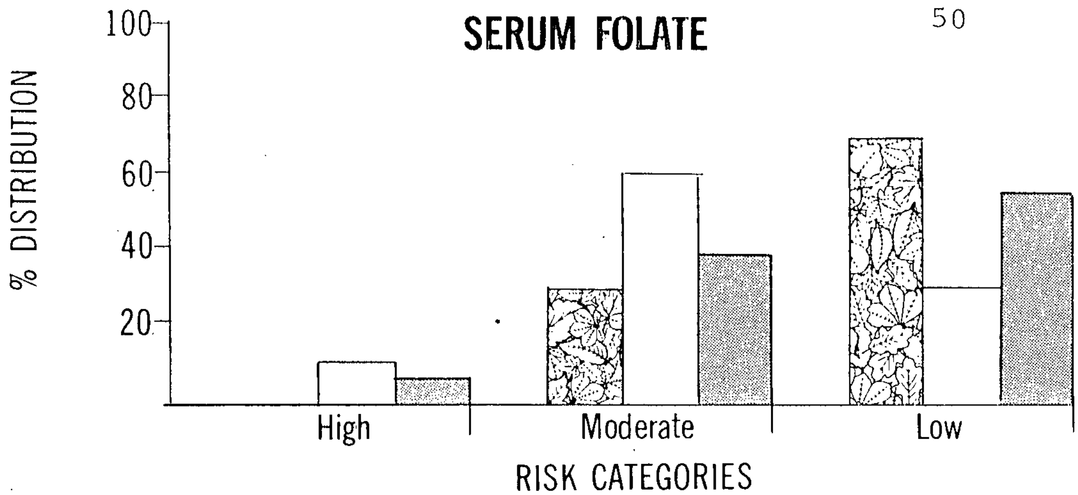
Results indicate that 70% of the subjects from Fort Ware are "at risk" with respect to serum folate with 10% high and 60% moderate risk values. Necoslie has 45% "at risk" with 6% high and 39% moderate risk values while Sechelt has 30% "at risk", with all values in the moderate risk category.

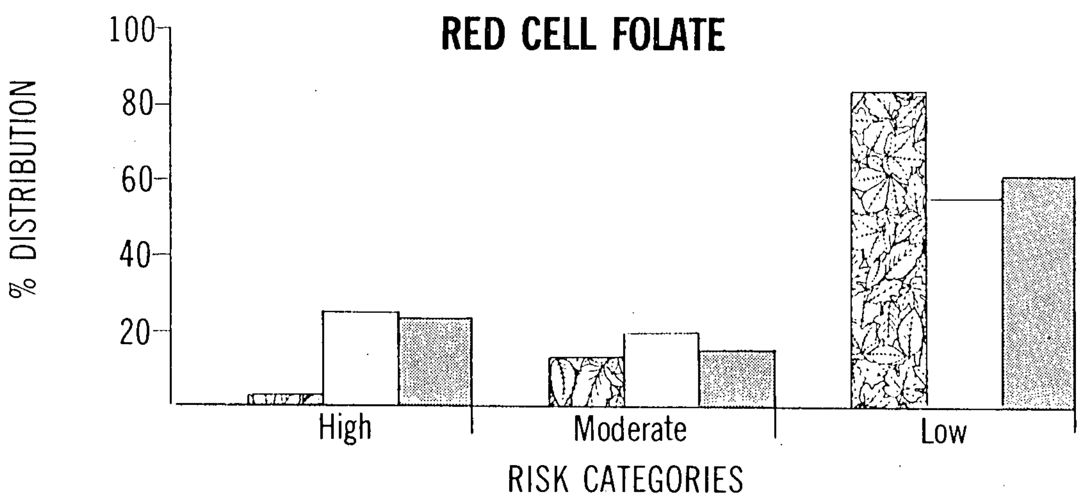
Forty-five percent of Fort Ware's subjects are "at risk" with respect to red cell folate, with 25% high and 20% moderate risk values. Necoslie has 24% and 15% of its values in high and moderate risk categories respectively, while Sechelt has just 3% in high risk and 13% in moderate risk categories.

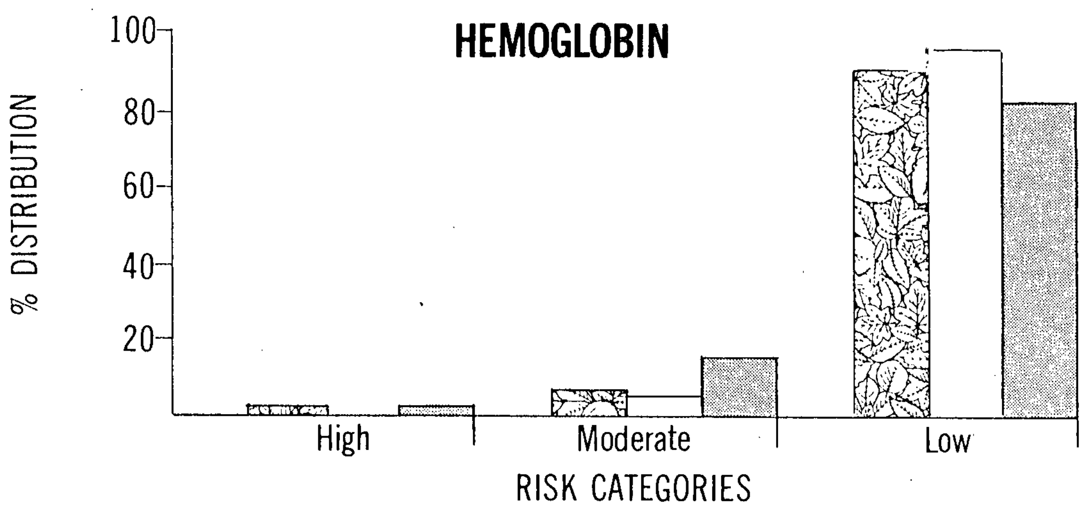
Although Fort Ware has the poorest folate status with the greatest percentage of subjects "at risk", it has the least subjects "at risk" with respect to hemoglobin (i.e. 5%). Ten percent and 18% of the subjects from Sechelt and Necoslie respectively are "at risk", with 3% from both reserves having high risk values.

Figure 4: Distribution of hematological variables into risk categories for the three reserves.

		N
	Sechelt	30
	Fort Ware	20
	Necoslie	33


SERUM FOLATE
% DISTRIBUTION
100
80
60
40
20
High
Moderate
Low
RISK CATEGORIES


RED CELL FOLATE
% DISTRIBUTION
100
80
60
40
20
High
Moderate
Low
RISK CATEGORIES


HEMOGLOBIN
% DISTRIBUTION
100
80
60
40
20
High
Moderate
Low
RISK CATEGORIES

Hematological variables are distributed into risk categories for subjects 0 to 17 years old from Sechelt Residential School and from the three reserves, as illustrated in Figure 5.

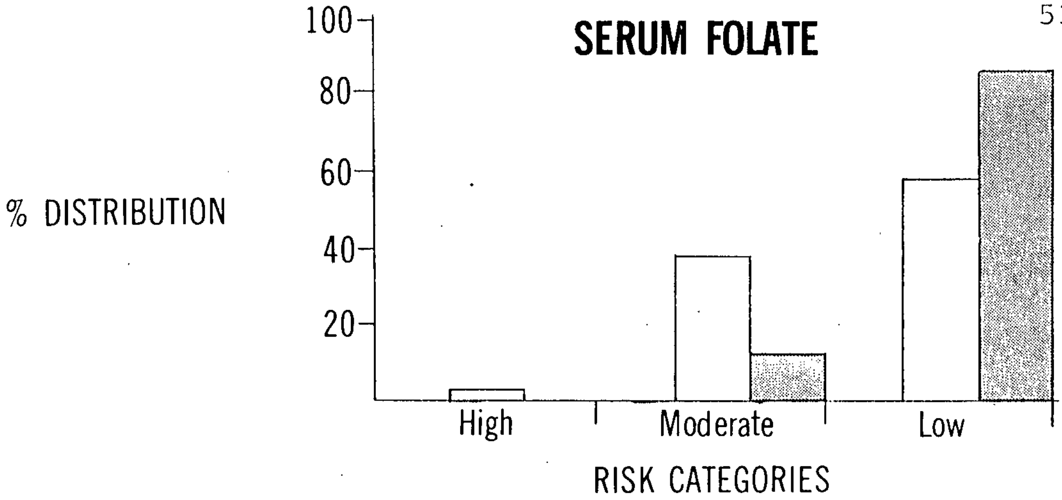
The percentage of reserve children with serum folate values in high and moderate risk categories are 3% and 38% respectively. Thirteen percent of the residence children are "at risk" with values in the moderate risk category only. Seventy-seven percent of the red cell folate values for residence children and 28% for reserve children are in high and moderate risk categories. No children have high risk hemoglobin values, although 25% of the residence children and 3% from reserves have values in the moderate risk category.

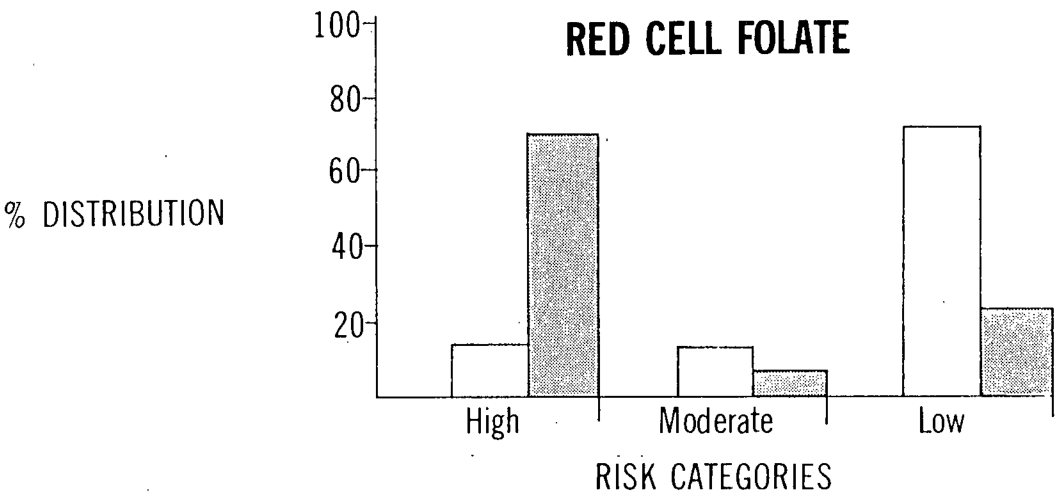
The risk distribution of serum folate values for this study and for two groups from the Nutrition Canada Survey (i.e. National Indian Survey and British Columbia Provincial Survey) is illustrated in Figure 6.

