NUTRITION AND ORAL HEALTH IN CAMEROON

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I. INTRODUCTION

Oral disease is a neglected area in the health of populations living in underdeveloped countries. This is attributable to the low priority it receives from the medical sector, the high prevalence and severity of periodontal disease and dental caries and the totally inadequate number of qualified persons to treat this health problem. This is the situation in the Cameroon which has recently initiated a program to improve medical health but has not implemented similar programs for oral health. Due to the severe magnitude of the problem it has to be concluded that the treatment of existing dental decay and periodontal disease would require a large influx of money and personnel which an underdeveloped country such as the Cameroon could not possibly carry out.

In spite of the overwhelming nature of the problem, periodontal disease and dental caries should be treated because they are focal areas of infection. Infection affects metabolism resulting in the breakdown of body protein and the loss of vitamins and minerals through increased excretion. This is of particular significance in malnourished individuals who have poor nutritional status, the consequence of infection resulting in further nutrient loss and possibly death. In addition to metabolic changes, periodontal disease and dental caries ultimately result in loss of teeth. This further aggravates poor nutritional status because of the inability to properly masticate food resulting in the choosing of foods less likely to be of benefit to nutritional status.

Oral disease consists of two components, the local factors such as dental plaque, calculus and food impaction, and a systemic component consisting of the host's resistance to the local factors. The ingestion
of certain foods such as carbohydrates favors the formation of local factors while nutrient deficiency results in a decreased ability of the host to mount a challenge to the local factors. In the Cameroon the reliance on carbohydrates as a primary food source and the deficiency of several nutrients are important factors in the high incidence and severity of oral disease. In addition to nutrient deficiency, another factor is the toxicity arising from cyanide as a result of the ingestion of cassava by the Cameroon population. Hydrocyanic acid is a protoplasmic poison which is liberated in the mouth and could affect periodontal tissue metabolism because of the high degree of permeability of gingival tissues. The relationship of cyanide toxicity to oral disease has not been studied although changes in the oral mucosa have been documented in those subsisting on this tuber.

A problem with the assessment of nutritional status is obtaining rapid, reliable data on blood status of nutrients. This will be accomplished by the use of high pressure liquid chromatography (HPLC) using methods we developed whereby different vitamins can be assayed from a single blood sample. High pressure liquid chromatography has not been used in nutritional surveys and its proposed use in this study will add to our knowledge of fat soluble vitamin deficiency in the Cameroon. Vitamin C status and cyanide toxicity will be assessed by spectrophotometric methods while radioassay techniques will be used to assess vitamin B₁₂, folic acid and iron status. These methods can be applied to rapidly assess the nutritional status of a population and add significant data to the relation of nutrition to systemic and oral health. This will result in the formation of methods to prevent oral and nonoral disease and therefore have a significant impact on the health of the population.
II. BACKGROUND

a) Periodontal Tissue Metabolism and Nutrition

According to the World Health Organization (1961) periodontal disease is a major health problem throughout all areas of the world. Its two major subdivisions are gingivitis, where disease is confined to the gingiva, and periodontitis, where disease is present in the gingiva and the supporting periodontal structures. Approximately 60 to 70 percent of tooth loss in the United States after age 40 is caused by periodontal disease, whereas in India, for example, periodontal disease is responsible for about 80 percent of teeth extracted after age 30 (Mehta et al., 1958). Although exact data is not available, the incidence of periodontal disease including both chronic and acute forms is high in the Cameroon (Personal Communication, G.L. Monekosso).

Although poor oral hygiene and plaque are regarded to be the cause of gingivitis, the correlation with periodontitis is not as clear. It is evident that not all cases of gingivitis lead to periodontitis which implies important differences in host resistance. With the advent of a better understanding of the role of certain nutrients in tissue metabolism and the realization that an optimum intake of nutrients is difficult to attain not only in underdeveloped but also developed countries, it is probable that nutrition plays a far greater role in the etiology of periodontal disease than was previously thought. In the Cameroon, populations with deficiencies of vitamins C, D, the B-complex group, iron and protein have been identified (Masseyeff et al., 1958; Masseyeff et al., 1959). Deficiencies of any of these nutrients could predispose to periodontal disease and be
a factor in the high incidence of oral infection and tooth loss.

