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Conjunctival Impression Cytology Assessment of Vitamin A Status of Migrant Children

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CONJUNCTIVAL IMPRESSION CYTOLOGY ASSESSMENT OF VITAMIN A STATUS OF MIGRANT CHILDREN

by

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ABSTRACT

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by

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Subclinical vitamin A deficiency was assessed in 65 Hispanic children attending four migrant Head Start programs in Utah. Subjects aged 2 to 6 years (median 3 years 10 months) were examined for evidence of vitamin A deficiency by conjunctival impression cytology. Biochemical indices for serum vitamin A, retinol-binding protein, zinc, and iron were performed.

Of eight children (12.5%) with subclinical vitamin A deficiency, one child had a marginal serum vitamin A of 11 μg/dl. Retinol-binding protein concentrations were significantly lower in two subjects with abnormal conjunctival impression cytology. Serum zinc, which when low can mimic signs of ocular vitamin A lesions, was normal for all 65 subjects. Fifteen children (23%) had iron-deficiency anemia.

Logistic regression was the central method of analysis used in this study. The results of the statistical analyses indicated there was a correlation value (0.31) between

abnormal conjunctival impression cytology and serum vitamin A, which supports the hypothesis that abnormal conjunctival impression cytology is concurrent with decreased serum vitamin A.

Assessment of vitamin A status of Hispanic migrant children by impression cytology was effective in identifying children at risk for hypovitaminosis A. Beyond vitamin A's role in vision and maintenance of epithelium, it is also required for growth and hematopoiesis. The children of migrant workers may be suffering physiologically important consequences of vitamin A and iron deficiency that can be prevented by screening with biochemical and histological testing. Nutrition intervention for deficient children is warranted.

(135 pages)

DEDICATION

This work is dedicated to my parents, Leonard and Jacqueline Nihan, in deep appreciation of their continuing support and encouragement in all aspects of my life, and to my daughter, Lauren, for her tolerance and willingness to endure when most children would have begged "Mom" to give it up. This project and degree are a compilation of support and friendship from everyone.

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Vitamin A is a fat-soluble nutrient required in adequate amounts in humans for normal vision, bone growth, maintenance of healthy epithelial cells, reproduction, and immunocompetence. Clinical signs of chronic retinol deficiency include poor night vision and characteristic changes in the eyes and skin. Acute vitamin A deficiency does not produce symptoms in otherwise well nourished individuals because the body can store vitamin A. Total body reserves of vitamin A, although low at birth, increase throughout the first few years of life to adult levels. Thus, dietary intakes of vitamin A that exceed immediate needs add to these reserves, which are stored primarily in the liver (Barker, 1976).

Background

In developing countries, vitamin A deficiency appears to be a preventable determinant of childhood morbidity and mortality, even at marginal levels where xerophthalmia is not evident. Likewise, deficiencies are rarely reported among healthy adults, although they are observed among children who are growing rapidly and who lack adequate fat stores. Inadequate vitamin A intake is considered the second most common nutritional deficiency in the world after protein-energy malnutrition (McLaren, 1986). Vitamin A deficiency, through adverse effects on eye epithelial tissues, is a major cause of blindness among children in many developing countries (Pitt, 1985). Vitamin A, or retinol, is needed for the maintenance of a wide variety of

body cells. As a result, a deficiency of this vitamin causes a variety of symptoms such as desiccation of the hair, skin, and conjunctiva of the eye; growth retardation; and depreciated resistance to infection, particularly in the respiratory, urogenital, and gastrointestinal tracts. Since many of these tissues form a barrier against infection that relies on vitamin A, deficiency of the vitamin is also responsible for substantial infections in youth. Foremost among classic vitamin A deficiency symptoms are night-blindness, or xerophthalmia, and keratinization of the epithelial cells lining the respiratory passages and alimentary tract. These internally keratinized tissues are reminiscent of keratinized squamous cells located on the epidermis. Morbidity and mortality due to respiratory infection are high and secondary to vitamin A deficiency. In 1986, Sommer et al. suggested that retinol supplementation may improve survival among children consuming inadequate levels of retinol or carotenes.

Vitamin A deficiency is closely associated with protein-energy malnutrition and arises when the diet contains practically no whole milk or butter and very limited amounts of fresh vegetables or fruit, therefore lacking dietary fat, retinol, and carotenes. Certain carotenoid pigments in food require fat in the diet to be successfully converted to vitamin A and absorbed in the body. A problem associated with vitamin A-carotene conversion is the difficulty of assessing the vitamin A value of the mixed carotenes in any general diet. Some carotenes are efficiently and promptly converted; others are not. Furthermore, carotenes from vegetable sources are not entirely available to the body due to incomplete intestinal cleavage or absorption.

Damage to the epithelial layer of the eye is one of the most important clinical signs of vitamin A deficiency in children. There is a drying and thickening of the conjunctiva; the tear ducts fail to secrete; keratinization results, with the epithelial cells of the cornea becoming opaque and sloughing off (Sommer, 1982). Infection and permanent blindness may follow if vitamin A is not administered.

It has been widely reported that vitamin A deficiency causes increased morbidity and mortality among preschool children in developing countries (Sommer et al., 1986). The signs of vitamin A deficiency are predominantly ocular, and the preschool child is especially vulnerable to the severe forms that cause blindness. Vitamin A deficiency afflicts more than five million children worldwide, inducing blindness, stunted growth, and infections. Zinc deficiency also retards growth and typically accompanies protein-energy malnutrition and vitamin A deficiencies. In terms of human suffering and economic loss, the cost of blindness is incalculable. In comparison, the cost of prevention is almost negligible (WHO, 1982).

Vitamin A Deficiency in the United States

Vitamin A deficiency, as suggested by low serum levels, was identified as a potential problem among Hispanics, children, and low-income groups in the United States population by the Ten-State Nutrition Survey conducted from 1968 to 1970 (U.S. DHEW, 1972a, 1972b). The Expert Panel on Vitamin A Nutriture of the Life Sciences Research Office recommended that vitamin A status should be examined in several United States population groups, particularly Blacks and Hispanics (Pilch,

1985). Since then, measurements of serum vitamin A levels in United States population groups have been performed in the first, second, and third National Health and Nutrition Examination Surveys (NHANES) (DHHS Pub. No. [PHS] 89-1255-1 1989; DHHS Pub. No. [PHS] 90-1307 1990; DHHS Pub. No. [PHS] 94-1308 1994), as well as in the Hispanic Health and Nutrition Examination Survey (HHANES) (DHHS Pub. No. [PHS] 85-1321 1985). An evaluation of the data from the various NHANES concluded that serum vitamin A levels alone are inadequate to provide estimates of the prevalences of vitamin A deficiency in the U.S. population (Pilch, 1985). Nonetheless, clinical and epidemiological data indicate that there are age-specific physiological correlates of low serum vitamin A levels related to deficiency (Pilch, 1985). The prevalences of serum vitamin A levels below 0.70 μmol/L (20 μg/dl) were relatively low (0-6.1%) for all age groups in all three NHANES. Comparisons among surveys are difficult because total serum vitamin A levels were measured in NHANES I and NHANES II, but serum retinol levels were measured in HHANES. Clinical signs of vitamin A deficiency were not seen in HHANES nor was there a significant prevalence of low serum vitamin A with the possible exception of 4- to 5-year-old Mexican Americans below the poverty level. Although clinical signs of overt vitamin A deficiency were not seen in Mexican Americans, 10.1% had marginal serum retinol levels below 0.70 μmol/L (20 μg/dl) (Expert Panel on Nutrition Monitoring, 1989).

Large national surveys of nutritional status are primarily concerned with the nutritional health of the overall United States population. While major subgroups of

the population are generally well represented in these surveys, small subgroups of the general population who are difficult to locate or sample are often inadequately represented or not included (Lepkowski, 1991). Unfortunately, many subgroups at high risk for malnutrition, such as low-income children, legal and illegal immigrants, homeless individuals, and migrant workers, are not adequately represented in national nutrition surveys.

Vitamin A Measurement

Vitamin A status is assessed by clinical, biochemical, or cytological methods. The measurement of serum retinol has been one of the most widely used methods of determining vitamin A status. Limitations of this technique include sensitivity and specificity. Serum retinol reflects liver stores only after moderate to severe depletion and remains unchanged across a wide range of dietary intakes due to homeostatic mechanisms (Underwood, 1984). The World Health Organization (WHO) recommends that vitamin A deficiency be considered a public health problem if more than 5% of a population has serum retinol levels below 10 μg/dl. A prevalence of $\geq$ 15% of "low" values has also been suggested as a cutoff for public health significance (Underwood, 1990).

Previous studies (ICEPO, 1988) have suggested that impression cytology represents the first simple, noninvasive, reliable test to detect mild subclinical xerophthalmia in young children (Natadisastra, et al. 1988; Amedee-Manesme, Luzeau, Wittpen, Hanck, & Sommer, 1988). Conjunctival impression cytology (CIC) is a

method of obtaining surface cells from the bulbar conjunctiva to stain and examine for histologic changes. Other methods to assess vitamin A status are invasive and difficult to perform in the field--these include relative dose response, serum retinol concentration, and liver biopsy.

Iron and Zinc Deficiency

In general, poor children experience more nutritional deprivation and overall illness than other children. For example, iron-deficiency anemia, indicated by the presence of an abnormally low concentration of hemoglobin in the blood, is much more common among poor children. Iron is required for the transport of oxygen and carbon dioxide, and is a component of various tissue enzymes essential for energy production and proper immune system function. Serum ferritin appears to be the best indicator of iron stores (Skikne, Flowers, & Cook, 1990). Hemoglobin concentration is used as a measure to determine the degree of iron deficiency once iron stores are depleted. Iron is interrelated with vitamin A. A microcytic anemia associated with vitamin A deficiency is due to decreased mobilization of iron from the liver (Staab, Hodges, & Metcalf, 1984). Vitamin A supplementation in anemic children elevates their levels of serum iron (Mejia & Chew, 1988).

Undernutrition in childhood can weaken resistance to infection. The immune function decreases when other micronutrients, such as zinc, are very low in a diet or show lower bioavailability because the food sources are primarily of plant origin

(Prasad, 1991). Poorly nourished children are then at risk for more frequent colds, ear infections, and other infectious diseases.

Zinc is a constituent of over 200 metallo-enzymes that participate in carbohydrate, lipid, and protein metabolism. Hence, zinc is essential for many diverse functions including immune function and stabilization of membranes (Cousins & Hempe, 1990). Zinc is necessary for the normal growth and development of children. The height of several groups of growth-retarded children in the United States, including Hispanic children in Colorado, has been shown to improve after zinc supplementation (Davis, 1984). Zinc is also important for the health of such diverse cells as those of the taste buds, the lining of the gastrointestinal tract, the immune system, and the retina of the eye (Russell, Cox, & Solomons, 1983; Solomons & Russell, 1980). Zinc deficiency has been associated with vitamin A deficiency. Retinol requires zinc for oxidation to retinol. Research shows that even a mild zinc deficiency can result in abnormal vision in darkness mimicking vitamin A deficiency (Prasad, 1991). Thus, it is critical to assess serum zinc when conducting CIC to rule out low zinc-induced versus low vitamin A-induced eye lesions. Symptoms of zinc deficiency include loss of appetite, poor night vision, impaired color discrimination, poor wound healing, increased susceptibility to infection, hair loss, and abnormalities in taste and smell (McClain, Antonow, Cohen, & Shedlofsky, 1986).

Surveys of the nutritional status of young children in Thailand demonstrated a synergistic effect between vitamin A and zinc deficiency (Tantipopipat, Banjong, Rojroongwasin, Dramer, & Smith, 1991). Zinc deficiency interferes with vitamin A

metabolism by reducing production of retinol-binding protein. Vitamin A and zinc supplementation normalized conjunctival epithelium in these children after six months treatment (Udomkesmalee et al., 1992). It has been shown that vitamin A repletion significantly reverses ocular cell degeneration, even in the presence of moderate zinc deficiency. However, zinc supplementation alone does not prevent ongoing ocular cell degeneration from severe vitamin A deficiency. Recovery with vitamin A repletion is enhanced when zinc is added to the diet. Normal vitamin A metabolism is dependent on an available supply of zinc (Leopold, 1978).

The absorption of zinc from foods is affected by its interaction with other trace minerals and by the existence of absorption-impairing substances in some foods--most specifically, whole-grain foods and soy protein (Cousins & Hempe, 1990). Zinc deficient diets are associated with the textured vegetable protein meat extenders, soy protein isolates, soy-based meat substitutes, and possibly soy-based milk formulas. Also, phytate, calcium, iron, phosphorus, and fiber-containing foods impair zinc absorption.

In general, protein foods contain higher levels of zinc than foods that are rich in carbohydrates. Good sources of zinc are found in meats, poultry, shellfish, eggs, dairy products, and legumes. Guidelines usually suggest a daily zinc intake of 10 mg for ages 1 through 10 years.

Purpose of the Study

The purpose of this study was to investigate the vitamin A status of preschool children of migrant workers in Utah by conjunctival impression cytology and biochemical means. Due to the interaction of vitamin A with zinc and its potential impact on conjunctival impression cytology, zinc status was evaluated using serum zinc. Iron, hematocrit, hemoglobin, ferritin, and TIBC were measured to determine the iron status of these migrant children. Information on typical daily food intake was collected in an effort to assess dietary intake of vitamin A.

This study attempted to answer the following four questions: (1) Are Hispanic migrant children at risk for vitamin A deficiency even though they attend Head Start programs? (2) What are effective and efficient methods to detect subclinical vitamin A deficiency in this population? (3) Do laboratory values for retinol-binding protein and serum retinol correspond to abnormal conjunctival impression cytology? (4) Is abnormal conjunctival impression cytology the result of a decreased serum zinc?

Design and Methodology

The prevalence of subclinical vitamin A deficiency was determined for the population group of children of migrant workers in Utah. Statistical analysis of factors that influence the presence of abnormal ocular cytology was conducted. Descriptive variables included age, gender, migrant center location, conjunctival impression

cytology, zinc, iron, hemoglobin, hematocrit, total iron-binding capacity, percent transferrin saturation calculated, vitamin A, and retinol-binding protein.

The study consisted of a nonrandom sample of 65 children of migrant Hispanic families between the ages of 2 and 6 years. These children were enrolled in the migrant Head Start program through the Institute of Human Resource Development (IHRD) at four school locations: Brigham City, Roy, Manti, and Salem, Utah. IHRD provided free preschool education, plus health and dental screening for migrant Hispanic children in Utah. The IHRD Head Start Program was federally funded for people who had relocated within 2 years and had at least 51% of their income from agriculture. Participants were required to meet the same financial income guidelines as food stamp recipients. Eligibility for participation in the Head Start program was not restricted by alien status. Head Start personnel employed a "don't ask" policy with regard to alien status. The IHRD allowed both legal and illegal alien children to participate. Regardless, Hispanic migrants frequently move, and this challenged the efforts of the Head Start program to provide continuous and long term services.

During July 1991, this study population of boys and girls represented 93% of all the Hispanic migrant children attending these four Head Start centers in Utah. Each weekday, the children arrived at the center for breakfast and remained through lunch. This provided a ready and convenient sample of migrant Hispanic children for assessing nutritional status using conjunctival impression cytology, anthropometric measurements, and biochemical evaluations of blood.

CHAPTER II

LITERATURE REVIEW

The body's absorption and utilization of carotenoids and retinol are influenced by many dietary and morbidity factors. Deficiencies of protein, alpha-tocopherol, iron, and zinc have been shown to adversely affect vitamin A transport, storage, and utilization (Olson, 1987). Also, the body's ability to absorb preformed vitamin A and precursors of vitamin A is greatly reduced when consumption of dietary fat is less than five grams per day (Olson, 1987). In addition, the presence of peroxidized fat and other oxidizing agents in food has been shown to impede the body's absorption and utilization of dietary vitamin A and its precursors (Olson, 1987). Individuals with diseases such as diarrhea and sprue have reduced intestinal absorption of vitamin A and carotenoids (Olson, 1987). Similarly, intestinal infections in children have been associated with lower human serum levels of vitamin A.

The Importance of Vitamin A

The best understood function of vitamin A is its role in human vision. Vitamin A also occupies essential roles in growth, bone development, maintenance of epithelial tissue, the immunity process, and normal reproduction.

Vitamin A is a component of the visual pigments and, as such, is essential to the integrity of photoreception in the rods and cones in the retina. During the process of vision, 11-cis-retinaldehyde, the aldehyde form of vitamin A, combines with the

protein opsin to form visual purple or rhodopsin, a photoreceptor pigment located in the rod cells of the eye's retina. When light hits the retina, the rhodopsin is bleached and all-trans-retinaldehyde is released from the opsin, which triggers a stimulus to the brain via the optic nerve. All-trans-retinaldehyde is then reduced to all-trans-retinol by dehydrogenase enzymes and transported out of the rod to the epithelial tissue. Most of the retinol is transported back to the rods and converted back to 11-cis-retinal to combine with opsin. However, some retinol is lost because poorly water soluble vitamin A is not transported efficiently and must be replaced. The rod cells are responsible for vision in dim light, but when the body has insufficient amounts of vitamin A, there is loss of functioning visual pigment, which causes night blindness. Night blindness takes years to develop in adults but occurs much sooner in children because they have fewer body stores.

Most often the victims of xerophthalmia are preschool children (Olson, 1987). When body stores become severely depleted of vitamin A, xerophthalmia results and leads to progressive changes in the conjunctiva and cornea of the eye, including nyctalopia (night blindness), Bitot's spots, corneal xerosis, corneal ulceration, keratomalacia, and corneal scar. The ocular signs of vitamin A deficiency are estimated to afflict five to ten million children worldwide and five to ten times that number are subclinically deficient (Underwood, 1990; Sommer, 1982). Consequences of subclinical vitamin A deficiency are increased risks of morbidity and mortality. Other processes in which vitamin A is known to be involved include fetal development, the immune response especially cell-mediated immunity, hematopoiesis through

interactions with iron, spermatogenesis, appetite, tooth formation, and physical growth (Mejia, Hodges, & Rucker, 1979). It is now thought that its fundamental role in cellular development may be implicated in most of these (Underwood, 1984).

Researchers observed dietary hypovitaminosis A was associated with follicular hyperkeratosis in 1931. Studies in the 1940s showed that patients with hereditary disorders of keratinization occasionally responded well to high-dose vitamin A therapy. Since then, the role of retinol in maintaining normal skin has been extensively studied. Degeneration of epithelial cells results in dry, scaly skin due to the inability to produce mucus. This occurs not only in epithelial cells, but also in the intestines and lungs, and can progress until the whole body is covered with flaky, scaly skin similar to dandruff. It is followed by follicular hyperkeratosis and increased likelihood of infections in the respiratory and GI tract. Because other nutritional deficiencies produce similar skin disorders, these changes are not useful as indicators of vitamin A deficiency.

Vitamin A in the form of retinoic acid plays a crucial role in embryo development (Smith & Eichele, 1991). Due to rapid protein synthesis during embryo development, a lack of vitamin A has been associated with teratogenesis. Deficiency of vitamin A causes a generalized retardation of cartilage and osseous growth. This is extremely important in infants; for example, if the skull bones do not grow and the brain continues to grow, increased intracranial pressure and papilledema can result (Muhilal, Murdiana, & Azis, 1988).