A greater percentage of subjects 5 to 9 years old were "at risk" in the National Indian Survey and British Columbia Provincial Survey as compared to this study, i.e. 60% and 58% as compared to 17%. Approximately 70% of the subjects 10 to 19 years old were "at risk" in the National Indian and B. C. Provincial Surveys as compared to 48% in this study. For subjects 20 years of age and older, 50% are "at risk" in this study as compared to 65% and 60% for the National Indian Survey and B. C. Provincial Survey respectively. Overall there was a greater incidence of high risk values for the two groups from the Nutrition Canada Survey as compared to this study, particularly for the National Indian Survey.

Figure 5: Distribution of hematological variables into risk categories for Residence and Reserve Subjects (0 to 17 Years old)

		N
☐	Reserve subjects (0 to 17 Years)	29
▒	Residence Subjects (0 to 17 Years)	69


SERUM FOLATE
% DISTRIBUTION
100
80
60
40
20
High
Moderate
Low
RISK CATEGORIES


RED CELL FOLATE
% DISTRIBUTION
100
80
60
40
20
High
Moderate
Low
RISK CATEGORIES

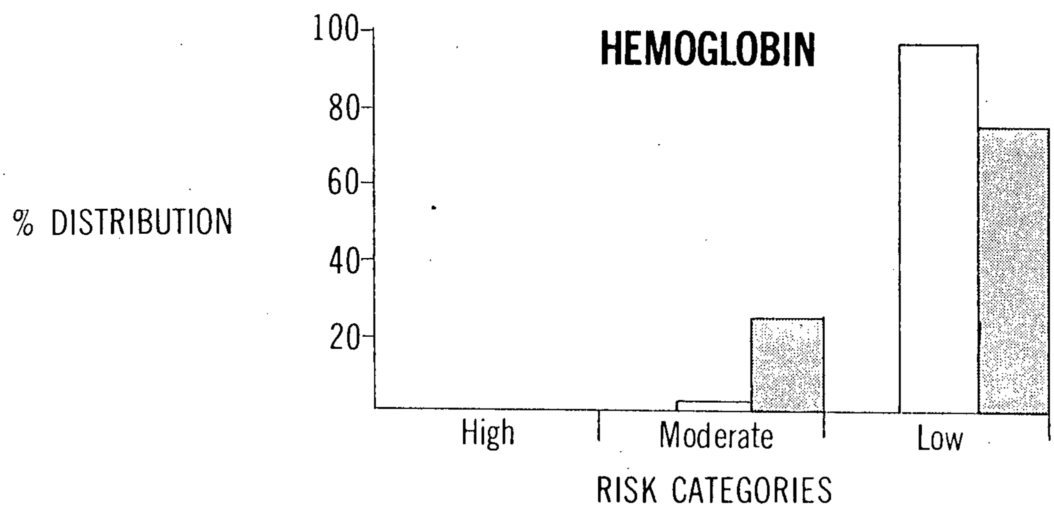
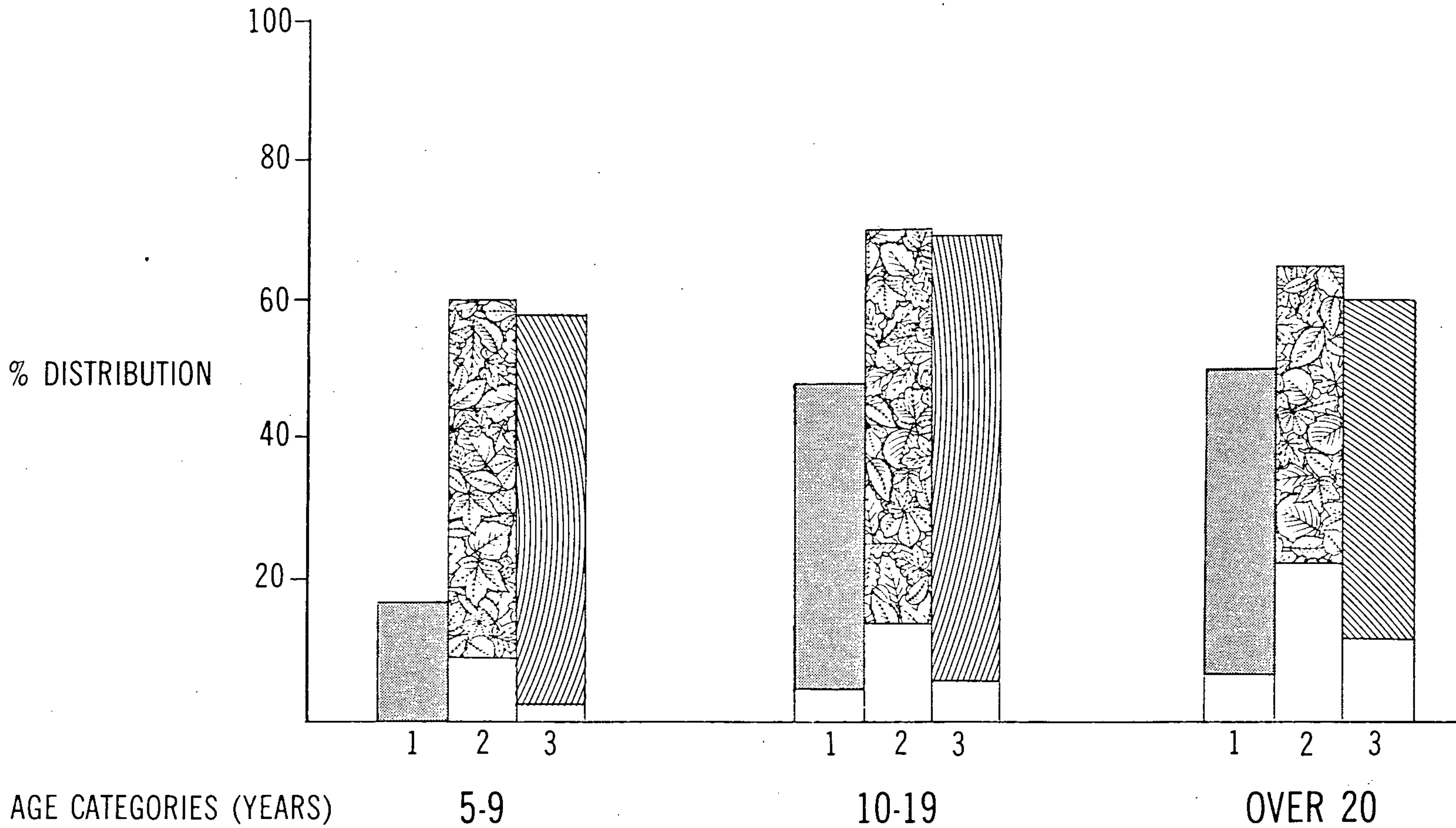

HEMOGLOBIN
% DISTRIBUTION
100
80
60
40
20
High
Moderate
Low
RISK CATEGORIES

Figure 6: Classification of serum folate values for reserve subjects from this study and for two groups from the Nutrition Canada Survey i.e. National Indian survey and B. C. Provincial survey.

	Risk Categories		N		
	High	Moderate	Age Categories (Years) 5-9	10-19	20+
1. This Study			7	26	50
2. National Indian Survey			217	437	815
3. B.C.Provincial Survey			253	548	1301


100
80
60
40
20
% DISTRIBUTION
1
2
3
AGE CATEGORIES (YEARS)
5-9
10-19
OVER 20

Regression Analysis

A SL-Test of regression equations was conducted to compare equations for different groups of subjects involved in the comprehensive part of the survey, with equations in the form of (y=A+Bx). Serum and red cell folate were set as the dependent variables and dietary folate the independent variable. Results from Table XV indicate there is not a common equation for the three reserves for both serum and red cell folate variables. A common regression equation is indicated between male and female subjects, for serum folate only ($P < 0.05$).

TABLE XV

SL-Test of comparison of regression equations with serum folate or red cell folate as dependent variables and dietary folate intake the independent variable, with equations in the form of (Y=A+Bx).

Dependent Variable (Y)	Group Equations Compared	F-Value*
Serum Folate	Sechelt vs. Fort Ware vs. Necoslie	-
	Reserve Males vs. Reserve Females	0.00
Red Cell Folate	Sechelt vs. Fort Ware vs. Necoslie	-
	Reserve Males vs. Reserve Females	-

*F-Value <0.05 indicates there is a common predicting equation for the groups compared. Dashes (-) indicate a common equation does not exist.

A regression analysis was performed to test the linear relationship between blood folate values (i.e. serum and red cell) and dietary folate intake for reserve subjects from Sechelt, Necoslie and Fort Ware. Results from Table XVI indicate there is no significant relationship between red cell folate values and dietary folate intake for the three groups tested. A significant relationship is indicated between serum folate values and dietary folate intake ($P<0.05$), for the subjects as a whole and for male subjects alone. Therefore, serum folate values are significantly and directly related to dietary folate intake.

TABLE XVI

Regression Line constants with serum folate or red cell folate as dependent variables and dietary folate intake as the independent variable, with equations in the form of ($y=A+Bx$).

Dependent Variable (ng/ml)	Sex	Intercept A (ng/ml)	Slope B (ng/ml) $(ng/Day)^{-1}$	F-Prob*
Serum Folate	M & F	4.344	.0085	.0133
	Male	3.898	.0110	.0172
	Female	5.095	.0032	-
Red Cell Folate	M & F	202.9	.0372	-
	Male	165.4	.1336	-
	Female	216.9	.0349	-

* Probability value $P<0.05$ indicates there is a significant relationship given by the equation. Dashes (-) indicate there is no significant relationship.