The periodontium includes the gingiva, periodontal ligament, alveolar bone and cementum. In addition to the unique anatomical relationship of soft to hard tissue in this part of the mouth, there are several aspects of periodontal tissue metabolism which are basic to an understanding of the interaction of nutrition within this area. First of all, the epithelium lining the gingiva apposed to the tooth is not covered by a keratin layer and therefore is a highly permeable area (Schroeder and Listgarten, 1971). This allows the products of bacterial plaque to gain access to the underlying tissues and as a result this area is in a constant state of stress and would be expected to be affected by nutrient deficiency before other areas of the body in less stressful situations.

A substance of particular toxicity to the periodontal tissues is hydrocyanic acid which is liberated in the mouth from linamarin a cyanogenic glucoside present in cassava (Oke, 1979). Populations ingesting the tuber have a high incidence of angular stomatitis, glossitis and dental caries (Osuntokun et al., 1969). In addition to oral findings, raised serum thiocyanate and depressed vitamin B₁₂ levels (Monekosso and Wilson, 1966) are found. The role of hydrocyanic acid in the etiology of periodontal disease has not been investigated. Given the high permeability of crevicular gingiva and the functions of hydrocyanic acid as a protoplastic poison, it is likely that it plays a far greater role in the initiation and progression of periodontal disease than previously thought.

In addition to its high permeability, the turnover time of crevicular epithelium has been estimated to be between 4 and 10 days (Schroeder and Listgarten, 1971). This is considerably shorter than other epithelia such as the skin, which has a turnover time of 28 days.
(Epstein and Maibach, 1965). On this basis the gingival crevicular epithelium is forming more cells per unit time compared to other epithelia and needs more nutrients. On the other hand, when malnutrition occurs, whether it be marginal or severe, this area would be affected before others with a slower rate of metabolism. This is of particular concern because nutrient deficiency could predispose the periodontal tissues to disease by altering their metabolism.

Periodontal disease, whether acute or chronic, is basically an inflammatory condition resulting in a state of destruction occurring simultaneously with repair. Infection anywhere in the body can have a generalized effect on protein metabolism. As a result of an infectious episode muscle protein is broken down and transported to the liver where it is utilized by the hepatic cells to synthesize glucose with the nitrogen portion excreted as urea (Scrimshaw, 1973). Although the precise mechanism is not understood, this operates through changes in the circulating levels of insulin, glucagon, cortisone and growth hormone (Scrimshaw et al., 1968). The end result is the careful maintenance of blood glucose levels which if allowed to fluctuate would result in the impairment of metabolism in certain tissues such as the brain. An important aspect of this shift is that the restoration of the protein broken down during the infectious episode takes a longer duration of time than the catabolic period. In addition to protein depletion, losses of ascorbic acid, vitamin A and iron occur as serum levels of these nutrients are lowered and increased urinary excretion occurs (Jacobs et al., 1954). Such losses would be expected to become important in a population such as the Cameroon where dietary intake is inadequate.
b) Role of Vitamin and Mineral Deficiencies in Periodontal Disease

Ascorbic acid is of major concern in the etiology of periodontal disease because of its role in the synthesis of collagen, the major structural protein of the periodontium. Man along with guinea pigs and the flying mammals have lost the capacity for its synthesis because of the inability to synthesize the enzyme responsible for converting L-gulonolactone to L-ascorbic acid (Chatterjee et al., 1975). The tissue levels of ascorbic acid are generally higher than those in the plasma, and gingival tissues vary from a fraction of a microgram to over 100 micrograms (Mallek, 1978). The level of the vitamin in this peripheral tissue has been found to correlate with the ascorbic acid content of the diet.