Vitamin A and Iron

Iron is required for the transport of oxygen and carbon dioxide and is a component of various tissue enzymes essential for energy production and proper immune system function (Vitamin A, 1989). Although the exact role of vitamin A in iron metabolism is not clear, Mejia and Chew have suggested that vitamin A status influences iron utilization and possibly hematopoiesis (1988). The hemoglobin values of children with vitamin A supplementation in this Indonesian trial rose by one gram per deciliter (Muhilal et al., 1988). The vitamin A deficiency ultimately results in anemia correctable by supplementation with vitamin A, iron, or both (Meija & Chew, 1988). This relationship may be important in areas where intakes of both nutrients are low. Hemoglobin concentration increased in children receiving vitamin A supplemented monosodium glutamate fortification trial in Indonesia (Muhilal et al., 1988). Furthermore, another supplementation study has shown that vitamin A improves growth (West, 1988). However, this effect has been attributed as secondary to decreased morbidity with a reported 45% reduction in mortality (Sommer, 1982). The results from a clinical trial in Africa demonstrated a 50% reduction in mortality caused by measles in children treated with vitamin A (Barclay, Foster, & Sommer, 1987).

Dietary iron deficiency is considered to be the most common nutritional deficiency in the United States (U.S. DHHS, 1989). The iron-deficient children have

an increased incidence of infectious diseases and respiratory infections. The symptoms most frequently seen are pallor, weakness, and fatigue (Dallman, 1977).

Vitamin A and Zinc

Research has linked night blindness and weakened immune response to zinc and vitamin A deficiencies. One hundred forty Thai children were given either placebos, zinc supplements, vitamin A supplements, or both zinc and vitamin A for 6 months. Compared with the placebo, zinc alone and zinc plus vitamin A improved the children's responses to functional eye tests, including their ability to see in dim light. But it took both zinc and vitamin A to raise their immunity to tuberculosis above that of the placebo-treated group (Udomkesmalee et al., 1992). Zinc-responsive night blindness has been observed in alcoholism and in patients with Crohn's disease (Morrison, Russell, Carney, & Oaks, 1978). Skin ulcerations, reduced resistance to infection, and growth failure are indications of zinc deficiency. Studies indicate that vitamin A metabolism and immune defense are especially sensitive to insufficient zinc intake (Leek, Keen, & Vogler, 1988). A combination of increased fiber intake, calcium, and phytic acid plus low bioavailable zinc lead to decreased serum zinc levels (Bindra, 1986).

Assessment of Vitamin A Status

The importance of all vitamin A functions in the human body has not been fully determined, but the known results of vitamin A deficiency definitely warrant

investigation into the causes of hypovitaminosis A. Assessing vitamin A status has been problematic for researchers because only a liver biopsy can accurately reflect vitamin A reserves in the body (Underwood, 1984). Vitamin A is depleted from the liver at the relatively low net rate of about 0.5% per day, but within the body it is in a highly dynamic state (Sauberlich, Hodges, & Wallace, 1974). On the basis of experimental animal and human studies the lower limit of a satisfactory liver vitamin A concentration is considered to be 0.07 μmol/g (20 mg/g) in both sexes (Blackfan & Wolbach, 1993).

The more common methods of assessment include ocular signs; serum retinol and retinal-binding protein levels; rapid dark adaptation test (RDAT); relative dose response (RDR) (Campos, Flores, & Underwood, 1987) and the modified relative dose response (MRDR); and conjunctival impression cytology (CIC). The clinical classification of vitamin A deficiency is based on the assessment of the signs and symptoms of xerophthalmia. Night blindness is often the earliest manifestation of vitamin A deficiency, resulting from impaired functioning of rod cells in the retina. Night blindness normally responds to vitamin A therapy within 24 hours (Sommer, 1982). A prevalence of night blindness that exceeds 1% is considered to reflect a public health problem (Table 1).

Conjunctival xerosis due to vitamin A deficiency may give rise to distinct, foamy, bubbly, or cheesy accumulations termed "Bitot's spots," which reflect more advanced vitamin A deficiency than night blindness alone. When these two conditions coexist, vitamin A deficiency is usually more severe, accompanied by a lower, average

serum retinol level than with either condition alone (Sommer, 1982). Bitot's spots tend to respond, although often not completely, within 2 weeks of treatment. In older children, Bitot's spots can be nonresponsive to vitamin A therapy (Sommer, Hussaini, & Muhilal, 1980). A prevalence of Bitot's spots greater than 0.5% is considered to be of public health significance (Table 1).

Corneal xerosis, ulceration, and keratomalacia represent a clinical spectrum of severe xerophthalmia that reflects an increasing probability of corneal destruction and blindness. Mild xerophthalmia may occur in children who do not appear acutely ill, whereas corneal disease is often precipitated by the combined stresses of chronic vitamin A deficiency, moderate-to-severe protein energy malnutrition, and recent infectious illness. Corneal xerosis normally fully responds to vitamin A treatment within 1 to 2 weeks leaving the cornea clear, in the absence of complications.

Table 1

WHO Indicators, Description, and Minimum Prevalence Criteria to Assess the Public Health Significance of Vitamin A Deficiency in Preschool-Aged Children

Indicator	Description	Minimum WHO prevalence criteria (%)
Clinical	Night blindness	1.0
	Bitot's spot	0.5
	Corneal xerosis	0.01
	Corneal ulceration/keratomalacia	0.01
	Corneal scar due to xerophthalmia	0.05
Biochemical	Serum retinol <10 μg/dl (<0.35 μmol/L)	5.0

Prompt treatment of corneal ulceration may preserve vision, depending on the size, depth, and location of the lesion and healed scar. More generalized necrosis of the cornea (keratomalacia) usually results in blindness but prompt therapy may spare the other eye and save the child's life (Sommer, 1982). Scarring of the cornea that is attributed to xerophthalmia accumulates from past severe disease and, therefore, serves as an indicator of blinding xerophthalmia. A prevalence of corneal xerosis or corneal ulceration (keratomalacia) of greater than 0.01% or of corneal scar due to xerophthalmia greater than 0.05% is a significant public health problem (WHO, 1982).

Methods of Measurements of Vitamin A Status

Measurement of serum retinol levels represent the most common biochemical measure of vitamin A status. However, serum retinol is not a reliable measure due to the fact that serum levels decrease late in progression of deficiency disease in the eye. Serum levels ranging from 10-19.9 μg/dl are considered marginal, and levels below 10 μg/dl put the individual at extreme risk for vitamin A deficiency. There is little evidence of an abnormal vitamin A status within the range of 20-80 μg/dl (WHO, 1982).

The World Health Organization (WHO) has recommended that vitamin A deficiency be considered a public health problem if more than 5% of a population has serum retinol levels below 10 μg/dl (Table 2). A prevalence of $\geq$ 15% of marginal levels has public health significance (Underwood, 1990).

Table 2

Plasma Vitamin A Levels and Vitamin A Status

Status	Plasma Vitamin A μmol/l	Plasma Vitamin A (μg/dl)
Deficient	< 0.35	(<10)
Marginal	0.35 - 0.70	(10 - 20)
Satisfactory	0.70 - 1.75	(20 - 50)

The rapid dark adaptation test (RDAT) was based on the early phase of the human eye's adaptation to the dark. The test involves recording the amount of time required by an individual to separate white, blue, and red disks in this sequence from a black surface in dim light (Solomons & Russell, 1980). This technique was found to be associated with vitamin A intake, but not to serum vitamin A levels in children. However, the lowest serum level in the study group was 30.7 μg/dl, a level considered adequate for serum vitamin A (Solomons & Russell, 1980).

Visual dark adaptation and pupillary adaptation measured with a prototype scotopic sensitivity machine was used to determine vitamin A status of children in Indonesia and Baltimore, Maryland. These methods correlated well with other indicators of vitamin A status but scotopic threshold testing was less useful in identifying deficient individuals (Congdon et al., 1995).

The relative dose response (RDR) test indirectly estimates the adequacy of hepatic retinol through measurement of the change in serum retinol following a

measured oral dose of vitamin A. The test is based on the principle that the carrier protein for vitamin A (retinol-binding protein or RBP) continues to be synthesized and accumulates in the liver in a vitamin A-deficient state (Loerch, Underwood, & Lewis, 1979). Holo-RBP is then rapidly mobilized following the test dose of vitamin A that results in an abnormal rise in serum retinol 5 hours later. The RDR value is obtained by dividing the difference between baseline and 5-hour serum retinol values by the 5-hour value (X100). A RDR of >20% is considered to reflect inadequacy of liver retinol stores (Underwood, 1990). A change in retinol value greater than 20% from baseline has been correlated with liver concentrations of retinol less than .07 μmol/g among hospital patients (Amedee-Manesme, Mourey, Hanck, & Therasse, 1987). A modified RDR test that uses only a single blood determination 4 to 8 hours after 3,4-dehydroretinol is administered orally to the subject. For the modified relative dose response (MRDR), only one blood sample is drawn four to eight hours after 3,4-dehydro-retinol is administered orally to the subject. This is a suitable field method for indirectly assessing relative hepatic retinol adequacy (Tanumihardjo et al., 1990). For relative dose response, the minimum prevalence criteria for assessing public health significance has not yet been established by the World Health Organization (WHO, 1982).

High performance liquid chromatography (HPLC) is then used to separate A1 from A2 and the molar ratio is inversely related to vitamin A status (Olson, 1987).

Conjunctival impression cytology (CIC) is a noninvasive method to detect early subclinical vitamin A deficiency (Keenum et al., 1990). Abnormal histological changes

of the conjunctiva, characterized by loss of goblet cells, and a reduced number, reduced size, distortion, and loss of integrity of epithelial cellsare indicative of early vitamin A deficiency and can be ascertained by this method. A disk of cellulose acetate filter paper is gently applied to the temporal surface of each eye for a second, fixed, and later stained. Specimens are evaluated under light microscopy for the presence and density of goblet cells and for the morphological appearance of epithelial cells. Abnormal specimens are characterized by loss of goblet cells and early squamous epithelial metaplasia (ICEPO, 1988). Because impression cytology can detect subclinical vitamin A deficiency, it identifies a larger proportion of indicator-positive individuals and thus requires a small sample size to estimate the prevalence of vitamin A deficiency (Amedee-Manesme et al., 1988; Kjolhede & Gadomski, 1989). The minimum prevalence criteria for abnormal conjunctival impression cytology has not yet been established by the World Health Organization (WHO, 1982).

Contributions to and Incidence of Vitamin A in Developing Countries

Of the numerous factors contributing to vitamin A deficiency, insufficient dietary intake is generally considered the most influential; however, few studies have reported consistent differences in dietary habits between xerophthalmic and normal children (Tarwotjo, Sommer, & Soegiharto, 1982). While diets deficient in vitamin A are always found with xerophthmalic patients, all individuals with diets deficient in vitamin A do not exhibit xerophthalmia. This has been attributed to the fact that the

ocular symptoms are a result of inadequate vitamin A stores in the liver and not to the individuals' dietary intakes at the time of study.

Blankhart, Carr, and Price (1967) reported the incidence of night blindness and the relationship of night blindness to children's food intakes in a 3-year study conducted in an urban area in western Java. Nutrition students weighed separately 156 children's food intakes for four consecutive days. The children were 8 years old and younger and were from 64 households. Breast milk intake was measured by weighing the infant before and after suckling. Evidence of night blindness in the children was based on their mothers' statements; the children identified by their mothers were then clinically examined. Except for one case, night blindness occurred only in children from 2 to 5 years old. Night blindness was reported in 17 households based on information from the interviewed mothers. The author noted that night blindness cases were often concurrent with other diseases, such as tuberculosis, hepatitis, otitis, amoebic dysentery, and diarrhea.

The dietary data were presented for the 2- to 5-year-old group because this was the only group with night blindness. The main sources of carotene for this group were dark leafy vegetables such as amaranth leaves. The subjects were stratified into three groups: healthy, malnourished with night blindness, and malnourished without night blindness. The authors found relatively little difference among the calorie and protein intake of the three groups. The per capita intake of the households indicated, according to the authors, a fair consumption of carotene; thus the investigators

concluded the children's low intakes were not from a lack in availability of food at the household level but rather to an unequal distribution of food within the household.

In northern India, Tandon examined the relationship of age, gender, dietary habits, and protein-calorie nutrition to vitamin A status measured by clinical examination. Subjects greater than 5 years of age, including adults, had a higher prevalence of all the ocular signs of vitamin A deficiency. By using a 24-hour recall, the authors found that patients with night blindness consumed significantly less vitamin A than matched controls in the 3- to 6- and 7- to 10-year-old age groups (Tandon, 1975).

Panamanian children infected with Ascaris lumbricoides had significantly lower mean plasma vitamin A than uninfected children (Taran, Chopra, & Kevany, 1987). When socioeconomic variables and the location of the children were controlled in a regression model, the presence of A. lumbricoides was still significant. Plasma carotenoid concentrations were also significantly lower in the infected children. However, when all the socioeconomic and location variables were controlled, there was no significant difference between carotenoid values of infected and noninfected children. Bhattacharyya, Milton, Reddy, and Naidu (1987) reported lower serum vitamin A in Indian children with ascaris infection compared to children without ascaris. The same investigators also reported a greater incidence of xerophthalmia in ascaris-infected children in India. Xerophthalmia ranged from night blindness to corneal scarring and blindness. Reddy, Bhaskaram, Raghuramulu, Milton, & Rao (1987), on the other hand, found no association between ascaris and serum vitamin A

levels of 487 Indian children. They also found no significant difference between the serum retinol levels of children who were dewormed and given a large amount of vitamin A supplement (200,000 IU) and those who were only given a vitamin A supplement. However, plasma values alone only indicate the possibility of the body's impairment to utilize vitamin A. Some small-scale clinical studies have provided evidence that infections do cause malabsorption of vitamin A (Mansour, Mikhair, Farid, & Bassily, 1979).

A study of child survival in Indonesia revealed a linear relationship between the severity of xerophthalmia and mortality rate (Sommer, Tarwotjo, & Katz, 1987). Another vitamin A intervention trial in Indonesia reported a 30% reduction in children's mortality (Sommer et al., 1986). Data from several field trials have reported on the impact of vitamin A on the incidence and severity of morbidity. In Thailand, the occurrence of respiratory infection was assessed by two consecutive 2-month histories of preschool children after a vitamin A supplement or a placebo. The incidence for respiratory infection was 39% lower in the vitamin A group (Bloem, Wedel, & Egger, 1990). In Indonesia, children with mild xerophthalmia had a greater incidence of respiratory disease and diarrhea (Sommer, Katz, & Tarwotjo, 1984). A significantly higher rate of respiratory infection, but not of diarrhea, was found in preschool children with xerophthalmia in India (Vijayaraghavan, Naidu, Rao, & Srikantia, 1989). Furthermore, the risk of developing mild xerophthalmia was greater for children who had respiratory and diarrheal disease (Sommer et al., 1987).

In four ecological zones in Cebu in the Philippines, 1,715 children between 1 and 16 years old were screened for xerophthalmia (Solon, Popkin, Fernandez, & Latham, 1978). Clinical, biochemical, and anthropometric measurements were collected from the children, and dietary and socioeconomic data concerning their respective households were obtained. Fifty-seven percent of the children had serum vitamin A values less than 20 μg/dl and 4.5% had either Bitot's spots, corneal xerosis, keratomalacia, night blindness, or corneal scars while 2% had both low serum values and clinical signs. Except in the urban areas, males had a greater prevalence of xerophthalmia, which was defined as a combination of both low serum levels of vitamin A and ocular symptoms. The lowest prevalence of xerophthalmia was found in the 1- to 3-year-old group and the highest in the 4- to 6-year-old group. By using multivariate analysis, the authors found a large inverse relationship between vitamin A intake and symptoms of xerophthalmia. Authors found a large variation in vitamin A intake between gender and age groups. Females consumed significantly more vitamin A than males and younger children tended to consume more than older children. Large household size was associated with decreased children's vitamin A intakes. In the analyses performed by the researchers, the presence of tuberculosis was positively correlated with xerophthalmia, but recent diarrhea, measles, and ascaris were not.

Qualitative differences between the diets of children with Bitot's spots and randomly matched children were examined in Indonesia (Tarwotjo et al., 1982). In a nationwide survey, field workers asked a responsible adult the approximate frequency with which the target children had consumed mango, papaya, green leafy vegetables,

and certain sources of retinol during the preceding 2 months. Normal controls between the ages of 12 and 36 months ate mangoes and papayas with significantly more frequency than did children with Bitot's spots. Among the 1-year-olds, 67% of the normal children consumed one or more of these fruits in a week while only 36% of the deficient children consumed these foods with the same frequency. The control subjects between 3 and 6 years old consumed dark leafy vegetables significantly more often. Eggs were consumed consistently more frequently by normal than by abnormal subjects. In a second controlled study, researchers compared consumption patterns of children diagnosed with corneal xerophthalmia and normal controls (Tarwotjo et al., 1982). Differences in the children's consumption were significant for carrots (which were not studied in the nationwide survey), leafy vegetables, and eggs. The relative risk of corneal disease was inversely related to the frequency of dark green leafy vegetables and egg consumption.

Tanzania, Pepping, Pinnock, & Badcock (1989) compared the food intake of 9 children with xerophthalmic eye lesions to the intake of 17 children without xerophthalmia. The children's intakes were estimated by a combination of the precise weighing method, the aliquot sampling technique, and the recall method. Intake for 4 days, two consecutive days for two periods, was recorded. The mean vitamin A intake over four days for the control group was 256 μg and for the children with Bitot's spots, 182 μg, but these values were not significantly different. The control group had a higher frequency of consumption of whole milk, butter, and dried vegetables.

Nutrition surveys have shown a high prevalence of vitamin A deficiency throughout Central America (Arroyave, Chichester, & Flores, 1982). In Guatemala between 1965 to 1967 dietary surveys were conducted that employed the 24-hour recall method of obtaining dietary data. These studies revealed that out of 200 Guatemalan families, 45% were consuming diets that were less than one fourth of the RDA (Arroyave, Aguilar, & Flores, 1979). The authors believed the prevalence of vitamin A deficiencies was verified by the percentage of children who had serum retinol values below 20 μg/dl, a level considered undesirably low. Guatemalan children between the ages of zero and 4 years old had a 26.2% prevalence of vitamin A deficiency while children 5 to 9 years old had a 16.2% prevalence. These findings in Guatemala prompted the Institute of Nutrition of Central America and Panama (INCAP) to initiate a program to fortify sugar with retinyl palmitate. The aim of the program was to increase the availability of vitamin A sources because the underlying cause of vitamin A deficiency was attributed to an inadequate intake of the nutrient. A longitudinal study undertaken by INCAP before and after the fortification program was initiated showed that serum retinol levels improved in preschool-aged children who initially had low serum levels (Arroyave et al., 1979). In 1978, the Guatemalan government approved legislation requiring sugar manufacturers to fortify sugar with retinyl palmitate. In 1986, the government reinstated the program since compliance with the previous mandate had waned.

More recently, Gadomski, Wittpenn, & Rosas (1989) examined several methods of assessing vitamin A status in Guatemala. Their study population included over 200

children from several areas in Guatemala, including an orphanage in Guatemala City, three barrios of Guatemala City, a village 30 kilometers from the capital, and another village in the eastern Guatemalan lowlands along the Rio Dulce. They found 18% of their population had serum retinol values below 0.70 μmol/l, which is a percentage that exceeds the public health recommendations of less than 15% set by International Vitamin A Consultive Group (IVACG, 1981). Serum retinol levels are not accurate indicators of vitamin A status except when extremely deficient or extremely excessive (Olson, 1987). In the study reported by Gadomski et al. (1989), the authors observed that 8% of their sample had relative-dose response (RDR) levels greater than 20%, a level considered to reflect inadequacy of liver retinol stores.