CHAPTER V

DISCUSSION

Total folate values for the 24-hour dietary recalls, calculated from food composition tables, are similar and are significantly correlated with the total folate values obtained by direct assay, for the 24-hour food sample collections. This offers confidence that our computed folate values are reliable for the assessment of dietary folate status on the seven reserves, and supports the 24-hour dietary recall as being a reliable method for estimating folate consumption for the Indian groups involved in this study. A close agreement between assayed and calculated folate values was also obtained by Moscovitch and Cooper (1973) and Pietarinen (1975) using similar methods.

Since most composition tables are inaccurate for calculating food folate, the importance of selecting the proper values and being confident that the folate values are reliable is fundamental to any study of this nature (Thenen, 1975 and Hurdle, 1973).

A comparison of total folate intakes for the seven reserves, as calculated from 24-hour recalls, reveals that the intake of dietary folate is significantly less on isolated reserves as compared to the non-isolated reserves. This is true for the subgroup involved in the comprehensive part of the study as well, where the mean folate intake at Fort Ware, an isolated reserve, is significantly less than mean intakes at Sechelt and Necoslie reserves, which are classified as non-isolated. Some authors have reported that foods known to be high in folic acid, such as fresh fruits and vegetables, are lacking or constitute a very small part of the Canadian Indian diet (Moore, 1946, Dong and Feeney, 1968 and Lee et al, 1971). This is especially true for Indians living in more isolated areas where the selection of fresh produce is restricted (Lee et al, 1971 and Smith, 1975) and canned fruits and vegetables are often unavailable (Smith, 1975). At one time, before the Indians moved to reservations, they obtained a great deal of food from the land. The Indians in British Columbia consumed a variety of berries such as red huckleberries, thimbleberries, blackberries, salalberries and salmonberries, a variety of vegetables, root crops, and other fruits such as clover root, fern root, skunk cabbage, wild turnips, wild peas, artichokes, choke cherries, crabapples and wild plums. Possibly many of these foods are still consumed but are not reported by the people, since they are not regarded as important food items, or conversely, these foods no longer make up a significant part of the Indian diet. Wild huckleberries and soapberries are gathered and preserved by some families in this study, although there was no mention of other wild fruits or vegetables being consumed.

Dietary folate intakes do not differ significantly with age for the group of subjects as a whole, however differences in folate consumption are observed between male and female subjects. For subjects from the total reserve population and for the reserve subgroup, the males consume significantly more dietary folate than the females; (i.e. 251 μg total folate as compared to 157.4 μg respectively for the total population). This is in agreement with other studies which report a higher dietary folate consumption in male adolescents as compared to females (Daniel <u>et al</u>, (1975) and adult males as compared to adult females (Levy <u>et al</u>, 1975).

In the study reported by Levy <u>et al</u>,(1975), low folate consumption in women of child-bearing age was related to the considerably higher incidence of folate deficiency anemia in women as compared to men. Lower dietary folate intakes for women in this study are likely placing them at higher risk in terms of folate status, especially for those who are considered to be particularly prone to folate deficiency; i.e. women taking oral contraceptives (Pietarinen, 1975); women who are pregnant (Sauberlich <u>et al</u>, 1974 and Herbert <u>et al</u>, 1975) or chronic alcoholics (Herbert and Zulusky, 1961 and Baker <u>et al</u>, 1975).

Patterns of dietary folate intake among the different groups tend to follow patterns of calorie consumption, in that groups with the highest calorie intakes (i.e. males from non-isolated and isolated reserves) also have the highest dietary folate intakes. Similarly, the lowest groups with respect to calorie consumption (i.e. women on isolated and non-isolated reserves) also have the lowest intakes of dietary folate. Therefore, if the foods which constitute the diet of these Indian people are adequate in calories, they are likely to contribute considerably more dietary folate than foods which constitute a low calorie diet. Either larger volumes of the same food are consumed in high calorie as compared to low calorie diets, or additional foods are introduced which increase the dietary folate and calorie intakes to more adequate levels. On the reserves where food selection is limited, men tend to consume larger volumes of the food which is available to everyone. Women reported omitting meals or eating very small amounts of breakfast and lunch, while men seldom missed a meal.

Results for the hematological measurements from the reserves and Sechelt Residential School point out important differences between the groups with respect to their folate status. Mean serum folate is significantly lower for Fort Ware, the isolated reserve, than for Sechelt or Necoslie reserves. Fort Ware also has the highest percentage of low serum values, with 70% of the subjects "at risk". Nutrition Canada (1973) also found that the incidence of low serum folate values was higher for Indians on isolated as compared to non-isolated reserves.

It is interesting to note that at Fort Ware, where dietary folate intakes are the lowest, the serum folate values are also the lowest. Conversely, the mean dietary folate intake for Sechelt Residential School is considerably higher than the mean intakes for the three reserves, while the serum folate values are also considerably higher. This is consistent with reports that serum folate values are reflections of recent dietary folate intakes (Herbert, 1962, Joint FAO/WHO Expert Group, 1970, Liu, 1974 and Hall et al, 1975). The results from the regression analysis further support this finding, indicating that serum folate values are directly and significantly related to dietary folate intakes. Similar results have been reported by Hurdle (1968) and Pietarinen (1975).

Nutrition Canada (1973) did not attribute low serum folate values in their study to low dietary folate intakes, since dietary folate status was not assessed, although Beaton (1975) proposed that this was likely a major factor, especially in isolated areas. Since serum folate values in this study are directly related to the level of folate consumed, the high incidence of low serum folate values at Fort Ware reserve can be attributed, in part, to low dietary folate intakes. Conversely, the significantly higher dietary folate consumption for subjects at Sechelt and Necoslie reserves is a factor responsible for their higher serum folate values.

The dietary intake of total folate is below the WHO Recommended Daily Allowance (200 µg for 1 to 12 year olds, and 400 µg for persons 13 years of age and older for almost everyone in each group of subjects, except children from Sechelt Residential School, yet the level of folate consumed is adequate to maintain normal serum levels in 70%, 65% and 30% of the reserve subjects from Sechelt, Necoslie, and Fort Ware respectively. Since there is uncertainty concerning the availability and utilization of different forms of dietary folates (Tamura and Stokstad, 1973), subjects consuming less total folate than recommended are not necessarily "at risk". There is further disparity between the folate intake required to maintain normal serum and erythrocyte levels of the vitamin, and the smaller quantity necessary to prevent clinical illness (Dietary Standards for Canada, 1975). This was shown by Cooper and Lowenstein (1966) in a study where patients with megaloblastic anemia responded clinically and hematologically to 100 µg per day of folic acid, but did not increase their serum or erythrocyte folate levels. It was estimated that considerably fewer than half of the general population with low serum folate will have clinical manifestations of deficiency (Dietary Standards for Canada, 1975), indicating that serum folate levels alone are not a good index of folate deficiency. If serum values of greater than 5 ng/ml are indicative of adequate dietary folate intakes, this study shows that 300 µg total folate daily is adequate for residence children, and according to dietary data from the reserves, even less is required. Further research is necessary, as this study indicates, to investigate the current standards for total folate requirements.

Although there is a lower incidence of subjects in this study as a whole "at risk" with respect to serum folate, as compared to the national Indian population, as reported in the Nutrition Canada Survey (1973), the incidence of risk values from Fort Ware alone is comparable, indicating folate inadequacy for this group of people. According to the national results for Nutrition Canada, serum values for males and females were similar, and the levels for children tended to be higher than adult levels. A similar pattern is apparent in this study, where mean serum folate values for males and females are not significantly different, and children 0 to 9 years old have a significantly higher mean serum folate than the older age categories. Daniel et al (1975) found that increased maturity in adolescents was associated with a decrease in plasma folate concentrations without a concurrent decrease in dietary folate intakes. Reduced folate levels were attributed, in part, to greater tissue and cellular folate demands. Possibly this could be related to this study, in that folate demands are greater during and after maturation, as WHO and Canadian recommendations indicate, yet dietary folate consumption does not increase to meet these demands, consequently the level of circulating folate in the serum decreases. The observation that males consume significantly more dietary folate than females yet their serum folate levels are not significantly higher, might also be explained by an increased folate need.

The incidence of low serum folic acid values is not as great in this study as in the Nutrition Canada Survey (1973), yet there is definite cause for concern in certain segments of the population, i.e. for isolated reserves (such as Fort Ware) and for the adult population, especially for women, whose dietary folate intakes are considerably lower than for men.

Mean red cell folate levels at Fort Ware are lower than at the other reserves, although not significantly lower than the mean at Necoslie. Twenty-five percent and twenty-four percent of the values from Fort Ware and Necoslie respectively were classified as high risk, and 20% and 15% as moderate risk values for red cell folate. Instead of reflecting recent folate status, red cell folate activity is a quantitative index of the severity of folate deficiency and is considered to be indicative of long-term folate status and tissue stores (Herbert, 1965 and Hoffbrand et al, 1966). On this basis, the results indicate that close to 50% of the subjects from Fort Ware and 40% from Necoslie have inadequate folate stores and are at risk of being deficient. Sechelt Reserve, on the other hand, has a significantly higher mean red cell folate than Necoslie or Fort Ware, with only 16% of the subjects classified as "at risk".

There is evidence that iron deficiency may increase folate requirements (Velez et al, 1966 and Taskes et al, 1974) and consequently alter serum and red cell folate levels (Omer et al, 1970, Roberts et al, 1971, Saraya et al, 1971, and Saraya et al, 1973). Population studies have demonstrated a high prevalence of folate deficiency concurrent with iron deficiency anemia; (i.e.) National Food and Nutrition Survey of Barbados (1972), Kaufman et al, (1975), and Levy et al, (1975). The low incidence of low hemoglobin and hematrocrit values for this study indicates that iron deficiency anemia is not an important problem for the subjects, and is therefore not likely a complicating factor in the etiology of the low blood folate values. Mean daily intakes of iron also support this conclusion.