Ascorbic acid has received more attention than any other nutrient in the etiology of periodontal disease. Studies using laboratory animals on ascorbic acid deficient diets have shown increased tooth mobility, gingival hemorrhage and alveolar bone resorption (Glickman, 1948). Gingivitis with enlarged hemorrhagic, bluish-red gingiva is the classic sign of human ascorbic acid deficiency. An important cause of low dietary intake is the prolonged cooking of vegetables which destroys the food content of the vitamin. Besides the development of deficiency as a result of inadequate intake, febrile diseases are known to interfere with ascorbic acid metabolism resulting in lowered serum levels (Scrimshaw, 1964). Urinary output increases and serum levels are lowered in subjects living in climates with high humidity and temperatures (Hindson, 1970). Marked decreases occur during common colds and of
particular significance is the fact that certain medications such as aspirin prevent the transfer of plasma ascorbic acid from the leukocytes to the tissues (Loh et al., 1973). The periodontal tissues are particularly sensitive to the ascorbic acid status and given the potential for deficiency in certain areas of the Cameroon (May 1968) it could be an important factor in the high incidence and severity of periodontal disease.

Ascorbic acid status is assessed by either leukocyte or plasma measurements. Although it is clear that both are poor indicators of the tissue status of the vitamin, the former is more reliable due to the function of leukocytes in the storage of ascorbic acid. Leukocyte ascorbic acid measurements will be carried out to assess vitamin C status using the procedure of Zannoni et al., (1974). This rapid spectrophotometric procedure is highly sensitive and has been found to produce reliable results in survey work (Mallek, 1978).

Vitamin A is involved in a number of functions including vision, reproduction, bone growth and the maintenance of epithelial tissues. The effects on bone and epithelium are of particular interest because these tissues comprise a part of the periodontium. When deficiency is present atrophy of certain epithelial tissues occurs which lowers the barrier to infective agents and as a result diminishes the body's ability to prevent infection (Moore, 1967). According to Wolbach (1942), the salivary glands are initially affected by vitamin A deficiency followed by the respiratory tract, eyes and skin respectively.

Studies of vitamin A status and periodontal disease have been conducted in underdeveloped countries. Although no statistical correlation could be made, vitamin A deficiency as manifested by low serum levels was characteristic of those populations with a
high incidence of periodontal disease (Radusch, 1940). Also, subjects with epithelial lesions such as follicular hyperkeratosis which is characteristic of vitamin A deficiency, have a higher incidence of periodontal disease (Day, 1944). In developing countries vitamin A deficiency is a problem of severe magnitude because the major source of the vitamin is β-carotene from green vegetables and maize, rather than preformed retinol. β-carotenes are converted in the intestinal mucosa to retinal which is reduced by either liver or intestinal mucosal enzymes to retinol, the active form of the vitamin. Absorption of carotene is variable and there are losses in the conversion to retinol so that 1 μg of β-carotene has the biological potency of 0.167 μg of retinol (Marks, 1974). Deficiency is widespread in developing countries because the vitamin A intake is in a form that yields low amounts of retinol and because of seasonal shortages of foods high in carotenes. Although vitamin A deficiency has not been found to occur in the Cameroon (National Nutrition Survey, 1977), this should be further investigated by measuring serum retinol levels. This will be carried out using high pressure liquid chromatography which offers several advantages for assessing vitamin A status. These include rapid methodology, quantitative recovery and extremely high sensitivity compared to traditional technique. Following sample preparation nanogram levels of serum retinol can be measured within a time span of a few minutes.

Vitamin D is important to biological systems utilizing calcium and phosphorus because of its role in regulating uptake and excretion of these minerals. Animal studies have shown periodontal changes during vitamin D deficiency which are consistent with inadequate bone mineralization (Glickman, 1972). Vitamin D deficiency can occur in those countries where food supplementation is not carried out. In
tropical climates the problem could become particularly acute during the rainy season because of reduced exposure to ultraviolet sunlight which is responsible for producing endogenous vitamin D (Shany et al., 1976). The status of this vitamin will be assessed by measuring serum 25 hydroxyvitamin D by means of high pressure liquid chromatography. 25 hydroxyvitamin D is an important metabolite of the vitamin and as with the measurement of vitamin A, rapid and reliable results can be obtained with high pressure liquid chromatography.