In three communities within Guatemala City, Solomons and Russell (1980) found the mean serum retinol levels of a total of 150 preschool children to be 28 μg/dl. Among the three communities, Santa Fe, Ciudad Kennedy, and Guajitos, low serum levels under 20 μg/dl were respectively found in 13%, 5% and 17% of the preschoolers. A CIC assessment of the children resulted in 33% of the preschoolers in Santa Fe having abnormal readings, 30% in Ciudad Kennedy, and 20% in Guajitos. Conjunctival xerosis was found in only 2% of the preschoolers in Santa Fe. No clinical signs were found in the other two communities.

In the past few years the whole emphasis of research into vitamin A deficiency has shifted away from xerophthalmia and now centers on child survival. A group working in Indonesia, and more recently in Nepal, has reported a greatly increased mortality risk and rates of respiratory and gastrointestinal disease in mild

xerophthalmia, and at least a 30% reduction in mortality due to vitamin A supplementation of subclinical deficiency along the lines used in prevention programs (Sommer et al., 1986; West, 1991). One study in India claimed a 60% reduction in mortality with a weekly dose of vitamin A (Rahmathulla, Underwood, & Thulasiraj, 1990) and another found no significant effect (Vijayaraghavan et al., 1989). Many studies have shown that the detection of subclinical vitamin A deficiency can reduce child morbidity and mortality with vitamin A supplementation. India, Bangladesh, Indonesia, Haiti, and Sri Lanka have developed effective programs which distribute large, prophylactic doses of vitamin A to preschool children (Sinha & Bang, 1973). Yet, experience with periodic megadose vitamin A supplementation suggests that this is not a feasible solution due to problems with logistics in distribution over the long term (Sommer, 1982).

Vitamin A Deficiency in the United States

Dietary deficiency of vitamin A occurs in industrialized nations as well, predominantly in Black and Hispanic populations in the United States (Pilch, 1985). Low serum retinol was identified as a potential problem among Hispanics, children, and low-income groups by the Ten-State Nutrition Survey (U.S. DHEW 1972a, 1972b). Although clinical signs of malnutrition or xeropthalmia are rarely seen in North American children, evidence that diets of children are inadequate in quantity or quality was reported in the Nationwide Food Consumption Survey (USDA, 1987). Vitamin or mineral supplementation was recommended for high-risk children from very

poor families, those with poor appetites and eating habits, and ill children (American Academy of Pediatrics, 1980).

One of the principal findings from the first Health and Nutrition Examination Survey (NHANES I) was a relationship between low family income and deficiency of vitamin A for children between the ages of 1 and 5. A study of nutritional status of WIC participants documented that intakes did not meet the RDA for vitamin A and iron (Smith, 1986). Furthermore, the nutrients iron, zinc, and vitamin A were identified as most frequently consumed below the recommended amounts (Expert Panel on Nutrition Monitoring, 1989).

Because NHANES I and II samples did not include a sufficient number of Hispanics to allow for estimates of their unique health and nutrition characteristics, a separate Hispanic HANES (HHANES) was conducted from July 1982 to December 1983. Vitamin A intake was lowest for Hispanic children when compared with other groups (HHANES). In addition, research has shown that vitamin A requirements for humans are closely related to growth rate (WHO, 1982). Thus, children in poverty groups tended to be at higher risk of inadequate nutrient intakes and poor growth. Growth for children between 1 to 5 years, as represented by height and weight for age, has been correlated to nutrition status (Grant & DeHoog, 1985). Findings from nationwide surveys, such as the National Health and Nutrition Examination Surveys (NHANES I, II, III) and Hispanic HANES (HHANES), show that many young children consume vitamin A intakes below their RDA. Furthermore, deficiencies of other nutrients such as protein, iron, alpha-tocopherol, and zinc adversely affect

vitamin A transport, storage, and utilization (Underwood, 1984). Efforts were made by the National Center for Health Statistics to reanalyze vitamin A using new methodology and stored serum levels from NHANES I and II to compare with HHANES. The data showed some relationships but were not reliable enough due to poor storage of earlier samples and lack of enough samples to adequately reassess the older blood samples.

Unfortunately, only limited data exist from past studies of visual acuity and ocular disorders in Hispanics (HHANES). In the 1971-1972 National Health and Nutrition Examination Survey for example, only the White and Black population visual acuity and eye disorders prevalences were obtained (NHANES I).

Data from the second National Health and Nutrition Examination Survey (NHANES II) indicated that the major sources of dietary vitamin A in the U.S. were liver, carrots, eggs, vegetable-based soups, whole milk products, and fortified food products (Block, Dresser, Hartmen, & Carroll, 1985).

Environmental factors of poverty, poor education, lack of health insurance, and migratory patterns affect migrant Hispanic children. Migrant Hispanics are at risk for nutritional problems from insufficient financial resources to meet basic food needs (Delgado, Johnson, Roy, & Trevino, 1990). Common dietary problems reported among Hispanic migrant children include overweight, delayed or slowed growth, iron-deficiency anemia, folate deficiency anemia, vitamin A deficiency, and dental carries (HHANES). It is difficult to distinguish the role of vitamin A deficiency as opposed to iron deficiency and poverty-related factors that influence growth.

CHAPTER III

METHODS AND MATERIALS

Sample Selection

The target population consisted of preschool-aged Hispanic children of migrant workers in Utah. A nonrandom sample of Hispanic Head Start participants from The Institute of Human Resource Development programs was selected because of their ages, ethnic background, and availability. The cooperation from the Hispanic Head Start staff was beneficial in contacting parents and obtaining subjects for this study. A written translation of the informed consent in Spanish was arranged by Dr. Windham, project supervisor, and Mr. Hector Cando of the Institute for Human Resource Development (Appendix A).

A statement of the proposed research describing the use of human subjects was approved by the Utah State University Institutional Review Board. In addition, written approval by the review committee was obtained for taking blood samples for determination of iron, zinc, ferritin, hemoglobin, hematocrit, total iron-binding capacity, cholesterol, triglycerides, cell blood count, retinol-binding protein, and vitamin A and for conjunctival impression cytology.

Support Staff

In Salt Lake City, the director of the Institute of Human Resource Development and staff from each of the four centers were briefed by the research team as to the

purpose of the study. The Head Start workers at each Head Start center distributed and collected consent forms and questionnaires, which were written in English and Spanish (Appendix A).

The research team consisted of the author, who is a registered dietitian, a phlebotomist from Logan Regional Hospital, a physician trained in China, and four research assistants, who were undergraduate dietetics students and spoke Spanish.

Consent Documents

A parent or guardian of the child was informed of the procedure including the conjunctival impression cytology, the nature and measurement of blood drawn by venipuncture on the child, the benefits for participating in the study, and that participation was voluntary. Parents could withdraw their child at any time, and failure to participate did not in any way affect their enrollment in migrant Head Start. The explanation was given in English, Spanish, or both and distributed and collected by the migrant Head Start staff (Appendix A and B). All participants had a consent form signed by the parent or guardian granting permission for the child to undergo conjunctival impression cytology and have blood drawn by venipuncture.

Laboratory Benchwork

Prior to beginning the field study, several chemical solutions were made for preserving and staining the samples. Fixative solution was made with 75 ml of 95% ethyl alcohol, 25 ml of distilled water, 5 ml of glacial acetic acid, and 5 ml of 37%

formaldehyde. Vials were filled to the top with fixative before going into the field. Each vial was labeled as specimens were obtained in the field.

Harris Hematoxylin was made using 5.0 g hematoxylin, 50.0 ml of 100% (absolute) ethyl alcohol, 100 g of aluminum ammonium sulfate (alum), and 2.5 g of mercuric oxide (red) in 1000 cc of distilled water. The hematoxylin was dissolved in alcohol with gentle heat. The alum was dissolved in distilled water with heat. When both solutions were dissolved completely, they were combined in a 2000-ml Pyrex Erlenmeyer flask. This solution was brought to a rapid boil, and then the flame was extinguished. While the solution still bubbled, mercuric oxide was slowly added, and the solution turned dark purple. The solution in the flask was then cooled evenly in a tub of cold water. The flask was lightly stopped with a gauze square and exposed to light for a week to ripen. Thirty-two ml glacial acetic acid were added to the solution to increase nuclear clarity. Schiff Reagent was made with 4.55 g PARA Fuscin, 86.5 g normal hydrochloric acid, and 4.55 g of potassium metabisulfite in 1000 cc distilled water.

Using gloves, the investigator prepared acetate millipore filter paper (HAWP 304FO, Pore size 0.45 μm: Millipore Corporation, Bedford, Massachusetts) by cutting it to size with a paper punch and storing papers in a small, sterile, covered Tupperware dish. This paper was used to obtain conjunctival cytology specimens. Slides were labeled, stained, and mounted for each specimen using Permount and a coverslip. After the slides dried, they were read by the author and the major professor. Another student photographed the slides under 100X magnification.

Data Collection Procedures

Measurements of subjects' height and weight were taken and recorded for each child by a research assistant; conjunctival impression cytology was done by the author; and serum was taken last by the phlebotomist.

Name, sex, age, height, weight, and laboratory data were recorded on carefully maintained forms (Appendix E). A self-reported "typical daily intake" and other health data were collected from parents by a questionnaire (CIC Participant Data Sheet) attached to the consent form (Appendix C).

Height was measured by a research assistant using a stadiometer. The child was in bare feet and instructed to stand erect ("stand up tall") with heels together, knees straight not bent, feet flat on the floor, back to the wall, and head in the Frankfort plane ("look straight ahead"). The plastic ruler was then brought snugly to the crown of the child's head with care to assure that it remained perpendicular to the ground. Height was recorded to the nearest centimeter.

Body weight was measured by a research assistant and recorded in each child's chart using a SECA electronic scale (calibration was accurate to 100 grams) that re-zeroed after each use. The child was instructed to remain still while weight was taken. Summer clothing weight was not deducted from recorded weight.

Conjunctival Impression Cytology

The author and major professor were trained at the Johns Hopkins University by experts in CIC and were provided with the latest equipment. The author took ocular impressions following the procedure studied at Johns Hopkins and described in the "Training Manual: Assessment of Vitamin A Status by Impression Cytology" (ICEPO, 1988). Each child was restrained on the lap of an assistant with the child's head tucked under the chin of the assistant and the assistant's arms wrapped around the child. A second assistant held the lids of the right eye apart using fingers. In one instance, the child was held by his mother to settle the child's fears. The child looked toward a toy or balloon to help fix his or her gaze and expose the temporal conjunctiva. Another assistant pumped the vacuum applicator continuously to maintain a steady pressure throughout the procedure of obtaining epithelial specimens. The author applied the 3/16-inch diameter disc of filter paper for one second to the bulbar conjunctiva using sterile technique. The specimen was placed in a vial with fixative before repeating the procedure for the left eye.

Staining Procedures

The specimens were transferred from fixative vials with forceps to a plastic well-plate with holes drilled to allow for drainage. Specimens were stained by a standard technique outlined in the ICEPO training manual (ICEPO, 1988) (Appendix

J). Specimens were progressively dehydrated and placed in xylene to make the paper transparent.

Slide Preparation

Two specimens were placed on each labeled slide. Slides were labeled by letter and number to correspond with the well-plate and xylene bottles. Immediately, several drops of Permount were put directly on the specimen using an eye dropper. Gently a coverslip was placed over the Permount to avoid creating bubbles. The slides were dried overnight and were cleaned with xylene the following day.

Histology

Interpretation of conjunctival impression cytology specimens was done by the author and the major professor. Specimens were graded as normal, abnormal, or unreadable. The analysis for normal was based on viewing sheets of small epithelial cells with goblet cells or mucin spots. Alternatively, abundant mucin spots in the absence of any adherent cells were identified as normal.

Abnormal conjunctiva had enlarged, separated, or separating epithelial cells throughout the specimen in conjunction with rare or absent goblet cells and absent mucin spots. Artifacts such as rolled epithelium, smeared mucin spots, overstaining, or variable staining made slides unreadable.

Diagnosis of subclinical vitamin A deficiency is based on the presence or absence of goblet cells or mucin spots in sufficient quantity. A child is considered

normal if either specimen is normal. Vitamin A deficiency is a systemic disease; therefore, both eyes will be affected. Lack of concordance between two specimens taken from the same child may be due to poor technique or the variation of goblet cell density across the conjunctival surface (Appendix N).

In order to be sure CIC data accurately reflected true results, the reliability was tested by having two trained staff members of Johns Hopkins interpret 25 randomly selected slides. In addition, the validity was assessed by comparing serum values with conjunctival impression cytology.

Serum Samples

Blood can deteriorate rapidly unless properly managed and preserved for transmission to the laboratory for assay. Optimal attention was given to specimen collection, preservation, and transportation. One child had an inadequate quantity of blood sample collected for serum tests.

Approximately 10 cc's of blood were drawn from each participant at the Head Start center by a phlebotomist from Logan Regional Hospital. Each test tube was labeled to identify the subject. Seven ml of blood were drawn into a "Tiger-Top" that contained gel that allowed the blood to clot freely without plasma. This vacutainer separated serum and cells and was used to obtain serum after centrifugation. Retinol-binding protein, retinol, cholesterol, triglyceride, and ferritin tests were done on serum.

Retinol was a critical frozen collection. The process separated serum from cells as soon as possible. The test tube was covered with aluminum foil so no exposure to light occurred and frozen with dry ice immediately. Freezing was very effective with dry ice in an insulated styrofoam container.

Hematocrit and hemoglobin were collected from 3 cc's whole blood drawn into the lavender top vacutainer by the phlebotomist as a screening test for iron-deficiency anemia. An anti-coagulant (EDTA) was in this test tube for whole blood measurements such as complete blood count (CBC).

Two cc's of blood for zinc testing were collected in a metal-free tube with a dark blue stopper. No additives were in the test tube. Serum was separated from cells by centrifugation and stored in a plastic vial at room temperature for later analysis.

Samples were transported to the laboratory at Logan Regional Hospital and stored for later analysis of vitamin A, retinol-binding protein, total iron-binding capacity, ferritin, hemoglobin, hematocrit, cholesterol, triglycerides, and zinc. Serum samples for vitamin A, retinol-binding protein, cholesterol, triglycerides, and zinc were analyzed by Associated Regional and University Pathologists, Inc., Salt Lake City, Utah. Logan Regional Hospital laboratory provided results of their analysis of iron status using hemoglobin, hematocrit, and CBC. The CBC consisted of a hemoglobin, hematocrit, red cell count, platelet count, the number and type of white blood cells (i.e., differential leukocyte count), and three red cell indices: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

Hematocrit and hemoglobin levels were measured to determine presence of iron-deficiency anemia. Hematocrit measured the amount (by percentage) of packed red blood cells in whole blood. Hematocrit falls during iron deficiency, but only after hemoglobin formation has become impaired. Measurement of the concentration of hemoglobin in whole blood was used as a screening test for iron-deficiency anemia. A low hemoglobin concentration was associated with hypochromia, a characteristic feature of iron-deficiency anemia. The red blood count was low in severe anemia and represents the number of red blood cells in one liter.

To assess hematocrit, whole blood was centrifuged, and the ratio of the height of the red cell column to that of the whole blood sample in a hematocrit tube was measured. This ratio represents the packed red cell volume and was expressed as the percent of the total blood volume.

A reduced saturation of transferrin in the plasma and a reduced serum iron level provide more specific evidence of iron deficiency. This test detected reduced iron stores before anemia developed.

Serum ferritin parallels the total amount of storage iron. Serum ferritin was the only iron status index that reflected a deficient or normal iron status. Furthermore, with iron deficiency, the total iron-binding capacity (TIBC) was elevated.

MCH refers to absolute hemoglobin content of an average RBC. Values under 25.4 pg indicated hypochromia with iron-deficiency anemia. Values over 30.4 pg indicated folate with B_{12} deficiency. MCHC values less than 25.4 g/dl indicate iron-deficiency anemia. MCV level under .76 fL indicates iron-deficiency anemia with

microcytosis. Values over 92 fL indicate macrocytic anemia. An RDW under 12% with low MCV indicates microcytic anemia (Table 3).

The sequence of events in iron deficiency were depletion of iron stores as serum ferritin decreased; TIBC and transferrin increased and serum iron decreased; hemoglobin and hematocrit levels decreased (i.e., anemia); and erythrocyte indices (MCV, MCH, and MCHC) decreased (Yip, Johnson, & Dallman, 1984).

Recent studies show evidence that early lesions of artherosclerosis begin in childhood and are related to high cholesterol concentrations. Children with elevated cholesterol levels have increased risk for coronary heart disease in adulthood. The best indicators of possible risk of hypercholesterolemia in children are elevated parental cholesterol levels, a family history of cardiovascular disease, and an elevated fasting cholesterol (Sutton, 1993).

In children, total cholesterol levels less than 170 mg/dl are considered optimal. Cholesterol between 170-100 mg/dl is considered "borderline high."

Table 3

Expected Changes in Red Cell Indices During Iron-Deficiency Anemia, Macrocytic Anemia for Children Aged 2-6 Years from ARUP

Red Cell Index	Iron-deficiency anemia	Macrocytic Anemia
MCV fL	under 76	over 92
MCH pg	under 25.4	between 25.4 - 30.4
MCHC g/dl	under 32.3	over 34.3

Fasting triglycerides are used to identify different forms of lipidemia. Elevated serum triglyceride concentrations are one of the risk factors in coronary heart disease. The range for healthy North American children is 35-200 mg/dl (U.S. DHEW, 1992). Lipids were tested only for children with turbid, creamy serum.

Limitations of Study

Dietary questions and other information reported by the parents were assumed to be reported correctly. However, there was no interview component, and some of the responses were too generalized to generate useful data. Perhaps the language barrier and fact that some parents were illiterate reduced the number of answers to written questionnaires.

This was a convenience sample; therefore, descriptive statistics were used to describe or characterize the vitamin A, zinc, and iron status and prevalence of deficiency for the participants at the four regional migrant Head Start programs in Utah.

CHAPTER IV

RESULTS AND DISCUSSION

Previous studies of Hispanic migrant children have identified a number of nutritional problems, most of which are related to the typically low income and low educational levels of migrant families. Hispanic children have been found to have an increased incidence of anemia, and in some areas, vitamin deficiencies (Acosta, Aranda, Lewis, & Read, 1974; Chase, Kuman, & Dodds, 1971; Fry, Eitelman, & Kelly, 1975; Larson, Doods, Massoth, & Chase, 1974; Yanochik-Owen & White, 1977; Zavaleta & Malina, 1980). Consequently, the research objectives of this study were to determine the likelihood of subclinical vitamin A deficiency, zinc deficiency, or iron deficiency among Hispanic migrant children attending Head Start programs.

Characteristics of the Population

This was a sample of convenience, and subjects were not randomly selected. However, the sample represents 93% of the total population of Hispanic migrant children attending the Institute of Human Resource Development migrant Head Start programs in Utah in July 1991. Furthermore, these subjects may not have necessarily been representative of typical migrant farm worker families, as they may represent a group that is more concerned with children's education and welfare than average.