Values for MCV and MCHC are within a normal range and the majority of the thin blood smears examined show no significant changes in cell morphology, indicating that overt megaloblastic anemia is not prevalent among the subjects. Although subjects are not anemic, the presence of low blood folate values represents, by definition, a folate deficiency (Herbert, 1965) and is important as an index of a degree of malnutrition (Hurdle and Williams, 1966) which could lead to megaloblastosis if the body is challenged by diseases, alcoholism, infections, or other pathological states which increase the folate requirements (Mathoth et al, 1964b and Hurdle and Williams, 1966). According to Butler (1975) the major current health problems of Indians in British Columbia are: accidents and violence, with alcohol abuse; perinatal mortality, with inadequate prenatal care; respiratory diseases, especially in the young; and gastro-intestinal diseases due, in part, to inadequate water and sanitation services. Simpson (1974) pointed out that there is an extremely high infection rate in the young which can be attributed to poor sanitation and health care. According to Simpson and Dormaar (1974) there is no question that Indian health is much poorer than that of the majority of the Canadian white population. The additional stress imposed upon the Indian, due to the factors just mentioned, could increase the folate demands of the body, consequently increasing dietary folate requirements.

Red cell folate values for the children from Sechelt Residential School are extremely low. Seventy-seven percent of these children are "at risk" as compared to only 28% of the children living on reserves. These low values are inconsistent with the serum folate, dietary folate, MCV, and MCHC values which indicate that the folate status of these children is adequate and likely superior to the status of reserve children. The thin blood smear preparations showed no evidence of changes in cell morphology characteristic of megaloblastic anemia. Although serum and red cell values reflect different aspects of folate metabolism, this cannot explain such a great discrepancy between the serum and red cell folate values for the children from Sechelt residence. Red cell folate values reflect folate status from at least four months previous to the time the blood sample is drawn (herbert, 1962), yet there is no reason to suspect that the residence diet had changed drastically in this space of time. Duplicate blood samples assayed in this laboratory and in an independent laboratory gave comparable values, suggesting that the assay method was not at fault. These residence blood samples were stored with all the other samples, so that if folate activity was lost in storage it would have occurred for all groups, which was not the case. Either the folate values for the blood samples are a true indication of the childrens' red cell folate status, or folate activity was, in some way, reduced before the residence samples were frozen.

Since the red cell folate values for Sechelt Residence subjects are questionable, very little weight has been put on them for interpreting the hematological results. As previously discussed, serum folate values, thin blood smear examination, blood cell counts, and dietary folate assessment indicates that dietary folate consumption for the residence children is adequate and there is no evidence of folate deficiency anemia. Since it is assumed that values from these measurements are reliable, the folate status of the residence children is considered acceptable.

In summary, this study shows that the folate status of Indians in British Columbia is variable, with more problems indicated in some groups than others. Dietary folate intakes are significantly higher in males than females and at non-isolated as compared to isolated reserves. Children from Sechelt Residential School consume considerably more dietary folate than reserve subjects. Low calorie diets are observed for women with low dietary folate intakes, while men with higher calorie diets tended to consume higher levels of dietary folate, indicating that generally low food consumption is one factor contributing to the low dietary folate values. Iron intake is generally adequate for both the reserve and residence subjects.

Serum folate values for the residence children are considerably higher than values for the children from the reserves. Serum folate values are significantly lower at Fort Ware reserve than at Sechelt or Necoslie. Red cell folate values are also the lowest at Fort Ware, significantly lower than values at Sechelt, although not significantly lower than Necoslie. Serum folate values are significantly and directly related to dietary folate intakes for the subjects as a whole. Therefore, low serum folate values are indicators of, or can be attributed in part, to low folate-containing diets. For example, the availability and consumption of folate-rich foods at Fort Ware is low and the serum values are also generally low.

Extensive nutrition education programs should not be the only approach to correcting nutritional problems for Indians at isolated locations such as Fort Ware. There is also a need for more resources, with a wider selection and availability of food stuffs, so that people have the opportunity to eat a balanced diet. Fresh fruits and vegetables which are rich in folic acid would ultimately improve their folate status. Different problems are apparent in non-isolated areas, such as Sechelt and Necoslie, where a wide variety of nutritious foods are readily available, but are often not consumed. Nutrition education programs should emphasize the importance of good nutrition for health and well being, and what foods can be selected and prepared at minimal costs, yielding optimal nutrition. The significance of good nutrition must be understood before people can be expected to improve nutritional practices. Nutrition counselling and services are needed to promote better nutritional practices among British Columbia Indians. The participation and enthusiasm of the Indian people themselves is essential if improvements in nutritional status are to be achieved. Large differences which exist in the levels of health of the Indian population and the general Canadian population have been documented, and, according to Butler (1975), many developments have been, and are being, introduced in an attempt to bring the health of the native people up to national standards. These developments should include expanded nutrition education and counselling services if this goal is to be attained.

CHAPTER VI

SUMMARY

This study was conducted to assess the folate status of native Indian groups in British Columbia. Dietary folate status was assessed, by use of a 24-hour recall, at three non-isolated and four isolated reserves throughout British Columbia. A subsample selected from the seven reserves, comprised of subjects from Sechelt, Necoslie and Fort Ware Reserves, was involved in a more comprehensive assessment of folate status, where dietary and hematological parameters of folate status were measured. Subjects from Sechelt Residential School were also involved in the comprehensive part of this study.

Dietary folate consumption is significantly lower on isolated as compared to non-isolated reserves, and is considerably higher for residence as compared to reserve subjects. Males consume significantly more dietary folate than do females. Serum folate values for the subjects as a whole are directly and significantly related to dietary folate intakes, and red cell folate values show no significant correlation. Serum folate values are lower at Fort Ware than at Sechelt or Necoslie reserves, and values for children living on reserves are lower than for children living in residence. On the basis of red cell values, 16 to 45% of the subjects at the three reserves are classified as "at risk", however no evidence of megaloblastic anemia is indicated from the hematological examinations.

Findings from this study are comparable to those from the Nutrition Canada Survey (1973), in that serum folate values for males and females are not significantly different, and are significantly higher for children than for adults, with a larger proportion of the adult population classified as "at risk". Folate status appears to be lower at isolated reserves than at non-isolated reserves, yet a need for improvement is indicated in both groups. Many individuals are either bordering on or are deficient with respect to folic acid. It is suggested that this appears to be a more serious problem at isolated reserves due to the poor selection and availability of foods.

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APPENDICES

Page

APPENDIX A

- CONSENT FORM -

THE PURPOSE OF THIS SURVEY IS TO ASSESS THE FOLIC ACID STATUS OF GROUPS OF INDIAN PEOPLE IN B.C.

IF YOU AGREE TO PARTICIPATE IN THE STUDY:

1. A 24-HOUR FOOD COLLECTION WILL BE TAKEN AND ANALYZED FOR FOLIC ACID

2. A BLOOD SAMPLE WILL BE TAKEN AND ANALYZED FOR FOLIC ACID AND PARAMETERS ASSESSING IRON STATUS

I HAVE HAD THE STUDY DESCRIBED ABOVE EXPLAINED TO ME AND AGREE TO PARTICIPATE. I UNDERSTAND THAT I MAY WITHDRAW FROM THE STUDY AT ANY TIME.

Signature: ____________________

Date: ____________________

APPENDIX B

Assayed and calculated results for total folate for the 24-hour food collections from Sechelt and Necoslie Reserves and Sechelt Residence.

Sample No.	Assayed Values	Values Calculated from Food Tables
	Total Folate (µg)	Total Folate (µg)
1	360	323
2	300	265
3	258	233
4	277	246
5	79	90
6	159	137
7	134	105
8	223	190
9	299	238
10	205	159
11	84	71
12	175	139
13	198	143
14	209	168
15	199	170
16	159	121
17	201	154
18	119	132
19	239	182
20	256	232
21	230	189

APPENDIX C

Canadian Recommended Daily Nutrient Intake*

Age (Years)	Sex	Energy (Kcal)	Iron (mg)	Free Folate (µg)
1 - 3	Both	1400	8	100
4 - 6	Both	1800	9	100
7 - 9	M	2200	10	100
	F	2000	10	100
10 - 12	M	2500	11	100
	F	2300	11	100
13 - 15	M	2800	13	200
	F	2200	14	200
16 - 18	M	3200	14	200
	F	2100	14	200
19 - 35	M	3000	10	200
	F	2100	14	200
36 - 50	M	2700	10	200
	F	1900	14	200
51 +	M	2300	10	200
	F	1800	9	200

* Committee for Revision of the Canadian Dietary Standard. Ottawa, 1975.

APPENDIX D

Guidelines for Interpretation of serum folate, red cell folate, and hemoglobin levels.

Variable		Less than acceptable (at risk)		
		Deficient (High risk)	Low (Moderate risk)	Acceptable (Low risk)
		ng/ml	ng/ml	ng/ml
Serum Folate[a] (All ages)		2.5	2.5-5.0	5.0
Red Cell Folate[b] (All ages)		140	140-159	160
		g/100 ml	g/100 ml	g/100 ml
Hemoglobin[c] Age Group (Years)	Sex			
0 - 1	M&F	9.0	9-10	10
2 - 5	M&F	10	10-11	11
6 - 12	M&F	10	10-11.5	11.5
13 - 16	M	12	12-13	13
13 - 16	F	10	10-11.5	11.5
17+	M	12	12-14	14
17+	F	10	10-12	12

a WHO Tech. Rep. Ser. No. 405 (1968)
b O'Neal et al, (1970) and WHO Tech. Rep. Ser. No. 405 (1968)
c ICNND Manual for Nutrition Surveys (1963)

LEGEND TO APPENDICES E, F, AND G

(Codes for isolation and sex)

Variable	Code
Isolation	0 = No classification
	1 = Non-Isolated Reserve
	2 = Isolated Reserve
Sex	6 = Male
	8 = Female

APPENDIX E

Raw Data - Individual Nutrient Values Calculated From 24-Hour Dietary Recalls For Subjects From The Seven Reserves.

Total Folate (μg/Day), Calories, and Iron (mg/Day)

CODE NUM	LOCATION	ISO LAT ION	SEX	AGE	* * * * DIETARY* * * * FOLATE UG/DAY	CALORIES	IRON MG/DAY
1001	SECHELT	1	8	41	245.7	1549	4.6
1002	SECHELT	1	8	12	175.5	1516	8.8
1003	SECHELT	1	6	14	193.8	2115	8.7
1004	SECHELT	1	6	15	383.0	2650	11.4
1005	SECHELT	1	8	42	106.7	1205	8.3
1006	SECHELT	1	6	52	137.1	1288	10.4
1007	SECHELT	1	6	15	125.0	1702	6.2
1008	SECHELT	1	8	66	105.2	1457	8.8
1009	SECHELT	1	6	16	204.4	1963	11.2
1010	SECHELT	1	6	28	255.5	2515	17.2
1011	SECHELT	1	8	27	136.4	1624	14.0
1012	SECHELT	1	6	4	134.6	1940	26.9
1013	SECHELT	1	8	5	99.8	1303	8.6
1014	SECHELT	1	8	26	93.1	1387	7.8
1015	SECHELT	1	8	7	63.5	1365	4.4
1016	SECHELT	1	6	6	140.4	1671	4.9
1017	SECHELT	1	8	25	180.9	965	7.2
1018	SECHELT	1	6	29	306.6	2546	17.5
1019	SECHELT	1	8	6	190.1	1445	8.2
1020	SECHELT	1	6	5	144.2	1026	5.7
1021	SECHELT	1	8	22	196.1	1548	7.2
1022	SECHELT	1	6	25	314.4	2600	16.8
1023	SECHELT	1	8	55	131.5	2019	17.0
1024	SECHELT	1	8	19	150.7	1650	16.7
1025	SECHELT	1	8	44	88.6	1444	8.1
1026	SECHELT	1	6	48	108.0	1407	10.1
1027	SECHELT	1	6	10	129.6	1554	9.6
1028	SECHELT	1	8	5	65.6	870	4.7
1029	SECHELT	1	6	13	157.0	1718	14.7
1030	SECHELT	1	6	15	140.0	1780	11.8
1031	SECHELT	1	8	16	202.2	1813	12.2
1032	SECHELT	1	8	21	63.6	788	6.9
1033	SECHELT	1	8	16	219.2	2533	11.9
1034	SECHELT	1	8	18	174.9	1987	12.5
1035	SECHELT	1	6	12	91.8	770	5.5
1036	SECHELT	1	6	13	142.9	2157	8.0
1037	SECHELT	1	8	45	164.4	1429	9.3
1038	SECHELT	1	6	44	422.0	4189	25.1
1039	SECHELT	1	6	18	370.4	3702	19.5
1040	SECHELT	1	6	8	309.9	2444	12.4
1041	SECHELT	1	8	19	159.7	1476	8.6
1042	SECHELT	1	6	2	184.7	1691	11.5
1043	SECHELT	1	8	1	108.3	850	2.1
1044	SECHELT	1	8	34	95.3	1245	11.0
1045	SECHELT	1	6	11	144.5	2630	12.6
1046	SECHELT	1	6	12	99.3	2570	13.9
1047	SECHELT	1	8	5	132.7	1671	7.9
1048	SECHELT	1	8	18	135.5	1736	15.1
1049	SECHELT	1	6	20	278.5	2400	24.4
1050	SECHELT	1	8	35	85.4	1822	14.5

CODE NUM	LOCATION	ISO LAT 1ON	SEX	AGE	* * * * DIETARY* * * * FOLATE UG/DAY	CALORIES	IRON MG/DAY
1051	SECHELT	1	8	22	132.3	1454	7.7
1052	SECHELT	1	6	21	417.5	4084	28.5
1053	SECHELT	1	8	1	56.7	641	2.8
1054	WARE	2	8	61	42.8	870	30.1
1055	WARE	2	6	69	42.8	870	30.1
1056	WARE	2	8	18	90.1	1976	25.7
1057	WARE	2	8	33	47.8	553	9.6
1058	WARE	2	6	48	144.6	1856	20.3
1059	WARE	2	8	7	61.3	892	9.2
1060	WARE	2	8	4	26.0	544	1.9
1061	WARE	2	8	86	80.2	988	10.8
1062	WARE	2	8	3	99.8	1007	10.6
1063	WARE	2	8	21	135.6	1433	17.5
1064	WARE	2	8	5	136.7	1711	13.7
1065	WARE	2	8	32	46.5	1800	10.0
1066	WARE	2	8	8	158.3	1709	10.4
1067	WARE	2	8	22	161.6	1728	18.3
1068	WARE	2	8	1	103.5	1242	15.9
1069	WARE	2	6	2	169.2	2270	15.0
1070	WARE	2	8	31	78.6	818	4.2
1071	WARE	2	8	50	136.9	1273	8.7
1072	WARE	2	8	31	187.7	2102	24.9
1073	WARE	2	6	8	212.4	2110	89.1
1074	WARE	2	6	10	299.0	3304	17.4
1075	WARE	2	8	14	162.8	3400	26.2
1076	WARE	2	8	22	156.5	1326	9.1
1077	WARE	2	6	25	180.8	1929	14.6
1078	WARE	2	8	5	130.7	1228	8.3
1079	WARE	2	8	33	168.4	2038	9.3
1080	NECOSLIE	1	8	30	104.3	1800	8.3
1081	NECOSLIE	1	8	7	222.8	2813	13.8
1082	NECOSLIE	1	6	8	436.6	4416	24.4
1083	NECOSLIE	1	8	21	150.5	1823	10.3
1084	NECOSLIE	1	6	26	251.3	2750	16.4
1085	NECOSLIE	1	8	25	131.9	1828	14.0
1086	NECOSLIE	1	6	37	274.6	3667	25.5
1087	NECOSLIE	1	8	2	141.3	1687	5.9
1088	NECOSLIE	1	8	21	117.2	1259	9.2
1089	NECOSLIE	1	6	36	174.0	1315	15.6
1090	NECOSLIE	1	8	4	148.1	1870	10.2
1091	NECOSLIE	1	8	61	121.0	1163	6.1
1092	NECOSLIE	1	8	44	177.7	1887	12.7
1093	NECOSLIE	1	6	44	289.2	3417	25.8
1094	NECOSLIE	1	8	27	141.5	1666	7.4
1095	NECOSLIE	1	6	36	311.4	3639	17.9
1096	NECOSLIE	1	8	8	183.0	2130	23.7
1097	NECOSLIE	1	8	51	153.7	1751	17.9
1098	NECOSLIE	1	8	24	232.2	2734	15.0
1099	NECOSLIE	1	8	2	165.5	1475	8.2
1100	NECOSLIE	1	8	4	187.6	1937	11.0

CODE NUM	LOCATION	ISO LAT ION	SEX	AGE	* * * * DIETARY* * * * FOLATE UG/DAY	CALORIES	IRON MG/DAY
1101	NECOSLIE	1	8	39	112.6	1316	18.2
1102	NECOSLIE	1	6	38	342.0	3781	36.7
1103	NECOSLIE	1	6	5	250.0	2888	35.3
1104	NECOSLIE	1	8	27	175.5	1159	8.5
1105	NECOSLIE	1	6	6	184.0	1601	6.6
1106	NECOSLIE	1	8	54	141.0	1955	18.5
1107	NECOSLIE	1	8	20	63.2	1179	7.0
1108	NECOSLIE	1	8	52	125.9	1803	9.4
1109	NECOSLIE	1	8	37	170.1	2337	14.7
1110	NECOSLIE	1	8	74	82.2	1096	17.4
1111	NECOSLIE	1	8	9	332.8	2799	18.0
1112	NECOSLIE	1	8	26	265.4	2545	14.1
1113	NECOSLIE	1	8	42	98.8	1914	10.6
1114	NECOSLIE	1	6	46	188.0	2867	16.9
1115	NANAIMO	1	8	28	214.9	2426	10.4
1116	NANAIMO	1	6	31	280.9	2292	11.6
1117	NANAIMO	1	6	3	215.5	2601	9.5
1118	NANAIMO	1	8	33	570.6	1773	18.8
1119	NANAIMO	1	6	38	799.4	2498	31.4
1120	NANAIMO	1	6	10	542.6	2922	24.5
1121	NANAIMO	1	6	8	388.3	2263	20.6
1122	NANAIMO	1	8	46	154.4	1175	7.0
1123	NANAIMO	1	8	25	177.6	2447	14.1
1124	NANAIMO	1	6	24	398.5	4479	27.3
1125	NANAIMO	1	8	24	130.5	2129	12.0
1126	NANAIMO	1	8	46	178.1	1956	9.2
1127	NANAIMO	1	8	25	211.2	2055	12.2
1128	NANAIMO	1	8	40	282.9	1618	4.7
1129	NANAIMO	1	6	42	280.6	1475	5.7
1130	NANAIMO	1	8	18	191.5	1918	9.3
1131	NANAIMO	1	8	13	178.3	2354	9.7
1132	NANAIMO	1	8	42	163.9	1470	9.4
1133	NANAIMO	1	6	44	463.1	3941	26.5
1134	NANAIMO	1	8	3	424.3	2505	13.0
1135	NANAIMO	1	8	5	424.5	1962	10.9
1136	NANAIMO	1	8	60	149.1	1728	6.4
1137	NANAIMO	1	6	64	174.7	1975	5.6
1138	NANAIMO	1	8	17	197.2	1767	6.8
1139	NANAIMO	1	8	84	110.1	1406	5.2
1140	NANAIMO	1	6	46	335.0	4302	19.0
1141	NANAIMO	1	8	61	228.8	1459	9.4
1142	NANAIMO	1	8	48	159.3	1961	18.0
1143	NANAIMO	1	6	50	300.7	3861	20.6
1144	NANAIMO	1	6	24	128.1	1548	7.0
1145	NANAIMO	1	8	38	65.2	1845	14.4
1146	NANAIMO	1	6	40	191.5	2586	19.9
1147	NANAIMO	1	6	17	155.8	3051	17.5
1148	NANAIMO	1	8	57	124.7	1113	11.8
1149	NANAIMO	1	8	85	102.4	1044	6.2
1150	NANAIMO	1	8	29	81.3	1520	3.9