The parents of 68 Hispanic migrant children provided informed consent for participation in this study. Only two parents did not provide consent, and three

children migrated. The subjects were from 62 different households and included three pair of siblings. Sixty-four serum samples and epithelial cells from the bulbar conjunctiva of both eyes were successfully collected. Three percent of the children were age 2 ($n = 2$); 35% were age 3 ($n = 23$); 37% were age 4 ($n = 24$); 23% were age 5 ($n = 15$), and one child was 6 years old ($n = 1$).

Occupation of Parents

Although the parents of these children were migrant workers, the types of labor differed. As could be expected, the typical occupation was laborer in the field, harvesting fruit and vegetables, with the exception of parents at the Manti site. In Manti, the migrants worked on large turkey farms or in plants that slaughtered and processed turkeys.

Site Location

Four children with abnormal impression cytology were from Brigham City, two were from Roy, and two were from Salem. No children with abnormal conjunctival impression cytology were found at the Manti Center (Table 4).

The logistic regression was done to determine if center location (as a classification variable) explained the occurrence of abnormal conjunctival impression cytology in this sample. Center location was found not to be statistically significant. In Brigham City, Roy, and Salem, one might postulate that some families and Head Start are not doing enough to encourage adequate dietary intake among these children.

Table 4

Conjunctival Impression Cytology by Site

Center	Abnormal CIC	Normal CIC	% Abnormal CIC
Brigham City[a]	4	28	12.5
Roy	2	12	14.3
Salem	2	4	33.3
Manti	0	13	0.0

[a] The child held by his mother at the Brigham City site had normal CIC.
CIC means conjunctival impression cytology.

Biochemical Analysis

The serum was drawn by a phlebotomist at each Head Start location and transported to Logan Regional Hospital laboratory for analysis. The serum tests in this study included zinc, retinol-binding protein, vitamin A, iron hemoglobin, hematocrit, ferritin, total iron-binding capacity, MCV, MCH, MCHC, and RDW. Trained personnel at the hospital used standardized procedures for all biochemical analysis. Results for lab data for children with normal and abnormal conjunctival impression cytology are compared in Table 5.

The laboratory standard for normal zinc was between 65-256 mcg/dl. The results of these tests indicate 64 subjects had normal values for serum zinc with a mean value of 108.5 mcg/dl (range 82-215 mcg/dl). The phlebotomist did not obtain enough serum from one child for blood tests.

Table 5

Lab Data for Children with Normal and Abnormal CIC

Variable	N	Mean	Range
Iron (Fe), μg/dl			
Lab Fe^{++} Normal Standard			(30 - 120)
CIC Abnormal Male	3	48	(21 - 68)
CIC Abnormal Female	5	92	(62 - 119)
CIC Abnormal Totals	8	76	(21 - 119)
CIC Normal Male	32	105	(35 - 243)
CIC Normal Female	25	101	(34 - 175)
CIC Normal Totals	57	103	(34 - 243)
TIBC (Total Iron Binding Capacity), μg/dl			(250 - 410)
Lab TIBC Normal Standard			
CIC Abnormal Male	3	259	(190 - 368)
CIC Abnormal Female	5	248	(220 - 304)
CIC Abnormal Totals	8	252	(190 - 368)
CIC Normal Male	31[a]	243	(173 - 370)
CIC Normal Female	25	242	(144 - 401)
CIC Normal Totals	56[a]	243	(144 - 401)

(table continues)

Variable	N	Mean	Range
Ferritin, ng/ml			
Lab Normal Standard			(7 - 144)
CIC Abnormal Male	3	22.1	(4.1 - 33.8)
CIC Abnormal Female	5	18.9	(11.4 - 32.0)
CIC Abnormal Totals	8	20.1	(5.1 - 33.8)
CIC Normal Male	32	15.5	(0.8 - 71.3)
CIC Normal Female	25	20.7	(3.4 - 55.6)
CIC Normal Totals	57	17.8	(0.8 - 71.3)
Zinc, μg/dl			(65 - 256)
Lab Normal Standard			
CIC Abnormal Male	3	122	(86 - 146)
CIC Abnormal Female	5	118	(95 - 161)
CIC Abnormal Totals	8	120	(86 - 161)
CIC Normal Male	32	105	(82 - 215)
CIC Normal Female	24[a]	110	(84 - 203)
CIC Normal Totals	56[a]	107	(82 - 215)

(table continues)

Variable	N	Mean	Range
Vitamin A, μg/dl			
Lab Normal Standard			(20 - 80)
CIC Abnormal Male	3	44	(37 - 51)
CIC Abnormal Female	5	51	(11 - 90)
CIC Abnormal Totals	8	48	(11 - 90)
CIC Normal Male	32	63	(34 - 107)
CIC Normal Female	25	75	(40 - 120)
CIC Normal Totals	57	68	(34 - 120)
RBP (Retinol-binding Protein), mg/dl			(3.0 - 6.0)
Lab Normal Standard			
CIC Abnormal Male	3	3.0	(2.4 - 3.9)
CIC Abnormal Female	5	3.4	(3.0 - 4.5)
CIC Abnormal Totals	8	3.2	(2.4 - 4.5)
CIC Normal Male	32	3.4	(2.3 - 6.6)
CIC Normal Female	25	3.1	(1.6 - 5.4)
CIC Normal Totals	57	3.3	(1.6 - 6.6)

[a] N is one less subject due to QNS quantity not sufficient for test.

A correlation was done to detect a relationship between CIC and RBP and vitamin A. The largest correlation value was between CIC and vitamin A, which supports the hypothesis that abnormal CIC reflects a decrease in serum vitamin A

(Table 6). However, inspection of the data for children with abnormal CIC provides only one case of marginal vitamin A deficiency (11 μg/dl). This child was the most depleted in serum vitamin A. She was a petite 3-year-old with extensive dental decay. She was 93.8 cm (37.5 inches) tall, weighed 13.6 kg (30 pounds), and appeared malnourished. She wore diapers. This supports other studies showing that serum vitamin A deficiency is only an appropriate indicator in advanced stages of vitamin A depletion.

Statistical Analysis

The results of a logistic regression of conjunctival impression cytology as the dependent variable on age, zinc, RBP, vitamin A, and center was done for 63 cases. The results of this statistical analysis were not significant for center effect ($p = .8852$ and Wald = 0.6489). No significant effects due to age, zinc, or RBP were detected.

Table 6

Correlation with CIC as the Dependent Variable with Vitamin A and Retinol-Binding Protein

Variable	CIC	Vitamin A	RBP
CIC	1.00	-0.31[a]	0.01
VITAMIN A	-0.31[a]	1.00	0.02
RBP	0.01	0.02	1.00

[a] The largest correlation value of -0.31 was between conjunctival cytology and vitamin A.

Even though vitamin A deficiency was infrequently observed, there was a significant vitamin A effect ($p = .002$). The only variable with a significant correlation was between vitamin A and CIC with a $p < .039$ for vitamin A and Wald = 4.2225. Overall, the model is very poor for predicting abnormal CIC from explanatory variables.

The comparisons of categorical variables were evaluated with 2 x 2 tables as illustrated in Table 7. Fewer than 50% of the abnormal cases were correctly classified. Serum zinc was not a sensitive test for abnormal CIC because zinc was normal for all subjects. Retinol-binding protein was normal for 75% with abnormal CIC ($n = 6$), which indicates RBP is irrelevant in advanced vitamin A deficiency. Vitamin A was normal for all subjects except one.

The sign of the coefficient of vitamin A was negative because the normal cases were coded 1 and abnormal were coded 2. As vitamin A decreases, there is a tendency for CIC to be abnormal. This supports the hypothesis that abnormal CIC reflects subclinical vitamin A deficiency. None of the other variables was statistically significant for explaining the variance in conjunctival impression cytology.

Sibling Data

The study included three pairs of siblings. The presence of siblings generated the question of whether the results of subclinical vitamin A deficiency were a familial-related trait. Coincidentally, these three pairs of siblings all occurred at the Brigham City center. All siblings had normal vitamin A, retinol-binding protein, zinc, and

Table 7

Classification Table for Chi-Square

	1	2	%
1	55	0	100
2	4	4	50
		Overall	93.65%

CIC (Table 8). The largest number of abnormal CIC cases were from the Brigham City Head Start program. While this is not adequate evidence to eliminate site as being linked to CIC, it does suggest the nutritional support from Head Start may not be adequate and sufficient to overcome familial dietary deficits. Because these pairs of siblings from the same center all had normal CIC and normal serum vitamin A, they apparently did not confound the data. Also, no values below normal range for vitamin A (20 μg/dl) were observed among siblings.

Iron Studies

In Utah, the low normal indices for each migrant Head Start center are based on the fact that altitude affects hemoglobin and hematocrit. Brigham City (4220 ASL), Roy (4370 ASL), and Salem (4807 ASL) have an adjusted cutoff for hemoglobin of 11.5 g/100 ml and an adjusted hematocrit cutoff for children below 35%. For Manti (5800 ASL), the hemoglobin cutoff is adjusted to 11.9 g/100 ml with a hematocrit cutoff for children below 36%. Head Start considers children aged two to six with

Table 8

Sibling Data

Siblings	Gender	Age	Vitamin A	CIC	Zinc
A.	male	3	70	normal	113
A.	male	4	64	normal	112
B.	female	4	45	normal	106
B.	male	5	55	normal	84
C.	female	2	82	normal	130
C.	female	4	66	normal	116

values below these adjusted cutoff levels at risk for anemia. Four children from Brigham City and Roy were at risk for iron-deficiency anemia based on hemoglobin values. Hemoglobin was low in a range between 9 - 11.3 g/100 ml (Table 9). Hematocrit was low in these same subjects as well as in five additional children in the range 23.5 - 35.0% (Table 10).

Table 9

Prevalence of Low Concentrations of Hemoglobin by Center

		Hemoglobin Concentration g/100ml				
Location	N	< 10.0	< 11.5	< 11.9	> 12.0	QNS[a]
Brigham City	32	N/A	1	5	26	N/A
Roy	14	1	2	2	9	N/A
Manti	13	0	N/A	N/A	12	1
Salem	6	0	N/A	N/A	6	N/A

[a] Serum quantity not sufficient for test. QNS means "Quantity not sufficient."

Table 10

Prevalence of Low Concentrations of Hematocrit by Center

		Hematocrit %					
Location	N	<33	<34	<35	<36	>36	QNS[a]
Brigham City	32	1	1	2	3	25	N/A
Roy	14	N/A	N/A	4	2	8	N/A
Manti	13	N/A	N/A	N/A	1	11	1
Salem	6	N/A	N/A	N/A	1	5	N/A

[a] Serum quantity not sufficient for test. QNS means "Quantity not sufficient."

Serum ferritin is the best indicator of iron status. In iron-deficiency anemia, serum ferritin levels are very low or zero. The cutoff for identifying abnormal lab values of iron status indices is shown by age in parenthesis (Table 11). Fourteen children had low iron stores and ferritin depletion, which ranged between 0.8-5.1 ng/ml for age 3 to 4 years and ranged between 3.4-8.2 ng/ml for subjects age 5.

Table 11

Number of Children by Age with Iron Deficiency Tests

Age	Ferritin[a] (ng/ml)		Transferrin[a] Saturation %		Iron[a] μg/dl		TIBC[a] μg/dl	
2	----	(n=0)	<12	(n=0)	<50	(n=1)	>410	(n=0)
3 - 4	<10	(n=11)	<14	(n=2)	<50	(n=5)	>410	(n=0)
5 - 6	<10	(n=3)	<15	(n=0)	<50	(n=1)	>410	(n=0)

[a] One child had insufficient quantity of serum for these tests.

Together, serum iron, total iron-binding capacity (TIBC), and percent transferrin saturation are useful studies for diagnosing iron-deficiency anemia. In iron depletion, serum concentration of iron decreases, and TIBC of serum increases. Serum iron was low for 7 subjects, and total iron binding capacity for all subjects was within normal limits. Transferrin saturation percentage was calculated as shown in equation 1.

$$Transferrin\ Saturation\ (\%) = \frac{Serum\ Iron\ (\mu mol/l)}{TIBC\ (\mu mol/l)}\ X\ 100\% \qquad (1)$$

Consequently, transferrin saturation is low in iron-deficiency anemia as seen for 2 subjects (a 3-year-old with a value of 11.05% and a 4-year-old with a value of 12.14%).

Three red cell indices--mean cell volume, mean cell hemoglobin concentration, and mean cell hemoglobin--are derived from measurements of hemoglobin, hematocrit, and red blood cell count. The indices MCH, MCHC, MCV, and RDW% were used to define cell size and the concentration of hemoglobin within the cell to diagnose different types of anemia. Four children had MCH values under 25.4 (21.9-25.3 range) with iron-deficiency anemia. Four had MCH values above 30.4 (30.8-33.6 range), which indicates folate with B_{12} deficiency. MCHC is the least useful value as it is the last to fall in iron deficiency. All participants had normal MCHC values in the range 32.4-41.3.

Three children with low MCV values of 67.4, 71.9, and 75.8 were diagnosed with iron-deficiency anemia. One child had macrocytic anemia with a MCV of 92.3, MCH of 32.1, MCHC of 34.7, and RDW of 11.4.

Nine children in this study had cholesterol and triglycerides examined due to creamy, turbid serum. One 3-year-old boy had elevated cholesterol of 276 mg/dl, which warrants follow-up fasting lipid testing. However, the family history for heart disease was not known, and none of the subjects were fasting. The other eight children had normal cholesterol levels (Table 12). All nine children had triglyceride levels within normal limits.

Assessment of Vitamin A Status

The results of conjunctival impression cytology showed a substantial percentage of young children of Hispanic migrant workers in this study had subclinical vitamin A deficiency. Eight children (12.5%) had subclinical vitamin A deficiency based on the results of conjunctival impression cytology. Conjunctival impression cytology for one child was unreadable. One child with abnormal conjunctival impression cytology also had low (11 μg/dl) serum vitamin A. Based on biochemical indices alone, all the subjects of this study exhibited normal status for serum zinc. Therefore, the presence of abnormal conjunctival impression cytology does not appear to be related to serum zinc in this population.

Table 12

Cholesterol Values in Children

Standard Values	Total Cholesterol (mg/dl)	N	Range
Acceptable	< 170	9	(84 - 164)
Borderline	170 - 199	N/A	
High	> 700	1	(276 mg/dl)

Note. Data from National Cholesterol Education Program (NIH pub N4161).

Incidental Observations

The "typical dietary intake" questionnaire completed by parents suggested that the overall nutritional quality of the children's diets was good. It was evident that the children had a high consumption of breakfast cereals, tortillas, and beans. Consumption of sweet foods was variable, with most children eating few desserts such as pie or cake and a moderate to high amount of candy, cookies, and ice cream. Soft drinks, Kool-Aid, and milk were frequently reported.

Each center received a 3-week cycle menu from the nutritionist at the Institute of Human Resource Development Central Office. This menu appeared to be followed closely at Salem and Manti the days we observed meal service. In Roy and Brigham City, the meals differed from the menus when the center was visited because of substitutions. Incidentally, the cook at Brigham City proudly showed two sets of menus. The cycle menu she developed used predominantly Hispanic foods. She also

had a separate set of menus that were not used except when "inspectors" from the Institute of Human Resource Development Central Office visited.

The menus from the IHRD nutritionist incorporated some ethnic Hispanic foods the children were accustomed to eating as well as typical American foods. Dietary sources for vitamin A noted on the menus included milk, meat, yellow and green vegetables, and butter. Meat and eggs were the best sources for iron in the meals observed.

Anthropometric Measurements

Stature and weight measurements provide additional clues about the health and nutritional well-being of these migrant children. Every measurement for height and weight was recorded on the child's chart. In this study, growth charts for Mexican Americans were used to separate children who were within average range of weight and height for age and gender from those who may be at risk for overweight, underweight, or delayed growth. The child's age was recorded in years and months on the date measurements were taken in order to plot data accurately.

The children's weights and heights, by gender, were compared to height-weight standard tables for age (Tables 13 and 14).

Three boys identified as primary ($\underline{n} = 1$) and secondary ($\underline{n} = 2$) had stunted growth, which corresponded to approximately the third percentile for one, and two below the third percentile, respectively, for height on the growth chart. Growth

Table 13

Height of Children

N	Age	Mean Height	Height Range	50% Percentile Height
		Normal CIC Males		
1	2	42"		34"
14	3	38.94"	(31.5" - 43.5")	38"
8	4	41.84"	(39" - 44.5")	41"
8	5	44.71"	(42" - 46.6")	43"
1	6	45.5"		45"
		Normal CIC Females		
1	2	39.25"		34"
4	3	38.92"	(36.5" - 42")	38"
13	4	40.52"	(37" - 44")	41"
7	5	42.25"	(41" - 45")	43"
		Abnormal CIC Males		
3	3	39.5"	(38.3" - 40.5")	38"
		Abnormal CIC Females		
1	3	37.5		38"
2	4	43.0	(41.3" - 44.8")	41"
2	5	44.3	(42.5" - 46.0")	43"

Table 14

Weight of Children

N	Age	Mean Weight	Weight Range	50% Percentile Weight
		Normal CIC Males		
1	2	58.80 lbs		28 lb
14	3	36.78 lbs	(27 - 64.2 lbs)	32 lb
8	4	42.59 lbs	(28.4 - 53 lbs)	36 lb
8	5	50.90 lbs	(36.3 - 76.5 lbs)	44 lb
1	6	47.2 lbs		50 lb
		Normal CIC Females		
1	2	30.6 lbs		28 lb
4	3	32.87 lbs	(30.4 - 36.9 lbs)	32 lb
13	4	36.50 lbs	(30.3 - 52.8 lbs)	36 lb
7	5	38.70 lbs	(36.9 - 44.7 lbs)	44 lb
		Abnormal CIC Males		
3	3	33.7 lbs	(33.2 - 34.6 lbs)	32 lb
		Abnormal CIC Females		
1	3	29.9 lbs		32 lb
2	4	43.3 lbs	(40.7 - 45.9 lbs)	36 lb
2	5	51.2 lbs	(42.9 - 59.5 lbs)	44 lb

retardation was evaluated by the percentage of standard height compared to expected height for age.

Undernutrition reflected by low weight in relation to height for age was calculated as shown below. Four girls and one boy were below the tenth percentile for their expected weight for height. In contrast, two boys were extremely obese (200% ideal body weight).

$$\frac{\textit{Actual Weight}}{\textit{50th Percentile Value of Weight of Child's Height}} X\ 100 \qquad (2)$$

The third and 97th percentiles represent unusual, though not necessarily abnormal, findings. It was uncertain whether short stature could be attributable to genetics or dietary deficiencies with this sample.

Stem-and-leaf plots were done to graphically summarize each variable in the data set. The stem-and-leaf plots for height appears to be approximately normal for children between the age of two to six (Figure 1). The mean height was 102.5 cm (41 inches). The stem-and-leaf plot for weight shows some skewness in the distribution of weights, and two outliers corresponding to two obese children (Figure 2). The mean weight was 18 kg (39 pounds) (Appendix N).

Stem and leaf of zinc shows all were within normal values for children with normal and abnormal CIC (Figure 3). Stem and leaf for RBP with abnormal CIC

shows 25% of the subjects (n = 2) with low RBP values (Figure 4). In comparison, 50% (n = 38) with normal CIC had low RBP values (Figure 5). RBP is not a good indicator of subclinical vitamin A as seen in Figure 6 with RBP for all subjects.

Figures 9 and 10 show normal vitamin A for all subjects except one with diminished serum retinol and abnormal CIC.