CODE NUM	LOCATION	ISO LAT ION	SEX	AGE	* * * * DIETARY* * * * FOLATE UG/DAY	CALORIES	IRON MG/DAY
1151	NANAIMO	1	6	29	431.5	3885	15.9
1152	NANAIMO	1	8	25	297.4	2296	17.1
1153	NANAIMO	1	6	24	373.3	2615	19.2
1154	NANAIMO	1	8	56	201.1	1438	8.0
1155	NANAIMO	1	6	63	415.3	3490	13.0
1156	NANAIMO	1	6	29	422.9	3047	17.6
1157	NANAIMO	1	8	27	169.9	1613	23.2
1158	NANAIMO	1	6	24	270.6	3466	13.9
1159	NANAIMO	1	8	26	265.7	1936	12.4
1160	NANAIMO	1	8	24	177.5	1216	8.6
1161	NANAIMO	1	8	4	232.1	1462	7.9
1162	NANAIMO	1	6	2	358.5	2493	14.0
1163	NANAIMO	1	8	17	277.5	2010	10.0
1164	NANAIMO	1	8	41	177.5	1895	12.9
1165	NANAIMO	1	6	41	323.0	4120	20.9
1166	NANAIMO	1	8	28	259.5	1420	13.3
1167	NANAIMO	1	8	27	141.2	1653	8.5
1168	NANAIMO	1	6	6	391.7	2713	14.2
1169	NANAIMO	1	8	23	217.1	1105	8.0
1170	NANAIMO	1	6	23	159.5	3269	17.7
1171	NAZKO	2	8	36	155.3	1633	16.9
1172	NAZKO	2	6	41	212.9	2234	21.9
1173	NAZKO	2	8	26	247.4	2443	24.2
1174	NAZKO	2	8	4	167.6	1346	8.2
1175	NAZKO	2	8	12	207.2	1234	11.0
1176	NAZKO	2	8	20	156.1	2332	19.4
1177	NAZKO	2	6	2	165.1	1443	10.7
1178	NAZKO	2	8	29	148.3	1679	11.2
1179	NAZKO	2	8	11	169.3	2359	19.3
1180	NAZKO	2	8	44	125.4	1410	8.3
1181	NAZKO	2	8	8	220.3	2769	12.8
1182	NAZKO	2	8	51	173.9	1046	11.0
1183	NAZKO	2	6	17	337.0	2627	14.7
1184	NAZKO	2	6	13	200.9	1658	10.5
1185	NAZKO	2	8	11	122.6	1621	10.5
1186	NAZKO	2	8	14	180.1	1592	13.4
1187	NAZKO	2	6	11	178.8	2483	11.8
1188	NAZKO	2	6	11	238.9	2067	13.9
1189	NAZKO	2	6	16	218.1	2640	17.7
1190	NAZKO	2	6	14	81.7	1800	5.5
1191	NAZKO	2	6	11	156.2	1850	7.3
1192	NAZKO	2	8	33	200.1	1945	8.8
1193	NAZKO	2	6	7	236.6	2557	11.1
1194	BABINE	2	8	32	146.9	1777	15.4
1195	BABINE	2	6	35	187.4	2305	40.3
1196	BABINE	2	6	10	291.5	2412	32.8
1197	BABINE	2	8	8	225.8	1702	27.8
1198	BABINE	2	8	59	116.6	1809	14.1
1199	BABINE	2	8	20	117.5	1924	17.3
1200	BABINE	2	8	10	86.8	1641	11.2

CODE NUM	LOCATION	ISO LAT ION	SEX	AGE	* * * * DIETARY* * * * FOLATE UG/DAY	CALORIES	IRON MG/DAY
1201	BABINE	2	8	71	110.7	1236	12.1
1202	BABINE	2	6	27	92.0	1616	11.5
1203	BABINE	2	6	20	219.5	2888	31.4
1204	BABINE	2	6	22	340.3	2911	31.6
1205	BABINE	2	6	28	635.7	3097	19.7
1206	BABINE	2	8	42	137.3	1464	13.6
1207	BABINE	2	8	14	217.6	2098	15.0
1208	BABINE	2	8	17	174.0	2143	20.9
1209	BABINE	2	8	9	209.0	2406	18.3
1210	BABINE	2	8	14	124.8	1025	15.0
1211	BABINE	2	8	35	151.5	2090	17.4
1212	BABINE	2	8	40	149.9	1612	17.2
1213	BABINE	2	8	2	178.7	1647	12.6
1214	BABINE	2	6	9	304.7	2289	26.7
1215	TAKLA	2	8	45	120.1	1673	13.8
1216	TAKLA	2	8	20	124.9	1618	16.7
1217	TAKLA	2	8	33	99.8	1234	9.9
1218	TAKLA	2	8	6	135.3	1615	10.8
1219	TAKLA	2	8	12	146.5	1839	14.3
1220	TAKLA	2	8	56	145.1	1667	19.2
1221	TAKLA	2	8	15	107.9	1215	16.0
1222	TAKLA	2	8	27	83.7	1103	4.9
1223	TAKLA	2	8	7	150.3	2493	11.2
1224	TAKLA	2	8	35	112.5	1377	19.2
1225	TAKLA	2	8	11	158.3	2027	14.1
1226	TAKLA	2	6	14	159.8	2009	18.1
1227	TAKLA	2	8	36	86.8	772	12.1
1228	TAKLA	2	8	9	122.8	1321	16.3
1229	TAKLA	2	8	46	162.4	2194	19.2
1230	TAKLA	2	6	60	175.6	2311	27.6
1231	TAKLA	2	8	6	176.4	1820	13.0
1232	TAKLA	2	8	23	204.4	2627	15.6
1233	TAKLA	2	8	3	161.0	2287	16.6
1234	TAKLA	2	8	28	167.8	1488	19.3
1235	TAKLA	2	8	50	133.5	1439	14.0
1236	TAKLA	2	6	52	159.0	1697	18.4
1237	TAKLA	2	8	28	121.5	1092	14.5
1238	TAKLA	2	8	35	237.1	1827	22.0
1239	TAKLA	2	8	12	288.6	2136	20.3
1240	TAKLA	2	8	26	141.3	1983	13.3
1241	TAKLA	2	8	20	173.8	2358	14.6
1242	TAKLA	2	8	13	172.8	2573	20.5
1243	TAKLA	2	8	53	152.5	1990	15.6
1244	TAKLA	2	6	10	223.8	2457	13.6

APPENDIX F

Raw Data - Individual Nutrient Values Calculated From 24-Hour Dietary Recalls For Subjects Participating In The Comprehensive Part Of The Study.

Total Folate (µg/day), Calories, and Iron (mg/day)

CODE NUM	LOCATION	ISO LAT ION	SEX	AGE	* * * * DIETARY* * * * FOLATE UG/DAY	CALORIES	IRON MG/DAY
11	SECHELT	1	8	41.14	245.7	1549	4.6
13	SECHELT	1	6	11.80	175.5	1516	8.8
14	SECHELT	1	6	13.78	193.8	2115	8.7
15	SECHELT	1	6	14.78	383.0	2650	11.4
41	SECHELT	1	8	27.42	136.4	1624	14.0
51	SECHELT	1	8	26.48	93.1	1387	7.8
53	SECHELT	1	8	7.78	63.5	1365	4.4
54	SECHELT	1	6	6.48	140.4	1671	4.9
61	SECHELT	1	8	24.56	180.9	965	7.2
62	SECHELT	1	6	29.62	306.6	2546	17.5
71	SECHELT	1	8	22.54	196.1	1548	7.2
91	SECHELT	1	8	44.46	88.6	1444	8.1
92	SECHELT	1	6	48.38	108.0	1407	10.1
93	SECHELT	1	8	9.62	129.6	1554	9.6
94	SECHELT	1	8	5.08	65.6	870	4.7
95	SECHELT	1	6	12.68	157.0	1718	14.7
96	SECHELT	1	6	14.88	140.0	1780	11.8
97	SECHELT	1	8	16.28	202.2	1813	12.2
98	SECHELT	1	8	20.82	63.6	788	6.9
103	SECHELT	1	8	16.02	219.2	2533	11.9
104	SECHELT	1	8	17.94	174.9	1987	12.5
105	SECHELT	1	6	11.74	91.8	770	5.5
106	SECHELT	1	6	13.08	142.9	2157	8.0
107	SECHELT	1	8	8.74	164.4	1936	9.1
108	SECHELT	1	8	23.92	71.0	2091	10.2
109	SECHELT	1	6	19.04	99.9	2380	14.0
121	SECHELT	1	8	19.18	159.7	1476	8.6
122	SECHELT	1	6	22.38	184.7	2197	13.3
161	SECHELT	1	8	21.60	132.3	1454	7.7
162	SECHELT	1	6	20.82	417.5	4084	28.5
174	WARE	2	6	12.60	90.1	2100	13.2
185	WARE	2	6	11.28	99.9	2098	11.2
211	WARE	2	8	85.92	80.2	988	10.8
215	WARE	2	8	20.90	135.6	1433	17.5
216	WARE	2	8	32.44	46.5	989	5.0
217	WARE	2	6	11.76	103.1	2298	13.7
231	WARE	2	8	21.68	161.6	1728	18.3
241	WARE	2	8	30.46	78.6	818	4.2
242	WARE	2	6	27.32	97.3	2615	16.1
251	WARE	2	8	50.00	136.9	1273	8.7
252	WARE	2	6	63.00	131.0	1672	10.2
253	WARE	2	6	20.00	107.9	2381	13.1
254	WARE	2	6	13.00	117.1	2381	13.1
264	WARE	2	6	10.24	299.0	3304	17.4
265	WARE	2	8	14.00	162.8	3400	26.2
266	WARE	2	6	19.32	101.3	2103	12.9
271	WARE	2	8	22.04	156.5	1326	9.1
272	WARE	2	6	25.30	180.8	1929	14.6
281	WARE	2	8	33.02	168.4	2038	9.3
283	WARE	2	6	13.62	93.6	2065	10.9