Stem and Leaf of Height for All Subjects N = 64
Leaf Unit = 0.10

35	8
36	55
37	0055
38	03333
39	033588
40	00035555888
41	00033335558
42	00003355
43	00355
44	03588
45	0055
46	05

Figure 1. Stem and leaf of height. This is a normal distribution for the height of sixty-four subjects. The height range was from 35.8 inches (89.5 cm) to 46.50 inches (116.3 cm).

Stem and Leaf of Weight for All Subjects N=64
Leaf Unit = 1.0

2	7899
3	0001111223333444444
3	5555666668889
4	0000000001122334
4	5578
5	11223
5	89
6	4
6	
7	
7	6

Figure 2. Stem and leaf of weight. This is a normal distribution for weight of subjects between age 2 to 6 (range between 27 to 76.5 pounds). The outliers are the two obese boys who weigh 64.2 pounds (29.1 kg) and 76.5 pounds (34.8 kg). The mean weight is 39 pounds (17.7 kg).

Stem and Leaf of Zinc for All Subjects N=64
Leaf Unit = 1.0

0	0
0	
0	
0	
0	88888888888899999999999999999
1	000000000000111111
1	222223333
1	4444
1	6
1	8
2	1
2	

Figure 3. Stem and leaf of zinc. Zinc was normal for all subjects in the range of 82 μg/dl to 215 μg/dl.

Stem and Leaf of Retinol-binding Protein for Abnormal CIC N=8
Leaf Unit = 0.10

2	4
2	6
3	0024
3	9
4	
4	5

Figure 4. Stem and leaf of RBP for abnormal CIC. For subjects with abnormal CIC, the range of RBP was between 2.4 to 4.5. Two subjects fell below the low normal range of 3.0 μg/dl for RBP.

Stem and Leaf of Retinol Binding Protein for Normal CIC N=57
Leaf Unit = 0.10

1	6
2	23334444
2	5556667777788889999
3	000111112223334
3	6678
4	0014
4	
5	014
5	8
6	2
6	6

Figure 5. Stem and leaf of RBP for normal CIC. There were 28 subjects with normal CIC and abnormal RBP below 3.0 μg/dl.

Stem and Leaf of Retinol Binding Protein for All Subjects N=64
Leaf Unit = 0.10

1	6
2	233344444
2	55566667777788889999
3	0000011111222233344
3	66789
4	001
4	5
5	014
5	8
6	2
6	6

Figure 6. Stem and leaf of RBP for all subjects. The range for retinol binding protein for all subjects was between 1.6 to 6.6.

Stem and Leaf Vitamin A for Abnormal CIC Subjects N=8
Leaf Unit = 1.0

1	1
2	
3	77
4	4
5	14
6	5
7	
8	
9	0

Figure 7. Stem and leaf of vitamin A for abnormal CIC.

Stem and Leaf Vitamin A for All Subjects N=64
Leaf Unit = 1.0

1	1
2	
3	45779
4	00455689
5	01113456789
6	012445566667899
7	0123556689
8	234569
9	01
10	039
11	0
12	0

Figure 8. Stem and leaf of vitamin A for all subjects. The one subject with low serum retinol of 11 μg/dl can be observed in Figures 9 and 10.

CHAPTER V

SUMMARY AND CONCLUSIONS

Vitamin A deficiency is one of the most widespread nutrition deficiencies in many parts of the world and contributes to a considerable portion of preventable blindness in children.

This study was designed to obtain data on the risk of subclinical vitamin A deficiency among preschool-aged children of Hispanic migrant workers. While the degree of vitamin A deficiency in this study was not of public health significance, the fact that eight (12.5%) of the children were subclinically deficient implies that intakes of vitamin A may be marginal or inadequate in some individuals. Although 12.5% may seem small in a country like the United States, this represents a highly significant public health problem. Children of migrant workers may not be at great risk for blinding xerophthalmia, but they are at increased risk for other manifestations of deficiency, including stunted growth and increased infectious diseases.

It is uncertain whether short stature is attributable to genetics or dietary deficiencies among these children. However, poor vitamin A status increases the incidence of intestinal infections, respiratory infections, and measles (Underwood 1984), and any one or combinations of these conditions could contribute to growth stunting. Vitamin A deficiency in itself is associated with growth retardation, and a decline in weight gain is one of the first signs of nutrition deficiency (Underwood 1984). Further, vitamin A deficiency is associated with poor growth. Though vitamin

A deficiency may not be the sole cause of growth stunting seen in study children, it may explain, in part, the low anthropometric measurements found in some of these children.

We can conclude from this study that Hispanic migrant children are at risk for nutritional deficiencies even though they attend Head Start programs in the United States. The prevalence of vitamin A deficiency was verified by one child who had serum retinol values below 20μg/dl, a level considered marginal, which corresponded to abnormal CIC data.

It appears CIC is the most effective and efficient method to detect subclinical vitamin A deficiency with the migrant population. In addition to CIC, other newly developed nonclinical indicators such as the modified relative dose response (MRDR) and rapid dark adaptation test (RDAT) could be effective and efficiently used with this population to assess vitamin A status. The most reliable assessment of vitamin A is likely when a combination of methods is used.

Findings showed that retinol-binding protein does not indicate the presence of abnormal CIC. Also, the abnormal CIC was not an effect of depleted zinc stores. The results showed that a substantial percentage of the subjects in this study also had iron-deficiency anemia.

The importance of this study rests not with the current size of the migrant Hispanic preschool population, but rather with the probable impact of the growth of this population in Utah as a result of Proposition 187 in California. An unintended outcome of this proposition is the inevitable shift of the immigrant population from the

unfriendly social climate of California to a near nonlitigenous neighbor, Utah. As the population increases, the medical problems documented in this dissertation will increase. The possibility that Hispanic migrant children not participating in Head Start may have higher incidence of subclinical vitamin A and iron deficiency is a concern.

In the 4 years that have passed since completion of this research, the size of the budget for migrant Head Start has increased, and they now serve more than 500 children in Utah. Health care needs of children of migrant workers are particularly challenging, given their migratory patterns, low incomes, poor education, and lack of health insurance. Certainly, nutrition education for parents and children would improve their chance of obtaining better dietary sources of vitamin A and iron.

In light of the outcome of this study and with the explosive growth in the Hispanic migrant Head Start population in Utah, this study should be repeated with a larger sample and a control group of nonmigrant Hispanic children. Research evidence clearly indicates that early detection of vitamin A and iron deficiency allows for appropriate intervention to correct problems before depletion leads to the appearance of clinical deficiency signs.

Twenty-four-hour dietary recalls with food frequency and parental interviews could benefit a follow-up study. Nutritional analysis of the menus actually used by Head Start could identify deficiencies in nutrient content. The data could be used to recommend dietary intervention via bilingual nutrition education for parents.

Vitamin A deficiency is a major nutritional problem of underprivileged populations of the world, affecting an estimated 124 million children each year.

Conjunctival impression cytology is an effective, noninvasive method to assess vitamin A status in field conditions for populations at risk in North America.

The goal of nutrition research is to improve the nutritional status of individuals and groups at risk for malnutrition. This study is valuable for providing data that may increase awareness of the problems of Hispanic migrants and increase efforts by Head Start to eradicate hypovitaminosis A by the year 2000 (U.S. Department of Health and Human Services, 1990).

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APPENDICES

Appendix A

English and Spanish Informed Consent

Informed Consent Statement

We are doing a research study to find out if children of migrant workers in Utah have enough vitamin A in their bodies and in the foods they eat, because some doctors and nutritionists think they do not. We want to see if this is true. We will do these tests on about 100 children of migrant workers in Utah to see how many of the children have problems.

If your child does not have enough vitamin A, (s)he may have a problem now or in the future with not seeing well at night, or may not grow as well as (s)he should, or may be sick too often. We can tell if your child does not have enough vitamin A in his/her body by checking how dry the outside of her/her eye is and by testing to see if there is enough vitamin A in the blood. If we find out that your child has any of these problems, we can tell you which foods (s)he can eat that might help to make it better.

To determine if your child has sufficient vitamin A we need to do three tests on your child that will only take about 5 minutes, and they only have to be done this one time. The first tests is to measure and weigh your child and to find out what (s)he usually eats at home and what kind of illness (s)he has very often. During the second test, we need to touch the side of each eye with a small piece of paper. This will not hurt your child's eye, but (s)he must try to be still and quiet to make it easier. For the third test, we need to take a small amount of blood from your child's arm. A nurse or technician who has been trained to do this and does this all the time will take this small amount of blood from your child's arm on the inside of his/her elbow. You do not have to pay for these tests; they are free.

The workers who are doing this study are from the University in Logan, Utah: Utah State University. They are people who study nutrition and what foods children need to eat to be healthy. They have been trained to test childrens' eyes and blood. The names, addresses and phone numbers of the workers in charge are written on the last page of these papers.

You do not have to have these tests made on your child. If you say you want the test for your child, you can change your mind at any time. If you do not want the tests on your child, there will be no problems with the Head Start Program. You will still receive the same services at the Migrant Head Start school.

We need you to sign this paper to give us permission to do these tests on your child. Your name, your child's name and the results of the tests will be confidential and will be kept locked in the office of Dr. Carol Windham. The results of the tests will only be shared with the Head Start school that assists your child. The results of this study will help us find out if there really is a nutrition problem for many children of migrant workers.

You can ask us as many questions as you want before you sign this paper. If you sign the paper and change your mind, you can tell us at any time, and your child will not have to have any tests. It also means that you will let us do the tests on your child's eyes and will let us take a small amount of blood from your child's arm.

People to contact for questions:

Dr. Carol T. Windham
Associate Professor
Department of Nutrition and Food Sciences
Utah State University
Logan UT 84322-8700
(801) 750-2121

Laura N. Butts, M.S., R.D.
Graduate Research Assistant
Dept of Nutrition and Food Sciences
Utah State University
Logan UT 84322-8700
(801) 750-2117

Información para obtener consentimiento de exámens de deficiencia de vitamina A.

Investigadores del Departamento de Nutrición de Utah State University estan llevando a cabo una investigación sobre la alimentación de los hijos de trabajadores agrícolas emigrantes en el Estado de Utah. Algunos médicos y nutricionistas piensan que los hijos de trabajadores agrícolas emigrantes no consumen suficiente vitamina A para garantizar un desarrollo normal. El estudio consiste, en determinar si los niños tienen las cantidades adecuadas de vitamina A en su organismo y si los alimentos que ellos consumen contienen suficiente vitamina A para sus necesidades básicas. Estos investigadores estan tratando de hacer exámenes en cien niños para determinar que porcentaje de ellos sufren deficiencia de vitamina A.

Si su hijo no consume suficiente vitamina A, él puede tener problemas en el futuro. Por ejemplo, el niño puede tener dificultades en su visión nocturna, no podrá crecer normalmente, o padecerá de enfermedades con mucha frequencia. Los encargados de este estudio puedan determinar si su hijo padece de deficiencia de vitamina A al examinar la humedad del exterior de los ojos, y con un examen de sangre. Si ellos determinan que el niño padece de deficiencia de vitamina A pueden recomendar los alimentos apropiados para mejorar la condición del niño.

Para poder determinar si su hijo tiene suficiente vitamina A es necesario hacer tres exámenes que toman 5 minutos cada uno. Los mencionados exámenes se realizarán solamente una sola vez. El primer examen es de obtener el peso, estatura, y saber los alimentos que el niño consume en casa. También, llenar un formulario conserniente a la salud actual de su niño. El segundo exámen es obtener una muestra de la humedad del exterior del ojo del niño que se consiste en tocar el interior del ojo con un pedacito de papel. Es necesario que el niño permanezca quieto mientras se toma la muestra para facilitar el procedimiento. El tercer examen requiere tomar una pequeña muestra de sangre del brazo del niño. Una enfermera o un técnico entrenado en el procedimiento se encargará de extraer la muestra de sangre. Los exámenes son gratuitos.

Los encargados de este estudio pertenecen a la universidad en Logan: Utah State University. Ellos son profesionales que estudian la nutrición que los niños deben consumir para su desarrollo normal. Estos investigadores han sido apropiadamente entrenados. Los nombres, direcciones y números telefónicos de las personas que estan encargadas de esta investigación están en la última página de este documento.

Estos examenes no son obligatorias. Usted no está obligado a que estos exámenes sean aplicados a su hijo. Es posible que usted autorize en primera instancia, pero usted está en libertad de cambiar de parecer en el futuro y negar la autorización. Usted seguirá recibiendo los mismos servicios de siempre en las escuelita (Migrant Head Start Program).

Si usted autoriza los exámenes es necesario que firme la primera página de este documento. Nosotros mantendremos su nombre, el de su hijo, y los resultados de los exámenes con llave y confidencial en las oficina de la Dra. Windham. Esta

información solamente será compartida con la escuelita donde asiste su niño. Los resultados de este estudio nos permitirá determinar si en verdad existe un problema con la alimentación de los hijos de los trabajadores agrícolas emigrantes.

Antes de su autorización, por favor háganos todas las preguntas al respecto de estos exámenes. Si usted firma la autorización y después cambia de parecer, usted solamente tiene que informarnos y su hijo no será contado en estos exámenes. Al firmar esta autorización significa que usted entiende las razones por las cuales nosotros estamos llevando a cabo estos exámenes.

Personas que pueden ser contactadas para responder mis preguntas sobre este estudio:

Dra. Carol T. Windham
Department of Nutrition & Food Sciences
Utah State University
Logan, UT 84322-8700
tel. (801)-750-2121

Laura N. Butts, M.S., R.D.
Department of Nutrition & Food Sciences
Utah State University
Logan, Utah 84322-8700
tel. (801)-750-2117

Appendix B

English and Spanish

Parent or Guardian Agreement Form

Parent or Guardian Agreement Form

I have been told about the purpose of this research study to find out if children of migrant workers in Utah have enough vitamin A in their bodies and in the foods they eat.

I understand that the workers doing this study will weigh and measure my child and ask questions about foods my child eats at home and how often my child is ill. I understand that the tests consist of touching my child's eyes with a small piece of paper and that they will take a small amount of blood form my child's arm. I understand that the chance that my child will be harmed are very small, because the workers who will touch the paper to their eyes and take the blood from their arm have been trained to do these tests.

I understand that these workers also need to use the information about what foods my child eats. I understand that my or my child's name will not be used with these tests and that all of the results are confidential and will be kept in the office of Dr. Carol Windham. I also understand that my name or my child's nane will not be used in any analysis or publications.

I understand that if I change my mind, I can withdraw my child from this study, and there will be no problems for me or my child and that I and my family can still receive services form the Migrant Head Start school.

I have been told who to talk to if I have any questions about the tests, the reasons for the tests, what my rights are, or any other information about the research study. I have been given the names, addresses and telephone numbers of the people to ask questions.

Name of child for whom permission is given to participant in these examinations for vitamin A deficiency_____

_____

Age of child for whom permission is given_____

Parent or guardian signature_____

Date _____

Permanent Address _____

City, State, Zip Code _____

Autorización del padre o persona responsable

He sido informado que el propósito de este estudio es para determinar si los niños de los trabajadores agrícolas emigrantes en el Estado de Utah poseen cantidades adecuadas de vitamina A en su organismo y si los alimentos que ellos consumen tienen suficiente vitamina A para el desarrollo normal de los mismos.

Entiendo que los trabajadores encargados de este estudio van a tomar el peso, estatura, y pedir información a cerca de los alimentos que mi hijo consume en casa. También, que tengo que llenar un formulario conserniente a la salud actual de mi hijo. También estos examénes consisten en tocar los ojos de mi hijo con un pedazo de papel y luego van a extraer una pequeña muestra de sangre del brazo. Entiendo que la probabilidad de que mi hijo sufra algún daño físico como consequencia de estos exámenes es muy mínima ya que los trabajadores encargados de estos examines han sido entrenados adecuadamente.

Entiendo que estos investigadores también necesitan usar la información acerca de los alimentos que mi hijo consume en casa. Entiendo que mi nombre o el de mi hijo no serán usados en los análisis o publicaciones, y que los resultados de los mismos son confidenciales además toda la información y los resultados del estos examénes seran asegurados en forma confidencial en la oficina de Dra. Windham.

También reconozco que si yo cambio de parecer puedo retirar a mi niño de estos exámenes, y no habrá ningún problema para mi o para mi hijo y que mi familia podrá seguir recibiendo los servicios de la escuelita.

He sido informado a quién dirigirme en el caso de que yo tuviese preguntas adicionales, acerca de las razones por las cuales estos exámenes están siendo realizados, cuales son mis derechos en este asunto, y cualquier otra información relacionada con este estudio. He recibido los nombres, direcciones y números telefónicos de las personas a quienes puedo dirigir mis preguntas.

****

Nombre del niño(a) a quién yo doy mi autorización para que participe en estos exámenes de deficiencia de vitamina A:

_____

Edad del niño(a) a quién yo doy mi autorización para que participe en estos exámenes de deficiencia de vitamina A:

_____

Firma del padre o persona responsable: _____

Fecha: _____

Dirección permanente: _____

Ciudad, Estado, Zona Postal: _____

Appendix C

English and Spanish

CIC Participant Data Sheet

CIC PARTICIPANT DATA SHEET

NAME (Nombre)_____ SEX (Sexo)_____

DATE OF BIRTH (Fecha de nacimiento) _____

HOW MANY BROTHERS/SISTERS?(Cuantos hermanos/hermanas)_____

HAS YOUR CHILD BEEN ILL THIS SUMMER?
(Ha estado tu hijo/hija enfermo este verano) ----------

WHICH ILLNESS HAS YOUR CHILD HAD RECENTLY?
(Que enfermedad ha tenido tu hijo/hija) ----------

COLD (CATARRO) _____ COUGH (TOS)_____

EAR ACHE
(DOLOR DE OIDO) ---------- EAR INFECTION
(INFECTION DE OIDO)-----

CONSTIPATION _____
(CONSTIPADO-NO PODER AL BANO) DIARRHEA _____
(DIARRHEA)

OTHER? (OTRO)_____

WHO PREPARES FOOD FOR YOUR CHILD?
(Quien prepara la comida para tu hijo) ----------

IS YOUR CHILD ALLERGIC TO ANY FOOD?
(Esta alergico ha algunas comida) ----------

WHICH FOODS? (Que comidas) _____

DOES YOUR CHILD TAKE VITAMINS?
(TOMA TU HIJO/HIJA VITAMINAS) ----------

WHAT KIND? (Que vitaminas) _____

WHAT DOES YOUR CHILD EAT ON A TYPICAL DAY? (Que come tu hijo/hija en undia regular) GIVE PORTION SIZES WHEN POSSIBLE

BREAKFAST (ALMUERZO)	LUNCH (COMIDA)	SUPPER (CENA)
SNACK (MERIENDA)	SNACK (MERIENDA)	SNACK (MERIENDA)

Appendix D

IHRD Letter

INSTITUTE OF HUMAN
RESOURCE DEVELOPMENT

IHRD

IHRD'S MIGRANT HEAD START PROGRAM
ADMINISTRATIVE OFFICE
700 SOUTH 205 WEST
SALT LAKE CITY, UTAH 84101
(801) 521 4473

Julio 22, 1991

Estmados Padres:

En el programa de Migrant Head Start nosotros estamos agradecidos a instituciones que desean mejorar el programa por medio de estudios. Esto estudios son beneficioso ya que nos proveen de recursos, información, y maneras en que podemos mejorar nuestros servicios para los niños inscritos en el programa. Este verano un equipo de investigadores de la Universidad Estatal de Utah (Utah State University) van a estudiar el porcentage de niños imigrantes quer sufren de deficiencia de vitamina A y Zinc. Estudios anteriores han demostrado qué deficiencias puedes ser altas y graves en la población imigrante.

Nosotros en las oficinas centrales estamos complacidos en participar de esta investigación, y poner a efecto las recomendaciones de los expertos. Mae Barela, Directora del Programa, Sr. José E. Martinez, el Director Ejecutivo, y yo hemos hablado con la Sra. Laura Butts, la Dra. Carol Windham, y el resto del equipo, y estamos complacidos de tener personas capaces en ayudarnos mejor el programa. Reconocemos que los esfuerzos van a ser de mucha ayuda para los niños que estan y se inscribirán en el programa en el futúro.

En uno o dos días usted recibirá información tocante a este estudio, por favor lealo muy cuidadosamente. Si tiene alguna pregunta, favor de comunicarse con la directora, o la encargada de la salud en el centro donde su hijo asiste. Si usted quiere hablar con migo concerniente a este asunto, for favor llameme. Para poder examinar a su hijo necesitamos que firme el permiso que estaremos adjuntando a la información. Yo le recomiendo que nos de su permiso, para que de esta manera nosotros podamos mejor ayudar a su niño.

Gracias por su ayuda y colaboración con respecto a este asunto.

Sinceramente,

Héctor E. Cando, Cordinador
Salud/Servicios para Niños con Disabilidades

La familia

Appendix E

Center Data Collection Tools

NAME _____ CODE# _____

AGE _____

GENDER _____

HEIGHT IN INCHES

WEIGHT IN POUNDS

GENERAL APPEARANCE

____ DENTAL CARRIES ____ CIRCLES UNDER EYES ____ RUNNY NOSE

____ UNDERWEIGHT ____ AVERAGE _____ OVERWEIGHT

OTHER _____

LAB VALUES

HEMATOCRIT _____

HEMOGLOBIN _____

SERUM RETINOL _____

RETINOL BINDING PROTEIN_____

SERUM ZINC _____

COMPUTED DATA

WEIGHT/HEIGHT _____

WEIGHT/AGE _____

HEIGHT/AGE _____

CIC (NORMAL/ABNORMAL) RIGHT EYE _____ LEFT EYE _____

PLATE WASTE STUDY

DATE _____ NAME OF CHILD _____

FACILITY _____ MEAL _____

CODE: CONSUMED 100% = 5 CONSUMED 50% = 3 CONSUMED NONE = 1

MENU ITEM _____ PORTION SIZE _____ CODE ____

MENU ITEM _____ PORTION SIZE _____ CODE ____

MENU ITEM _____ PORTION SIZE _____ CODE ____

MENU ITEM _____ PORTION SIZE _____ CODE ____

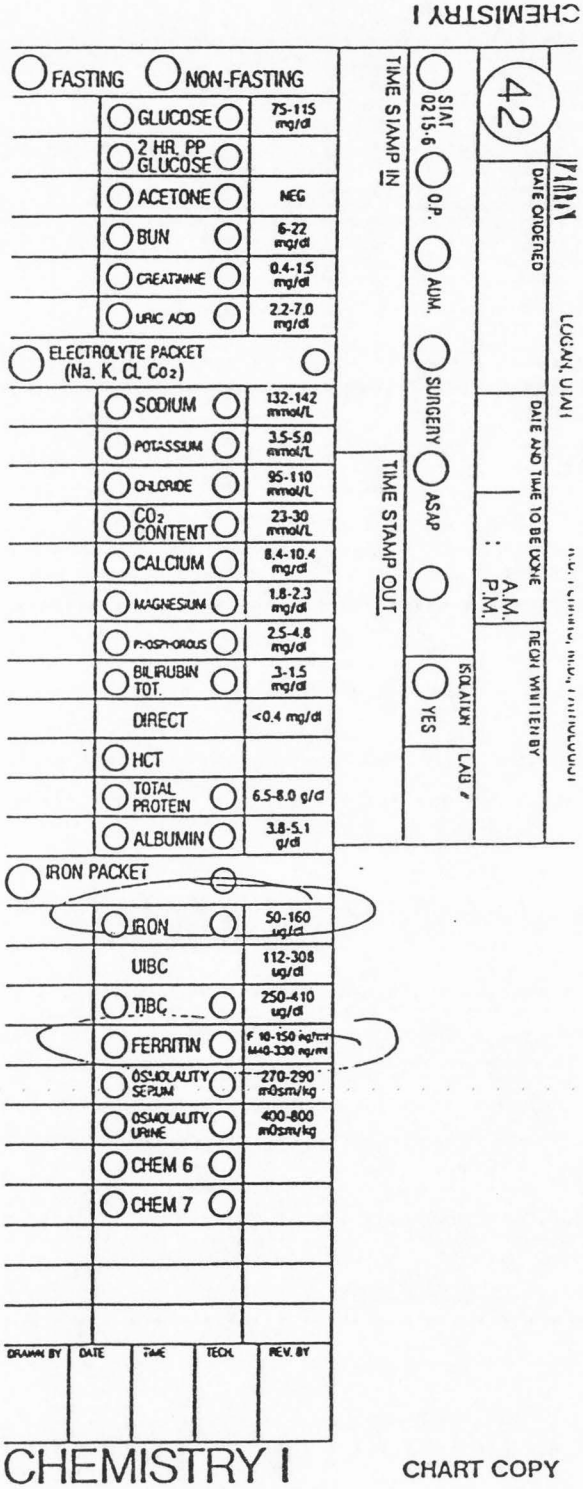
MENU ITEM _____ PORTION SIZE _____ CODE ____

MENU ITEM _____ PORTION SIZE _____ CODE ____

Write a short evaluation of your study which analyzes the results. Consider portion size, appearance, menu, time to eat, product temperature, weather, etc. Incorporate children's response if possible. Use the back of this sheet for your response.

Appendix F

Normal Laboratory Values


CHEMISTRY I
42
DATE ORDERED
DATE AND TIME TO BE DONE
: A.M. P.M.
TECH WRITTEN BY
SIAT 0215-6
O.P.
AUM.
SURGERY
ASAP
ISOLATION
YES
LAB #
TIME STAMP IN
TIME STAMP OUT
FASTING
NON-FASTING
GLUCOSE 75-115 mg/dl
2 HR. PP GLUCOSE
ACETONE NEG
BUN 6-22 mg/dl
CREATININE 0.4-1.5 mg/dl
URIC ACID 2.2-7.0 mg/dl
ELECTROLYTE PACKET (Na, K, Cl, Co2)
SODIUM 132-142 mmol/L
POTASSIUM 3.5-5.0 mmol/L
CHLORIDE 95-110 mmol/L
CO2 CONTENT 23-30 mmol/L
CALCIUM 8.4-10.4 mg/dl
MAGNESIUM 1.8-2.3 mg/dl
PHOSPHORUS 2.5-4.8 mg/dl
BILIRUBIN TOT. .3-1.5 mg/dl
DIRECT <0.4 mg/dl
HCT
TOTAL PROTEIN 6.5-8.0 g/dl
ALBUMIN 3.8-5.1 g/dl
IRON PACKET
IRON 50-160 ug/dl
UIBC 112-308 ug/dl
TIBC 250-410 ug/dl
FERRITIN F 10-150 ng/ml M40-330 ng/ml
OSMOLALITY SERUM 270-290 mOsm/kg
OSMOLALITY URINE 400-800 mOsm/kg
CHEM 6
CHEM 7
DRAWN BY
DATE
TIME
TECH.
REV. BY
CHEMISTRY I
CHART COPY

☐ CBC
☐ CBC W/O DIFF

DATE ORDERED:

ORDERED BY

DATE AND TIME TO BE DONE

• A.M.
• P.M.

CASS. NO.

PARTIAL ASP.

TIME

I.D.

TEST NO.

PATIENT IMPRINT AREA:

SA	/	NORMAL VALUES	OP CODES
		WBC x 10³ M F 4.8-10.8	
		RBC x 10⁶ M 4.7-6.1 F 4.2-5.4	
		Hgb g/dl M 14-16 F 12-16	
		Hct % M 42-52 F 37-47	
		MCV fL M 80-94 F 81-99	
		MCH pg M F 27-31	
		MCHC g/dL M F 32-36	
		RDW % M F 11.5-14.5	
		PLT x 10³ M F 130-400	
		MPV fL M F 7.4-10.4	
		LYMPH % M F 20.5-51.1	
		MONO % M F 1.7-9.3	
		NEUT % M F 42.2-75.2	
		EOS % M F 0.0-10.0	
		BASO % M F 0.0-0.8	
		LYMPH x 10³ M F 1.2-3.4	
		MONO x 10³ M F 0.11-0.59	
		NEUT x 10³ M F 1.4-6.5	
		EOS x 10³ M F 0.0-0.7	
		BASO x 10³ M F 0.0-0.2	

W B C				LYMPHO-PENIA		LYMPHO-CYTOSIS
				NEUTRO-PENIA		NEUTRO-PHILIA
				VAR LYMPHS		MONO-CYTOSIS
				BLASTS		EOSINO-PHILIA
				BAND GRAN BANDS		BASO-PHILIA
R B C				NRBC		ANISO
				POIK		MACRO
				RBC FRAG		MICRO
				RBC AGG		HYPO
P L T				PLT CLUMPS		LARGE PLTS
				GIANT PLTS		SMALL PLTS

DIFFERENTIAL	
	Myelo.
	Meta.
	Band
	Seg.
	Baso.
	Eos.
	Lymph.
	Mono.
	PLATELET ESTIMATION

LOGAN REGIONAL HOSPITAL
LOGAN, UT

REMARKS:

DRAWN BY	DATE	TIME	TECH.	REV. BY

FILE COPY

LA 016 R 9/91

Carl R. Kjeldsberg, M.D.
Laboratory Director

Associated Regional and University Pathologists, Inc.
500 Chipeta Way Salt Lake City, Utah 84108
583-2787 IN UTAH 800-522-2787 OUTSIDE UTAH 800-242-2787

ARUP

CLIENT NUMBER

FINAL

LOGAN REGIONAL MED CTR
1400 NORTH 500 EAST
LOGAN, UT 84327

NAME/I.D.# UNIVERSITY, STUDY

ARUP REF. I.D.# (1046)000-25-0433

DATE COLLECTED 13AUG91 TIME 0846
DATE RECEIVED 13AUG91 TIME 0846
DATE REPORTED 14AUG91 TIME 2023

REFERRING PHYSICIAN

TEST	RESULT	H/L	REFERENCE RANGE	UNITS
	IMMUNOLOGY-SEROLOGY			
SPECIFIC PROTEINS				
RETINOL BND PRO			(3.0-6.0)	MG/DL
	SPECIAL CHEMISTRY			
VITAMIN STUDIES				
VITAMIN A			(50-220)	ug/dL
	METALS			
ZINC			(65-256)	MCG/DL

UNIVERSITY, STUDY

END OF CHART

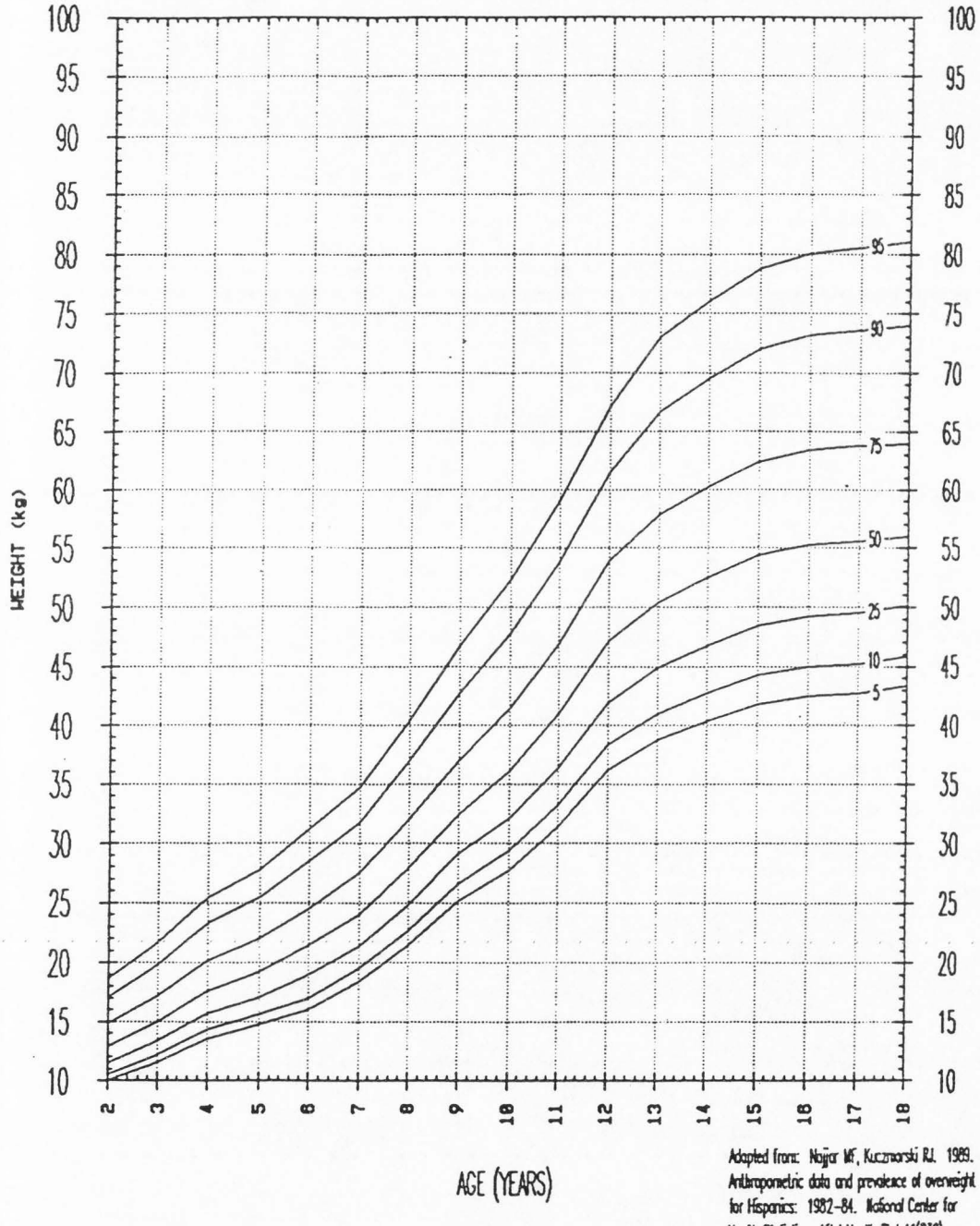
Appendix G

Physical Growth

Mexican American Girls and Boys

MEXICAN AMERICAN GIRLS: 2 TO 18 YEARS
PHYSICAL GROWTH
NCHS PERCENTILES*

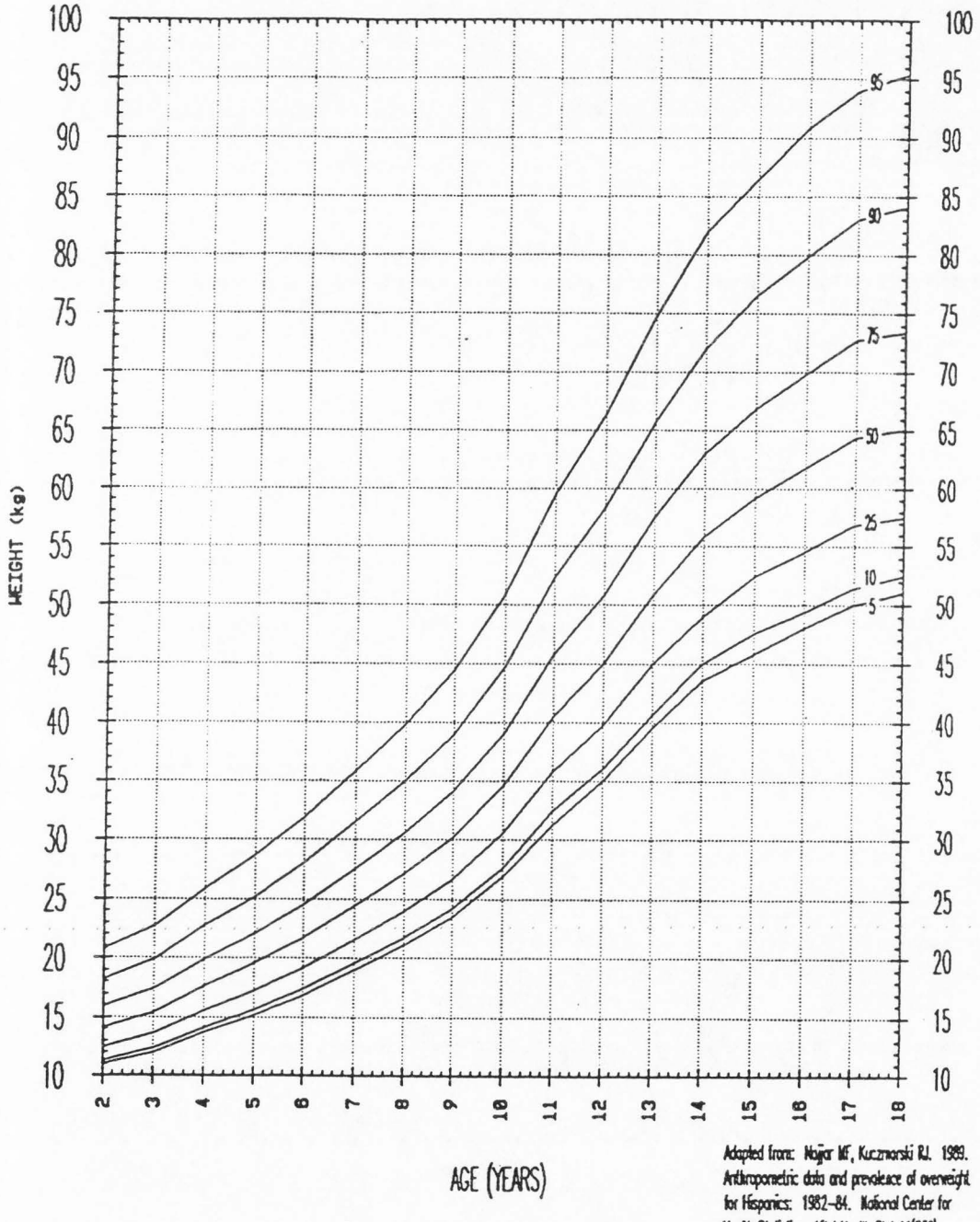
NAME _____ RECORD # _____
MOTHER'S STATURE _____ FATHER'S STATURE _____



Adapted from: Najjar MF, Kuczmarski RJ. 1989. Anthropometric data and prevalence of overweight for Hispanics: 1982–84. National Center for Health Statistics. Vital Health Stat 11(239).

MEXICAN AMERICAN BOYS: 2 TO 18 YEARS
PHYSICAL GROWTH
NCHS PERCENTILES*

NAME _____ RECORD # _____
MOTHER'S STATURE _____ FATHER'S STATURE _____



Adapted from: Najjar MF, Kuczmarski RJ. 1989. Anthropometric data and prevalence of overweight for Hispanics: 1982–84. National Center for Health Statistics. Vital Health Stat 11(239).