CODE NUM	LOCATION	ISO LAT ION	SEX	AGE	* * * * DIETARY* * * * FOLATE UG/DAY	CALORIES	IRON MG/DAY
293	NECOSLIE	1	8	7.14	222.8	2813	13.8
294	NECOSLIE	1	6	8.42	436.6	4416	24.4
301	NECOSLIE	1	8	21.30	150.5	1823	10.3
311	NECOSLIE	1	8	25.12	131.9	1828	14.0
314	NECOSLIE	1	8	24.00	151.8	1860	13.9
321	NECOSLIE	1	8	20.58	117.2	1259	9.3
322	NECOSLIE	1	6	36.06	174.0	1315	15.6
331	NECOSLIE	1	8	61.32	121.0	1163	6.1
333	NECOSLIE	1	6	25.00	187.3	2375	15.2
341	NECOSLIE	1	8	43.66	177.7	1887	12.7
343	NECOSLIE	1	8	16.00	99.9	1988	9.8
344	NECOSLIE	1	6	11.66	251.3	1937	9.8
351	NECOSLIE	1	8	27.04	141.5	1666	7.4
361	NECOSLIE	1	8	51.06	153.7	1751	17.9
362	NECOSLIE	1	6	50.00	99.9	2410	16.1
363	NECOSLIE	1	8	25.00	171.6	1975	13.8
364	NECOSLIE	1	8	10.78	143.4	2015	10.2
371	NECOSLIE	1	8	24.28	232.2	2734	15.0
381	NECOSLIE	1	8	39.42	112.6	1316	18.2
384	NECOSLIE	1	8	45.00	149.1	1510	17.0
385	NECOSLIE	1	6	16.00	342.0	2418	16.7
402	NECOSLIE	1	6	58.36	211.0	2892	15.7
403	NECOSLIE	1	8	23.00	63.0	1179	7.0
404	NECOSLIE	1	8	25.00	103.3	1581	11.0
405	NECOSLIE	1	6	10.32	143.1	1636	8.1
406	NECOSLIE	1	6	11.86	131.8	1636	8.1
411	NECOSLIE	1	8	51.90	125.9	1803	9.4
421	NECOSLIE	1	8	37.42	170.1	2337	14.7
423	NECOSLIE	1	8	73.50	82.2	1096	17.4
431	NECOSLIE	1	8	25.68	265.4	2545	14.1
433	NECOSLIE	1	8	24.00	281.7	2481	13.7
441	NECOSLIE	1	8	42.44	98.8	1914	10.6
442	NECOSLIE	1	6	45.44	188.0	2867	16.9
851	RESIDENCE	0	8	10.38	310.1	2227	13.4
861	RESIDENCE	0	8	12.82	310.1	2227	13.4
871	RESIDENCE	0	8	12.26	310.1	2227	13.4
881	RESIDENCE	0	8	10.66	310.1	2227	13.4
891	RESIDENCE	0	8	11.50	310.1	2227	13.4
901	RESIDENCE	0	8	13.24	310.1	2227	13.4
911	RESIDENCE	0	8	11.34	310.1	2227	13.4
921	RESIDENCE	0	8	14.54	310.1	2227	13.4
931	RESIDENCE	0	8	13.24	310.1	2227	13.4
941	RESIDENCE	0	8	14.28	310.1	2227	13.4
951	RESIDENCE	0	8	13.12	310.1	2227	13.4
961	RESIDENCE	0	8	11.50	310.1	2227	13.4
971	RESIDENCE	0	8	13.90	310.1	2227	13.4
981	RESIDENCE	0	8	13.56	310.1	2227	13.4
991	RESIDENCE	0	8	12.50	310.1	2227	13.4
1001	RESIDENCE	0	8	11.72	310.1	2227	13.4
1011	RESIDENCE	0	8	12.76	310.1	2227	13.4

CODE NUM	LOCATION	ISO LAT 10N	SEX	AGE	* * * * DIETARY* * * * FOLATE UG/DAY	CALORIES	IRON MG/DAY
1021	RESIDENCE	0	8	11.20	310.1	2227	13.4
1031	RESIDENCE	0	8	12.84	310.1	2227	13.4
1041	RESIDENCE	0	8	9.04	310.1	2227	13.4
1051	RESIDENCE	0	8	16.48	310.1	2227	13.4
1061	RESIDENCE	0	8	15.30	310.1	2227	13.4
1071	RESIDENCE	0	8	9.75	310.1	2227	13.4
1081	RESIDENCE	0	8	8.32	310.1	2227	13.4
1091	RESIDENCE	0	8	9.08	310.1	2227	13.4
1101	RESIDENCE	0	8	10.08	310.1	2227	13.4
1111	RESIDENCE	0	8	8.38	310.1	2227	13.4
1121	RESIDENCE	0	8	8.92	310.1	2227	13.4
1131	RESIDENCE	0	8	6.88	310.1	2227	13.4
1141	RESIDENCE	0	6	14.08	310.1	2227	13.4
1151	RESIDENCE	0	6	11.62	310.1	2227	13.4
1161	RESIDENCE	0	6	12.98	310.1	2227	13.4
1171	RESIDENCE	0	6	15.00	310.1	2227	13.4
1181	RESIDENCE	0	6	15.46	310.1	2227	13.4
1191	RESIDENCE	0	6	13.16	310.1	2227	13.4
1201	RESIDENCE	0	6	10.78	310.1	2227	13.4
1211	RESIDENCE	0	6	13.36	310.1	2227	13.4
1221	RESIDENCE	0	8	6.64	310.1	2227	13.4
1231	RESIDENCE	0	6	10.00	310.1	2227	13.4
1241	RESIDENCE	0	6	10.46	310.1	2227	13.4
1251	RESIDENCE	0	8	9.06	310.1	2227	13.4
1261	RESIDENCE	0	6	11.42	310.1	2227	13.4
1271	RESIDENCE	0	6	12.62	310.1	2227	13.4
1281	RESIDENCE	0	8	7.84	310.1	2227	13.4
1291	RESIDENCE	0	8	5.54	310.1	2227	13.4
1301	RESIDENCE	0	8	7.84	310.1	2227	13.4
1311	RESIDENCE	0	8	7.94	310.1	2227	13.4
1321	RESIDENCE	0	8	9.48	310.1	2227	13.4
1331	RESIDENCE	0	6	10.48	310.1	2227	13.4
1341	RESIDENCE	0	6	11.34	310.1	2227	13.4
1351	RESIDENCE	0	6	11.00	310.1	2227	13.4
1361	RESIDENCE	0	6	11.76	310.1	2227	13.4
1371	RESIDENCE	0	6	11.42	310.1	2227	13.4
1381	RESIDENCE	0	6	9.00	310.1	2227	13.4
1391	RESIDENCE	0	6	10.14	310.1	2227	13.4
1401	RESIDENCE	0	6	9.48	310.1	2227	13.4
1411	RESIDENCE	0	6	9.12	310.1	2227	13.4
1421	RESIDENCE	0	6	10.24	310.1	2227	13.4
1431	RESIDENCE	0	6	10.26	310.1	2227	13.4
1441	RESIDENCE	0	6	8.36	310.1	2227	13.4
1451	RESIDENCE	0	6	9.14	310.1	2227	13.4
1461	RESIDENCE	0	6	9.44	310.1	2227	13.4
1471	RESIDENCE	0	6	7.38	310.1	2227	13.4
1481	RESIDENCE	0	6	9.88	310.1	2227	13.4
1491	RESIDENCE	0	6	9.54	310.1	2227	13.4
1501	RESIDENCE	0	6	11.18	310.1	2227	13.4
1511	RESIDENCE	0	6	8.16	310.1	2227	13.4

CODE NUM	LOCATION	ISOLATION	SEX	AGE	* * * * DIETARY* * * * FOLATE UG/DAY	CALORIES	IRON MG/DAY
1521	RESIDENCE	0	6	6.14	310.1	2227	13.4
1531	RESIDENCE	0	6	7.98	310.1	2227	13.4
1541	RESIDENCE	0	6	7.94	310.1	2227	13.4

T=0.50 [illegible]

$SIGNOFF

APPENDIX G

Raw Data - Individual Hematological Values For Subjects Participating In The Comprehensive Part Of The Study.

Serum Folate (ng/ml) Red Cell Folate (ng/ml),

Hemoglobin (g/100 ml), Mean Corpuscular Volume (μ^3),

and Mean Corpuscular Hemoglobin Concentration (%)

CODE NUM	LOCATION	ISO LAT ION	SEX	AGE	SERUM FOL- ATE	RED CELL FOL.	HB	MCV	MCHC
11	SECHELT	1	8	41.14	5.8	519	13.4	84	34.0
13	SECHELT	1	6	11.80	6.1	152	12.6	79	34.2
14	SECHELT	1	6	13.78	7.7	330	12.8	80	35.0
15	SECHELT	1	6	14.78	4.8	171	14.8	80	34.4
41	SECHELT	1	8	27.42	4.5	187	12.7	80	34.5
51	SECHELT	1	8	26.48	4.3	294	14.2	78	34.1
53	SECHELT	1	8	7.78	5.4	248	13.5	73	34.0
54	SECHELT	1	6	6.48	7.0	323	12.6	74	34.9
61	SECHELT	1	8	24.56	5.0	374	9.8	77	31.9
62	SECHELT	1	6	29.62	10.2	203	15.3	85	34.1
71	SECHELT	1	8	22.54	6.7	281	13.0	78	34.4
91	SECHELT	1	8	44.46	3.5	209	14.1	79	33.4
92	SECHELT	1	6	48.38	3.2	188	14.0	78	34.3
93	SECHELT	1	8	9.62	6.4	352	12.2	78	34.4
94	SECHELT	1	8	5.08	10.2	241	11.7	72	34.6
95	SECHELT	1	6	12.68	5.0	147	14.0	79	35.0
96	SECHELT	1	6	14.88	6.1	233	13.6	81	34.6
97	SECHELT	1	8	16.28	3.9	179	12.2	76	34.0
98	SECHELT	1	8	20.82	6.7	259	14.1	86	34.2
103	SECHELT	1	8	16.02	3.9	139	14.3	83	34.2
104	SECHELT	1	8	17.94	8.5	354	11.2	71	32.2
105	SECHELT	1	6	11.74	8.5	288	14.2	73	34.8
106	SECHELT	1	6	13.08	6.4	274	13.1	74	34.1
107	SECHELT	1	8	8.74	10.2	221	13.2	74	34.2
108	SECHELT	1	8	23.92	6.7	306	13.6	81	33.3
109	SECHELT	1	6	19.04	6.4	259	15.6	84	34.8
121	SECHELT	1	8	19.18	9.8	245	13.0	80	34.4
122	SECHELT	1	6	22.38	9.8	148	14.5	83	34.5
161	SECHELT	1	8	21.60	8.5	147	12.2	82	34.0
162	SECHELT	1	6	20.82	12.0	249	14.9	86	34.7
174	WARE	2	6	12.60	3.0	125	15.6	85	34.4
185	WARE	2	6	11.28	4.0	177	14.1	77	34.3
211	WARE	2	8	85.92	6.3	265	13.7	89	33.8
215	WARE	2	8	20.90	3.2	185	14.0	81	34.7
216	WARE	2	8	32.44	3.2	183	12.8	83	33.0
217	WARE	2	6	11.76	2.3	179	13.9	88	33.0
231	WARE	2	8	21.68	2.3	159	16.0	82	33.0
241	WARE	2	8	30.46	6.0	220	14.2	84	32.7
242	WARE	2	6	27.32	6.3	126	14.7	81	33.1
251	WARE	2	8	50.00	2.6	187	12.0	78	32.3
252	WARE	2	6	63.00	4.0	123	14.4	82	33.6
253	WARE	2	6	20.00	2.8	102	15.8	86	33.8
254	WARE	2	6	13.00	5.3	272	99.9	80	99.9
264	WARE	2	6	10.24	7.3	294	13.0	76	34.3
265	WARE	2	8	14.00	3.2	147	13.8	82	34.0
266	WARE	2	6	19.32	3.7	156	16.1	83	33.8
271	WARE	2	8	22.04	5.6	236	13.3	81	32.5
272	WARE	2	6	25.30	4.2	141	14.8	83	33.0
281	WARE	2	8	33.02	2.8	126	12.7	80	33.2
283	WARE	2	6	13.62	3.2	171	14.0	80	33.5