Appendix H

Johns Hopkins CIC Evaluation

CIC-A GRADING COMPARISON

		SCORING:	N = NORMAL	
			A = ABNORMAL	
			U = UNREADABLE	
Slide	Laura's Score	CTW's Score	Lilly's Score	Rick's Score
A1	N	N	N	N
A2	N	N	N	N
A3	N	N	N	N
B2	N	N	N	N
B3	A	U	A	A
B5	N	U	N	N
C2	N	U	N	N
C3	A	U	A	A
C4	N	U	N	N
C6	U	U	U	U
E1	A	U	A	A
E4	N	N	N	N
F4	N	N	N	N
F5	N	A	N	N
G2	N	U	N	N
G4	N	U	N	N
G5	A	U	A	A
G6	A	N	A	A
I2	N	N	N	N
J3	N	N	N	N
J4	A	U	A	A
K3	N	N	N	N
K4	A	A	A	A
K5	N	N	N	N
K6	A	U	A	A

Appendix I

Appendum to CIC Study

APPENDUM TO CIC STUDY

TO: SYDNEY PETERSON 1 JULY 1991

FROM: LAURA NIHAN BUTTS

RE: ADDITIONAL CLIENTS TO BE STUDIED

Mr. Hector Condo, Health Coordinator for the Institute of Human Resource Development (IHRD) and Ms. May Barrela, Center Director of IHRD in Salt Lake City, Utah have agreed to let us study Hispanic Migrant children in the Headstart program at the following locations in Utah.

Brigham City Center
40 North 100 East
Brigham City

Roy Center
4228 South 2175 West
Roy

Salem Center
10 West Center Street
Salem

Manti Center
425 East Union
Manti

We have sent consent forms to Mr. Condo in Spanish and English. The consent forms will be sent home with all eligible children between the age of three to five to be signed. Again, if the parent does not wish to have his child participate it will not reflect upon their eligibility to participate in the Headstart program.

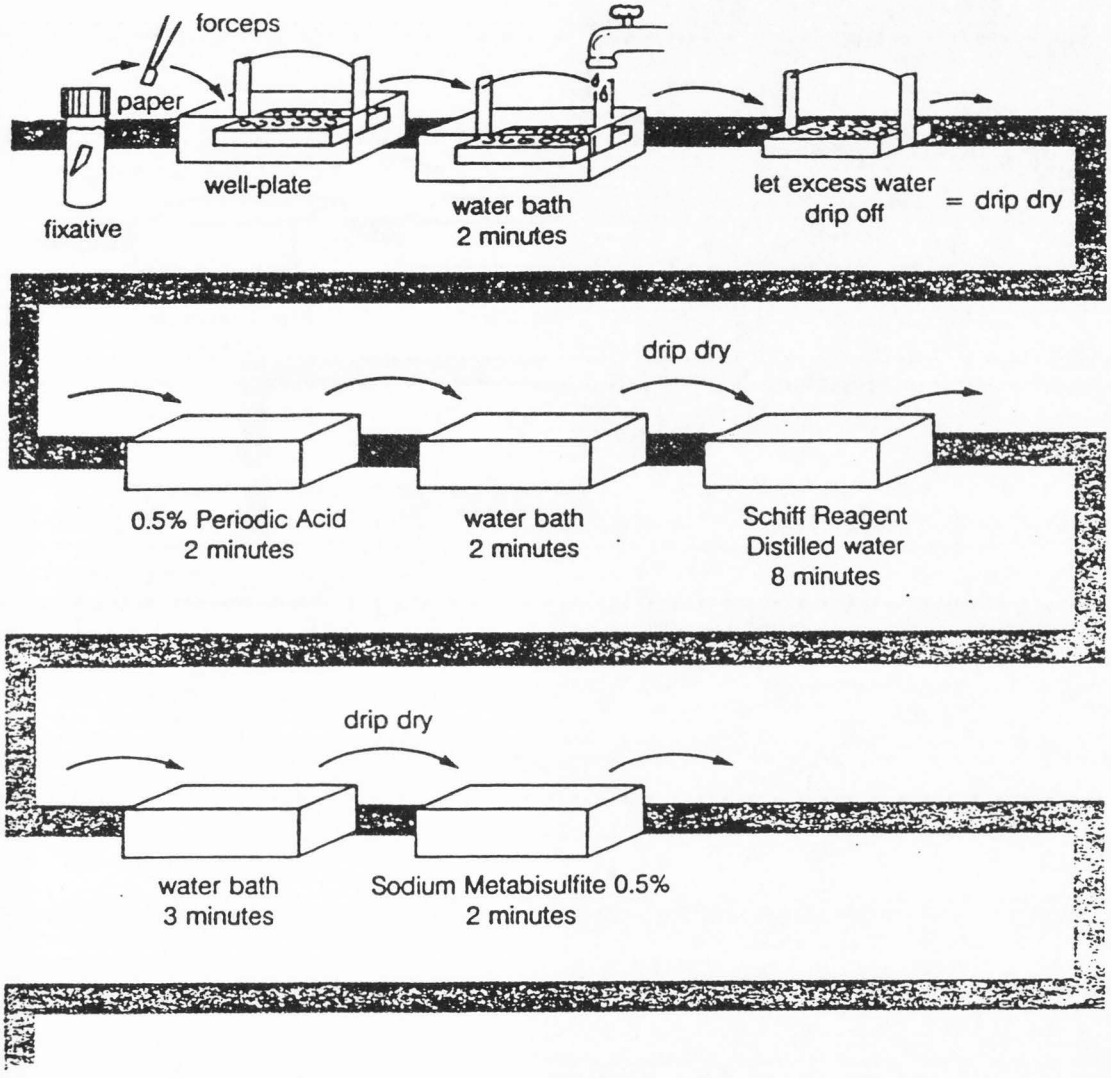
Thank you. If you have further questions, please call me.

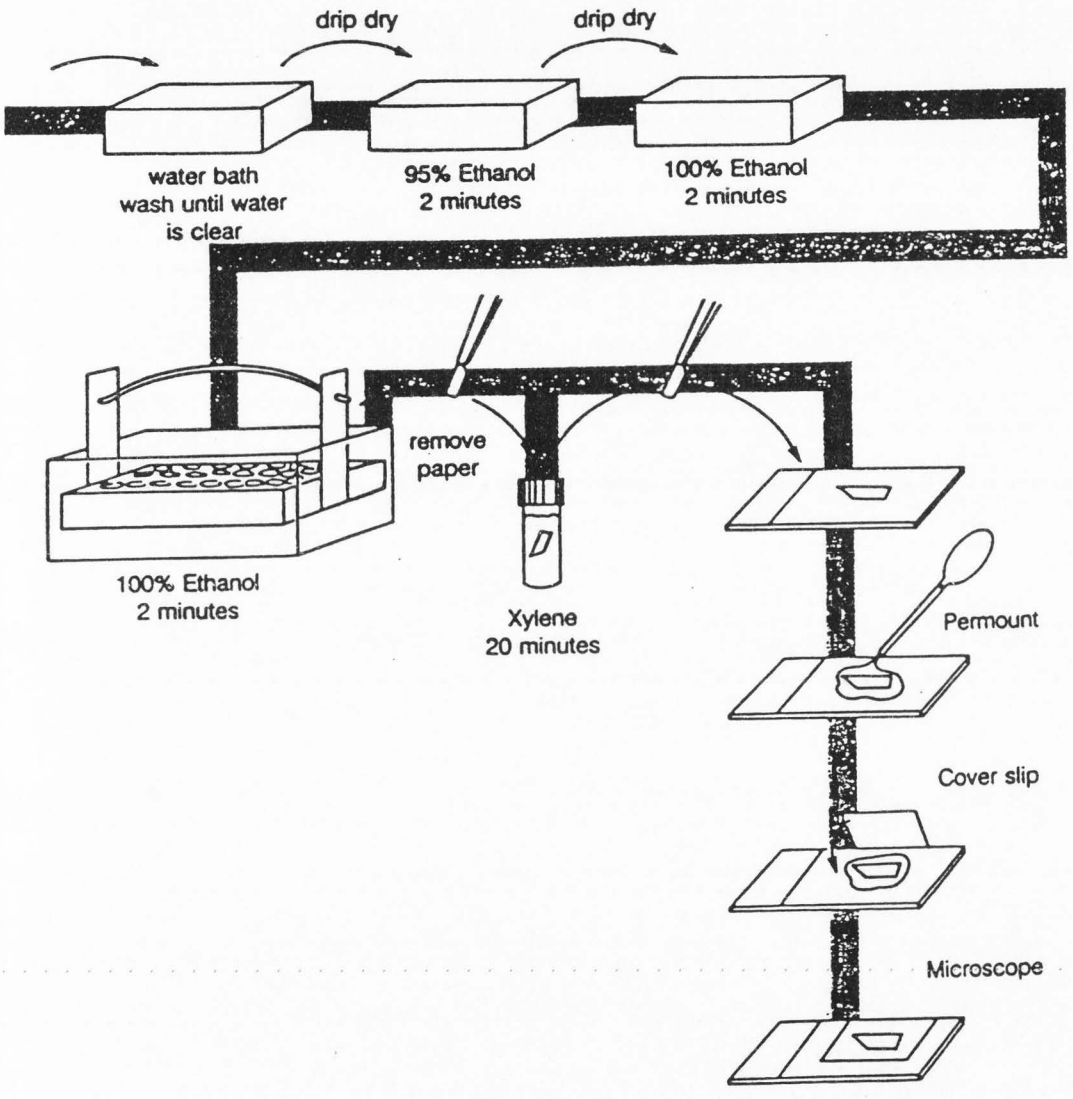
Sincerely,

Laura Nihan Butts

Appendix J

Staining Procedure

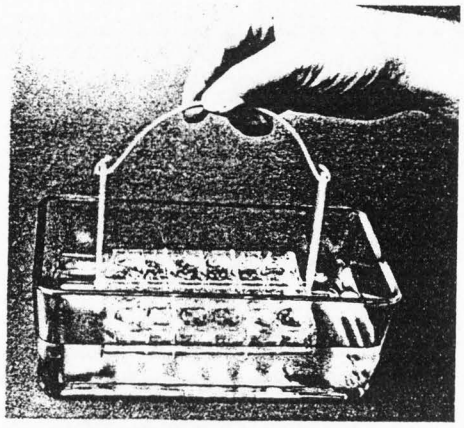

forceps
paper
well-plate
fixative
water bath
2 minutes
let excess water
drip off
= drip dry
drip dry
0.5% Periodic Acid
2 minutes
water bath
2 minutes
Schiff Reagent
Distilled water
8 minutes
drip dry
water bath
3 minutes
Sodium Metabisulfite 0.5%
2 minutes



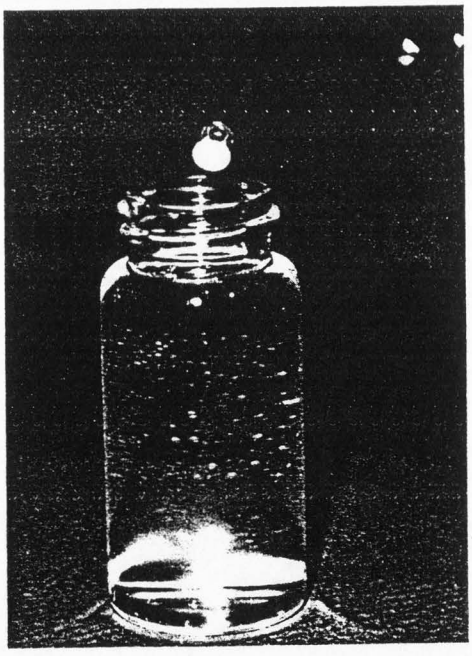
Flow chart used with verbal permission from Dr. Keith P. West, Division of Human Nutrition, Department of International Health, Johns Hopkins School of Public Health.

Appendix K

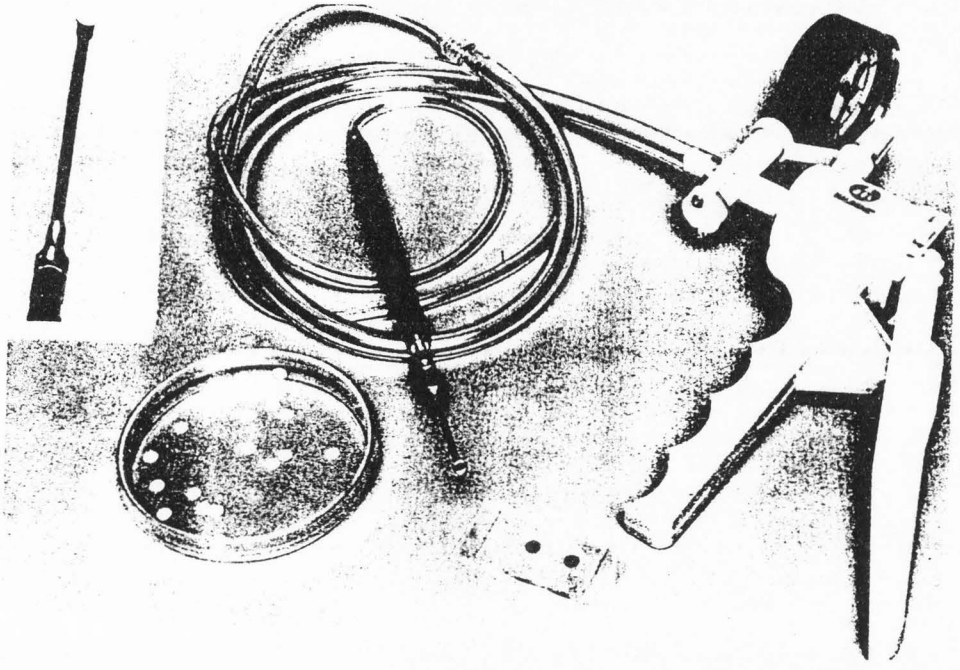
Equipment for CIC Procedure



Well-plate in water bath.



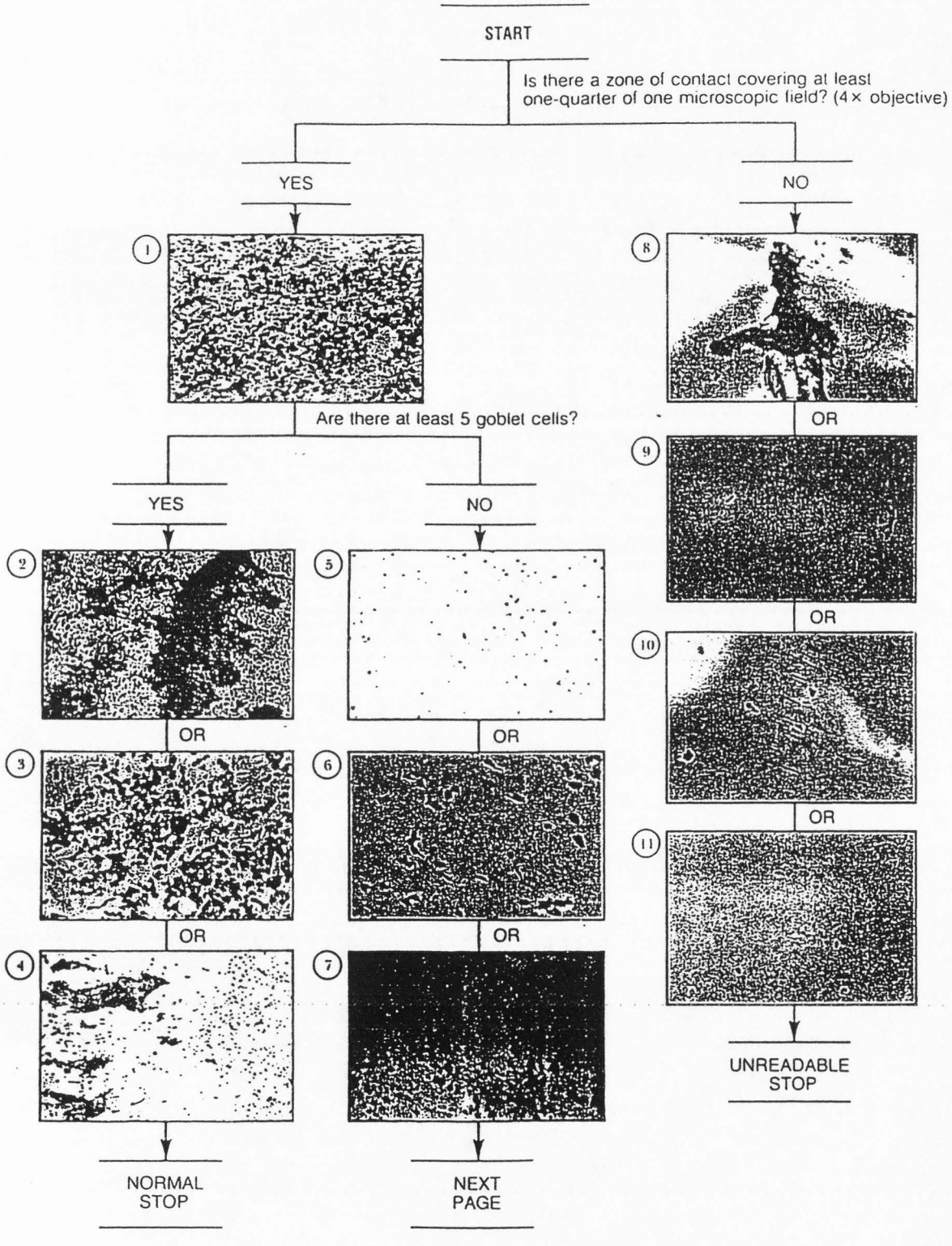
Holding applicator tip with paper disc over open fixative vial, lift finger from by-pass hole to remove suction, allowing disc to drop into vial.