CODE NUM	LOCATION	ISO LAT ION	SEX	AGE	SERUM FOL-ATE	RED CELL FOL.	HB	MCV	MCHC
293	NECOSLIE	1	8	7.14	8.3	198	13.1	86	32.9
294	NECOSLIE	1	6	8.42	4.6	226	12.8	83	34.4
301	NECOSLIE	1	8	21.30	5.0	259	11.1	74	32.6
311	NECOSLIE	1	8	25.12	5.2	168	14.0	85	34.3
314	NECOSLIE	1	8	24.00	6.5	191	11.8	86	34.2
321	NECOSLIE	1	8	20.58	6.0	321	13.7	91	33.6
322	NECOSLIE	1	6	36.06	11.0	97	15.8	91	34.3
331	NECOSLIE	1	8	61.32	5.0	216	12.9	87	33.3
333	NECOSLIE	1	6	25.00	6.9	141	15.8	87	34.2
341	NECOSLIE	1	8	43.66	6.9	274	11.7	83	31.9
343	NECOSLIE	1	8	16.00	5.2	99	13.4	85	32.8
344	NECOSLIE	1	6	11.66	3.5	158	13.8	76	34.2
351	NECOSLIE	1	8	27.04	5.7	194	13.4	83	33.6
361	NECOSLIE	1	8	51.06	7.3	197	13.8	92	33.2
362	NECOSLIE	1	6	50.00	5.7	206	15.5	89	34.6
363	NECOSLIE	1	8	25.00	9.7	400	11.1	95	33.9
364	NECOSLIE	1	8	10.78	11.5	275	12.8	83	33.7
371	NECOSLIE	1	8	24.28	6.3	127	12.9	88	33.8
381	NECOSLIE	1	8	39.42	4.3	207	15.4	96	33.6
384	NECOSLIE	1	8	45.00	4.0	149	13.9	91	33.6
385	NECOSLIE	1	6	16.00	10.3	212	14.6	85	34.3
402	NECOSLIE	1	6	58.36	3.2	131	15.5	89	33.4
403	NECOSLIE	1	8	23.00	2.0	275	12.6	79	32.9
404	NECOSLIE	1	8	25.00	2.0	171	13.8	83	33.7
405	NECOSLIE	1	6	10.32	8.3	198	13.8	79	33.8
406	NECOSLIE	1	6	11.86	5.0	137	12.9	81	33.8
411	NECOSLIE	1	8	51.90	3.2	85	12.7	93	33.9
421	NECOSLIE	1	8	37.42	4.3	109	14.1	91	33.2
423	NECOSLIE	1	8	73.50	5.2	145	13.0	92	33.0
431	NECOSLIE	1	8	25.68	5.2	153	12.1	81	32.4
433	NECOSLIE	1	8	24.00	3.7	214	9.9	82	32.9
441	NECOSLIE	1	8	42.44	4.3	184	12.8	81	32.5
442	NECOSLIE	1	6	45.44	4.0	127	13.7	87	33.5
851	RESIDENCE	0	8	10.38	12.2	195	13.1	79	33.7
861	RESIDENCE	0	8	12.82	2.7	114	13.1	79	33.9
871	RESIDENCE	0	8	12.26	5.2	104	12.2	84	34.3
881	RESIDENCE	0	8	10.66	6.5	197	12.9	80	34.8
891	RESIDENCE	0	8	11.50	8.5	100	12.1	83	34.0
901	RESIDENCE	0	8	13.24	7.1	100	11.5	75	33.5
911	RESIDENCE	0	8	11.34	8.2	65	12.8	81	34.6
921	RESIDENCE	0	8	14.54	4.1	999	12.2	80	34.3
931	RESIDENCE	0	8	13.24	6.5	207	11.8	74	33.6
941	RESIDENCE	0	8	14.28	7.8	85	12.5	81	33.9
951	RESIDENCE	0	8	13.12	6.1	81	12.9	82	35.0
961	RESIDENCE	0	8	11.50	9.8	88	12.8	84	34.7
971	RESIDENCE	0	8	13.90	3.0	77	11.6	83	33.9
981	RESIDENCE	0	8	13.56	4.1	71	12.6	83	33.7
991	RESIDENCE	0	8	12.50	6.5	165	11.7	82	34.3
1001	RESIDENCE	0	8	11.72	5.8	999	12.9	81	33.9
1011	RESIDENCE	0	8	12.76	5.8	84	12.2	84	33.6

CODE NUM	LOCATION	ISO LAT ICN	SEX	AGE	SERUM FOL-ATE	RED CELL FOL.	HB	MCV	MCHC
1021	RESIDENCE	0	8	11.20	3.9	119	11.0	81	33.3
1031	RESIDENCE	0	8	12.84	8.2	102	12.2	86	34.1
1041	RESIDENCE	0	8	9.04	6.9	191	12.0	79	34.6
1051	RESIDENCE	0	8	16.48	8.2	114	12.1	82	34.3
1061	RESIDENCE	0	8	15.30	9.3	133	12.5	82	33.9
1071	RESIDENCE	0	8	9.75	7.8	170	13.0	76	34.9
1081	RESIDENCE	0	8	8.32	9.7	119	12.5	82	34.2
1091	RESIDENCE	0	8	9.08	9.2	246	11.7	81	33.9
1101	RESIDENCE	0	8	10.08	7.6	111	12.4	83	34.0
1111	RESIDENCE	0	8	8.38	8.4	177	12.0	81	33.9
1121	RESIDENCE	0	8	8.92	6.5	89	12.4	82	34.0
1131	RESIDENCE	0	8	6.88	8.0	241	12.5	83	34.1
1141	RESIDENCE	0	6	14.08	9.2	86	12.3	83	33.8
1151	RESIDENCE	0	6	11.62	8.8	132	12.2	82	34.5
1161	RESIDENCE	0	6	12.98	6.1	129	12.9	75	34.3
1171	RESIDENCE	0	6	15.00	8.8	86	13.5	84	34.5
1181	RESIDENCE	0	6	15.46	9.1	130	13.3	86	34.3
1191	RESIDENCE	0	6	13.16	6.1	191	12.3	81	34.5
1201	RESIDENCE	0	6	10.78	8.8	266	12.2	78	34.8
1211	RESIDENCE	0	6	13.36	6.5	222	12.5	74	34.2
1221	RESIDENCE	0	8	6.64	11.8	152	11.6	76	33.9
1231	RESIDENCE	0	6	10.00	8.8	127	11.9	84	34.6
1241	RESIDENCE	0	6	10.46	8.4	110	12.6	81	34.8
1251	RESIDENCE	0	8	9.06	3.5	170	12.3	80	35.1
1261	RESIDENCE	0	6	11.42	8.8	112	11.4	84	34.3
1271	RESIDENCE	0	6	12.62	11.5	140	12.6	81	34.3
1281	RESIDENCE	0	8	7.84	10.0	120	11.0	74	33.9
1291	RESIDENCE	0	8	5.54	3.8	68	11.4	77	34.9
1301	RESIDENCE	0	8	7.84	10.5	84	11.3	79	35.4
1311	RESIDENCE	0	8	7.94	5.8	158	12.2	81	35.3
1321	RESIDENCE	0	8	9.48	10.0	204	11.7	79	33.7
1331	RESIDENCE	0	6	10.48	9.2	90	12.1	81	33.5
1341	RESIDENCE	0	6	11.34	5.8	95	11.2	79	34.5
1351	RESIDENCE	0	6	11.00	14.0	92	12.3	82	35.6
1361	RESIDENCE	0	6	11.76	15.5	71	12.4	83	34.6
1371	RESIDENCE	0	6	11.42	10.2	74	11.6	74	34.8
1381	RESIDENCE	0	6	9.00	7.8	96	11.3	78	34.0
1391	RESIDENCE	0	6	10.14	9.5	122	12.2	81	35.1
1401	RESIDENCE	0	6	9.48	5.8	146	12.4	81	35.5
1411	RESIDENCE	0	6	9.12	12.5	123	11.8	81	35.2
1421	RESIDENCE	0	6	10.24	4.7	98	12.6	77	35.4
1431	RESIDENCE	0	6	10.26	12.2	63	11.9	81	35.4
1441	RESIDENCE	0	6	8.36	4.7	99	11.1	78	34.5
1451	RESIDENCE	0	6	9.14	5.8	105	13.0	77	35.8
1461	RESIDENCE	0	6	9.44	18.5	124	12.1	79	34.7
1471	RESIDENCE	0	6	7.38	11.3	107	12.0	81	34.5
1481	RESIDENCE	0	6	9.88	9.2	91	12.3	83	34.2
1491	RESIDENCE	0	6	9.54	9.5	64	12.1	75	34.4
1501	RESIDENCE	0	6	11.18	10.7	110	11.7	76	35.2
1511	RESIDENCE	0	6	8.16	12.5	140	11.4	75	34.6

CODE NUM	LOCATION	ISO LAT ION	SEX	AGE	SERUM FOL- ATE	RED CELL FOL.	HB	MCV	MCHC
1521	RESIDENCE	0	6	6.14	10.7	55	11.3	82	34.2
1531	RESIDENCE	0	6	7.98	9.5	88	11.5	77	34.4
1541	RESIDENCE	0	6	7.94	10.2	116	12.3	77	33.9