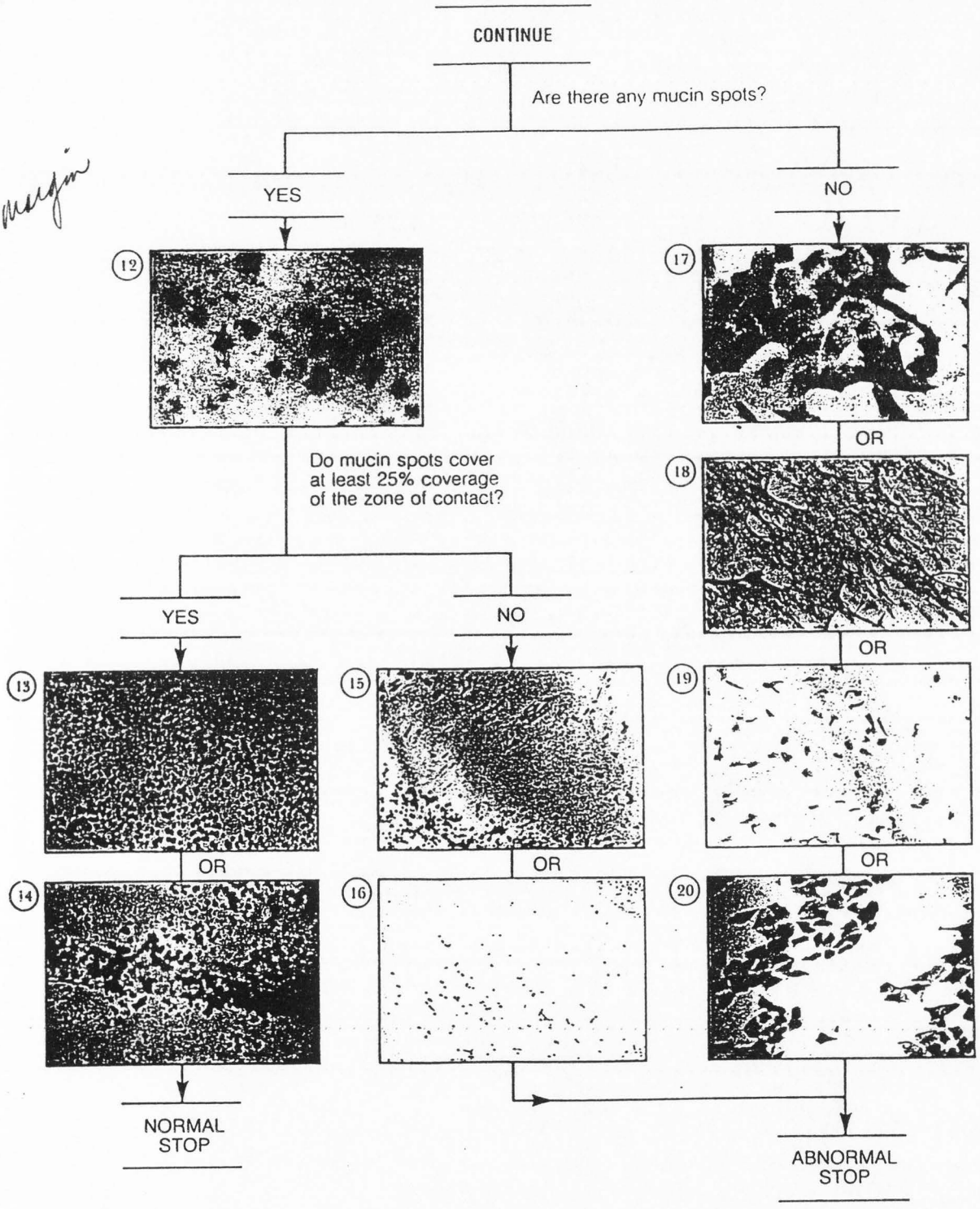


Vacuum pump disc applicator

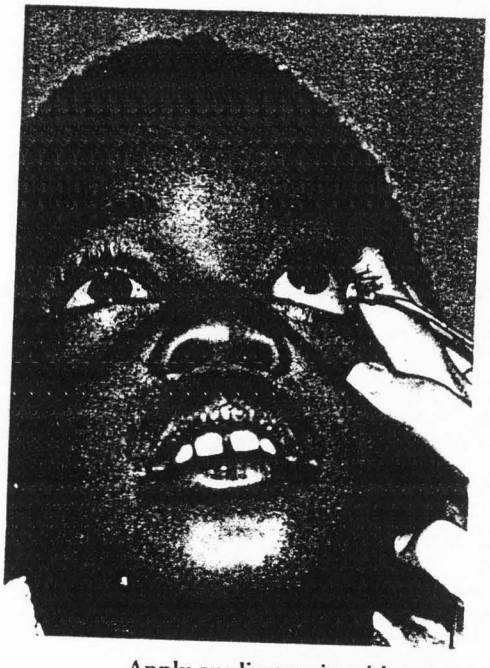
Appendix L

Obtaining Specimens/Reading Slides


START
Is there a zone of contact covering at least one-quarter of one microscopic field? (4× objective)
YES
NO
1
8
Are there at least 5 goblet cells?
OR
9
OR
10
OR
11
YES
NO
2
5
OR
OR
3
6
OR
OR
4
7
UNREADABLE STOP
NORMAL STOP
NEXT PAGE



Photos used with verbal permission from Dr. Keith P. West, Division of Human Nutrition, Department of International Health, Johns Hopkins School of Public Health.



Apply applicator tip with paper disc to the inferior temporal conjunctiva.

Appendix M

Data Printout

SAT %

NO.	CENTER	HEIGHT	WT	AGE	CIC	RBP	VITA	ZINC	HCT%	HCT%	FE++	FER	TIBC	CULATED	RBC	MCV	MCH	MCH	RDW
1	BC	41.0	39.3	5	UNREAD	4.4	70	115	13.6	40.8	195	15.1	329	59.27%	5.14	79.4	26.5	33.4	12.7
2	BC	43.0	42.2	4	NORMAL	6.2	64	112	13.5	39.9	243	21.2	QNS	QNS	5.06	78.8	26.6	33.8	12.5
3	BC	39.8	38.3	4	NORMAL	2.6	45	106	12.6	37.6	96	9.7	243	39.51%	4.67	80.5	27.1	33.6	12.5
4	BC	43.0	44.7	5	NORMAL	5.4	109	140	13.5	38.7	115	14.3	271	42.44%	4.55	88.8	30.9	34.8	11.9
5	BC	42.0	39.5	4	NORMAL	5.1	40	98	11.2	33.4	158	9.5	287	48.08%	3.89	85.7	28.8	33.5	12.6
6	BC	44.5	51.4	4	NORMAL	2.7	69	105	13.1	38.6	109	9.9	269	40.52%	4.66	84.7	28.6	33.8	13.1
7	BC	44.0	52.8	4	NORMAL	4.0	69	86	13.8	41.1	117	14.7	255	45.88%	4.81	85.4	28.7	33.6	12.1
8	BC	42.5	42.9	5	ABN	3.4	90	95	13.5	40.2	119	32.0	233	51.07%	4.67	86.0	28.9	33.5	12.2
9	BC	45.5	47.2	6	NORMAL	3.2	54	86	11.7	34.2	55	58.8	198	27.78%	4.34	78.6	26.9	34.2	12.1
10	BC	41.5	40.2	3	NORMAL	3.6	46	93	12.7	37.5	72	17.2	181	39.78%	4.57	81.9	27.9	34.0	12.4
11	BC	45.5	45.1	5	ABN	5.8	55	84	13.9	41.1	192	15.3	298	66.21%	4.73	86.9	29.4	33.9	13.0
12	BC	37.5	29.9	3	ABN	4.5	11	96	12.5	36.9	102	17.1	220	46.36%	4.36	84.7	28.3	34.0	14.0
13	BC	40.5	33.2	3	NORMAL	3.9	51	86	11.8	34.7	68	33.8	218	31.19%	4.37	79.3	26.9	33.9	13.6
14	BC	42.0	36.9	3	NORMAL	2.8	60	127	13.9	41.1	106	13.5	208	50.96%	5.01	81.9	27.7	33.8	11.7
15	BC	39.3	30.6	2	NORMAL	2.3	82	130	13.3	39.0	54	28.1	144	25.61%	4.48	87.0	29.6	34 0	12.1
16	BC	42.0	58.8	2	NORMAL	3.3	75	111	12.8	37.1	83	31.1	173	47.98%	4.29	86.5	29.9	34.6	12.1
17	BC	36.5	34.9	3	NORMAL	2.5	59	94	12.3	36.9	116	15.0	195	59.49%	4.38	84.2	28.1	33.3	12.4
18	BC	41.5	40.0	5	NORMAL	2.6	65	89	12.4	37.0	114	21.1	179	63.69%	4.31	85.7	28.7	33.4	12.0
19	BC	40.5	43.9	4	NORMAL	4.1	66	101	12.7	37.9	89	6.1	185	48.11%	4.49	84.5	28.3	33.5	12.4
20	BC	44.8	43.5	6	NORMAL	2.3	107	91	12.9	33.0	160	11.5	255	62.75%	4.46	85.4	29.0	33.9	12.3
21	BC	41.0	32.6	5	NORMAL	3.2	91	109	13.3	39.5	146	3.4	246	59.35%	4.46	88.5	29.9	33.8	11.3
22	BC	40.0	34.4	4	NORMAL	2.4	75	98	13.5	39.0	97	52.6	189	51.32%	4.68	83.4	28.9	34.6	12.6
23	BC	40.5	35.4	4	NORMAL	2.3	66	116	13.3	39.7	77	55.6	148	52.03%	4.77	83.1	27.9	33.6	12.8
24	BC	46.5	52.5	5	NORMAL	3.8	86	157	15.5	45.5	158	11.3	204	77.45%	5.74	79.3	27.0	34.0	13.5
25	BC	45.0	40.5	5	NORMAL	2.7	66	97	12.4	37.1	175	7.2	229	76.42%	4.38	84.7	28.4	33.5	12.5
26	ROY	37.5	31.9	3	NORMAL	2.7	59	86	12.8	39.0	58	0.8	241	24.07%	6.1	76.5	25.1	32.9	15.9
27	ROY	44.5	51.5	5	NORMAL	3.0	62	107	12.9	38.7	90	8.2	269	33.46%	4.65	83.3	27.8	33.3	12.3
28	ROY	45.0	76.5	5	NORMAL	4.0	76	93	3.2	58.3	111	47.3	292	38.01%	4.29	89.3	30.8	34.4	13.0
29	ROY	42.5	41.1	4	NORMAL	3.2	53	125	12.1	35.3	96	16.4	254	37.80%	4.34	81.3	27.8	34.2	12.4
30	ROY	44.8	45.9	4	ABN	3.0	73	135	9.0	36.3	62	19.6	238	26.05%	3.19	82.4	28.1	34.1	11.7
31	ROY	39.8	34.6	3	ABN	2.4	44	146	11.3	34.6	21	5.1	190	11.05%	4.3	71.9	23.6	32.7	12.6
32	ROY	38.3	32.4	3	NORMAL	2.4	49	120	11.3	34.7	35	0.8	227	15.42%	5.14	67.4	21.9	32.4	15.4
33	MAN	38.3	30.4	3	NORMAL	2.9	68	126	13.5	41.3	151	14.0	261	57.85%	5.01	82.3	26.9	32.7	12.3
34	MAN	40.8	36.5	3	NORMAL	2.9	84	215	QNS	QNS	259	10.6	370	64.59%	1.98	82.7	27.6	33.3	13.5
35	MAN	39.3	34.7	3	NORMAL	2.5	75	87	12.6	38.8	64	4.4	208	30.77%	4.62	82.1	27.2	33.1	14.2
36	MAN	36.5	31.7	3	NORMAL	2.2	85	93	11.9	35.8	65	28.2	257	26.58%	4.25	84.2	28.0	33.2	12.2
37	MAN	38.8	34.7	4	NORMAL	2.8	110	91	12.9	38.1	58	23.0	192	19.79%	4.51	84.3	28.5	33.8	13.3
38	MAN	41.5	36.5	4	NORMAL	5.8	103	90	13.5	40.1	161	20.7	289	55.71%	4.86	82.4	27.7	33.6	12.8
39	MAN	43.3	53.3	4	NORMAL	3.1	89	91	12.6	37.6	82	20.7	210	39.05%	4.64	81.1	27.1	33.4	13.6
40	MAN	37.8	31.0	4	NORMAL	2.8	61	84	12.8	36.8	132	5.1	243	54.32%	4.75	77.3	25.3	32.7	11.9
41	MAN	40.0	40.3	4	NORMAL	2.7	87	82	13.5	40.3	85	8.9	200	42.50%	5.04	79.9	26.8	33.5	13.2
42	MAN	43.5	64.2	3	NORMAL	3.6	58	88	14.2	41.3	74	15.0	205	36.10%	4.97	83.1	28.5	34.2	12.6
43	MAN	40.8	38.0	4	NORMAL	3.7	120	115	13.6	42.0	111	10.1	342	32.46%	5.21	80.5	26.2	32.5	12.5
44	MAN	40.8	35.0	3	NORMAL	3.4	66	101	13.2	40.3	63	3.0	219	28.77%	4.86	82.8	27.2	32.8	12.2
45	SAL	41.3	40.7	4	ABN	2.6	37	133	12.8	37.9	55	27.5	368	14.95%	4.54	83.5	28.1	33.7	11.8
46	SAL	41.3	40.3	4	NORMAL	3.1	70	145	13.0	38.2	80	36.0	334	23.95%	4.54	84.0	28.6	34.0	12.2
47	SAL	46.0	59.5	5	ABN	3.2	65	161	13.3	38.7	91	11.4	304	29.93%	4.79	80.7	27.7	34.3	13.4
48	SAL	41.3	36.9	5	NORMAL	2.8	76	183	12.8	37.5	89	19.5	401	22.19%	4.42	84.8	28.9	34.0	11.8
49	MAN	39.5	35.8	3	NORMAL	6.6	50	104	13.8	39.7	163	16.5	270	60.37%	4.29	92.5	32.1	34.7	11.4
50	SAL	41.3	35.3	4	NORMAL	2.5	64	147	12.9	38.2	42	32.1	346	12.14%	4.85	78.8	26.6	33.7	13.8
51	SAL	38.3	29.5	3	NORMAL	3.1	57	109	12.4	37.0	64	19.2	337	18.99%	4.51	81.9	27.4	33.4	13.0
52	SC	40.3	31.9	4	NORMAL	2.9	100	91	12.5	36.7	80	23.6	210	38.10%	4.14	88.7	30.3	34.2	11.9
53	BC	42.3	38.0	5	NORMAL	1.6	51	QNS	12.8	37.8	56	24.4	187	19.25%	4.39	86.1	29.2	33.9	12.3
54	BC	43.5	40.2	4	NORMAL	3.1	48	114	13.5	39.2	103	27.1	242	42.56%	4.8	81.7	28.1	34.3	13.4
55	BC	38.3	33.2	3	ABN	3.8	54	101	11.7	35.8	87	14.5	247	35.22%	4.28	81.8	27.5	33.6	13.5
56	BC	37.0	33.0	3	NORMAL	2.4	40	100	9.7	23.5	43	26.3	210	20.48%	2.89	81.3	33.6	41.3	15.2
57	BC	41.0	33.2	4	NORMAL	3.3	79	103	13.2	38.8	109	23.3	265	41.13%	4.67	83.1	28.2	33.9	12.9
58	BC	42.3	43.2	4	NORMAL	2.9	78	116	12.1	35.7	92	26.5	228	40.35%	4.23	84.2	28.6	34.0	12.0
59	ROY	41.0	40.6	3	NORMAL	2.6	45	104	13.7	39.6	105	15.3	235	44.68%	4.66	85.2	29.5	34.6	12.4
60	ROY	35.8	27.0	3	NORMAL	2.4	35	91	11.6	34.7	102	13.6	234	43.59%	4.39	79.0	26.3	33.3	15.1
61	ROY	39.0	28.4	4	NORMAL	3.0	72	122	13.1	37.7	109	23.7	252	43.25%	4.75	79.3	27.5	34.7	11.6
62	ROY	40.0	34.8	3	NORMAL	2.7	56	89	11.7	34.4	111	18.4	256	43.36%	4.25	80.9	27.4	33.8	12.2
63	ROY	42.0	36.3	5	NORMAL	3.1	71	92	12.6	37.3	51	71.3	187	27.27%	4.57	81.6	27.6	33.8	12.2
64	ROY	41.8	38.2	5	NORMAL	3.3	83	91	12.2	35.9	97	10.7	232	41.81%	4.48	80.0	27.3	34.1	12.2
65	ROY	40.5	41.4	3	NORMAL	3.8	51	84	13.0	37.4	72	11.4	215	33.49%	4.93	75.8	26.4	34.8	14.8

Appendix N

Statistics

```
MTB > BOXPLOT C3

                          ------------------------
        ------------------------I          +          I------------------------
                          ------------------------
    ----+----------+----------+----------+----------+----------+---HEIGHT
      36.0       38.0       40.0       42.0       44.0       46.0
```

```
MTB > BOXPLOT C4

                    --------------------
          ----------I      +       I----------    **    *          0
                    --------------------
    -------+----------+----------+----------+----------+----------+WEIGHT
          30         40         50         60         70         80
```

Figure 11. Boxplots for height and weight of all subjects

VITA

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EDUCATION

University of Massachusetts, Amherst	B.S. Dietetics	1974
United States Air Force	Dietetic Internship	1975
University of Southern California	M.S.Ed.	1986
Utah State University	Ph.D.	1995

FOREIGN LANGUAGES

French intermediate reading and speaking
Japanese elementary reading and speaking

PROFESSIONAL EXPERIENCE

1974- present — Lieutenant Colonel, USAF, MacDill AFB, FL
Published Healthy Heart Shopping Guide by the US Government Printing Office. Published Nutrient-Drug Interactions for Air Force Medical Facilities staff education programs. Developed and implemented policies, procedures, budgets, standards of care, and continuous quality improvement programs. Developed and supervise the USAF dietetic externship program for Eastern Kentucky University.

1992- present — Consultant Dietitian, Federal Correctional Institution Manchester, KY
Supervised personnel to provide nutrition support.

1992- present	Assistant Professor, Eastern Kentucky University Richmond, KY Teach nutrition and food service management at the graduate and undergraduate levels.
1991	Consultant Dietitian, Sierra Tucson, Tucson, AZ Dietitian for children and adolescents in recovery from addiction and psychiatric care facility.
1991	Consultant Dietitian, Godfrey's Nursing Home, Brigham City, UT Nutrition care for patients.
1988-91	Consultant Dietitian, CPC Olympus View, Salt Lake City, UT Nutrition care for children and adults in recovery from addiction in a psychiatric care facility.
1988	Clinical Dietitian, VA Medical Center Salt Lake City, UT Managed outreach community nutrition programs. Preceptor for dietetic interns. Supervised dietary activities for patient care.
1987	Woman, Infant, Children Nutritionist, Weber & Morgan Counties, UT Provided nutrition education for women, infant, and child nutrition.
1986	United States Navy Education Counselor, Misawa, Japan Arranged testing, training and apprenticeships. Recommended course of study to obtain advancement through education. Coordinated student programs with four Universities and provided executive support to faculty.
1983	Woman, Infant, Children Nutritionist, Yuba County, CA Instituted a program for distribution of vouchers for women, infants, and children. Developed training materials for client education and taught classes. Trained staff.

1983	Senior Nutrition Program Director, Area 4, Auburn, CA Supervised meals-on-wheels staff at seven sites, wrote menus, ordered equipment, taught nutrition programs.
1979 - 1983	Sierra College, Instructor, Rocklin, CA Taught clinical nutrition and food service management courses.
1979-1983	Private Practice, Sacramento, CA Consultant to Hewelett-Packard Corporation, Take Care Clinics, Sutter Memorial, Sutter General, Fremont Hospitals, New West dialysis clinics and three nursing homes. Wrote and published Renal Diets and Recipes.
1979	Nutrition Education Training Coordinator, Sacramento, CA Wrote curriculum and taught safety, sanitation, nutrition, menu planning, and management. Wrote a training manual for teachers and students as well as handbook for new food service personnel.

PROFESSIONAL AFFILIATIONS

American Dietetic Association 401504
Kentucky Bluegrass Dietetic Association
Bluegrass Dietetic Nomination Committee
American Society for Parenteral and Enteral Nutrition

HONORS AND AWARDS

1986	Air Force Outstanding Officer, Biomedical Science Corps
1987	Japanese-American Cultural Exchange, Aomori Prefecture
1988	Utah State University Nutrition Assistantship
1989	Utah State University Nutrition Fellowship
1991	Commendation Medal, United States Air Force
1992	Phi Upsilon Omicron
1993	Meritorious Service Medal, United States Air Force

DISSERTATION

Nihan, Laura. Conjunctival impression cytology for the assessment of vitamin A status of children of migrant workers. 1995.

THESIS

Butts, Laura Nihan. Interdisciplinary approach to weight loss using behavior modification in USAF weight management program. 1986.