Deficiency of thiamine, riboflavin, niacin, folic acid, pyridoxine, vitamin B_12, pantothenic acid and biotin have been shown to cause glossitis, cheilosis, angular stomatitis, inflammation of the buccal mucosa and changes in the periodontal tissues (Schour and Massler, 1945). These signs and symptoms are usually the first to be noticed during the onset of deficiency. Although it is possible that several of these vitamins are deficient in the diet of the Cameroon population, this proposal will study the status of only folic acid and vitamin B_12. Deficiency of these have been shown to cause anemia, a problem which has shown to have a high incidence in the National Nutrition Survey (1978) of the country.

Folic acid deficiency occurs as a result of inadequate dietary intake and when the body's requirement for the vitamin is increased. Although folic acid is widely distributed in foods there is considerable loss during cooking and storage. Conditions associated with increased needs of the vitamin are infancy, adolescence, pregnancy and infection (Herbert, 1959). The epithelial and hematologic tissues are particularly susceptible to folic acid deficiency due to their rapid turnover times. Cellular changes in the buccal mucosa occur in humans during deficiency of the vitamin (Boddington, 1959) and animal studies have shown the development of severe gingivitis
and periodontitis in the absence of dietary folates (Dreizen et al., 1970).

Although vitamin $B_{12}$ deficiency can occur due to lack of intrinsic factor, pregnancy and during malabsorption syndromes (Beck, 1962); deficient dietary intake can occur when meat and eggs are not eaten. Vitamin $B_{12}$ is required for the release of folate from body stores hence the two deficiencies often occur together. Patients with pernicious anemia exhibit a marked pallor of the gingiva, with atrophy of the filiform papillae of the tongue and difficulty with swallowing (Winter, 1940). As with folic acid, the tissues most susceptible to vitamin $B_{12}$ deficiency are those with rapid turnover times such as oral epithelium.

The roles of vitamin $B_{12}$ and folic acid in anemia and oral disease will be assessed by measuring serum levels of these vitamins. These can be assayed together from a single sample of serum using Radioassay techniques (Becton Dickinson SIMULTRAC). Two radioactive tracers, $^{57}$Co for vitamin $B_{12}$ and $^{125}$I for folic acid are measured on a two channel gamma counter. Radioassay methods have for simplified reagent preparations and pipetting, short incubation and counting times and provide an accurate measure of total vitamin $B_{12}$ and serum folates.

Iron deficiency anemia is the most common nutrition disorder, not only in developing but also industrialized countries. The causes include parasitic infections of the gastrointestinal tract, pregnancy, lactation and menstruation (Beaton, 1974). Apart from these factors, considerable iron is lost each day from sweating and exfoliation of cells from the gastrointestinal tract and epidermis. Adding to the problem of iron deficiency is the fact that a number of dietary factors mitigate against sufficient iron intake. These are oxalate, phosphate, phytate and cellulose which interfere with the absorption of vegetable
iron by the process of chelation (Forth and Rummel, 1966). Iron status is likely to be poor in those populations avoiding meat and fish as iron in these foods is more readily available for absorption than iron in vegetables.

Iron deficiency occurs in three stages: pre-latent, latent and iron deficiency anemia. These are characterized by progressive loss of iron from body stores to changes in red blood cells with deficient erythropoiesis. An important consequence of iron depletion is the affect on the activity of iron containing enzymes. The activity of such enzymes as cytochrome oxidase in the buccal mucosa has been shown to correlate with the different stages of iron deficiency (Jacobs, 1961). A decrease in activity of any of several iron containing enzymes could compromise tissue metabolism and lead to a decrease in host resistance. For the periodontal tissues this could be an etiologic factor in disease as this area is in a high state of stress from plaque and could be more susceptible to iron deficiency.

Iron status of the subjects in this study will be determined by radioassay determination of subjects' serum iron. By using this method, it will be possible to determine the stage of iron deficiency and obtain a much more accurate assessment of iron nutriture compared to red blood cell determination. This will provide new information on the role of iron deficiency in anemia and oral disease.
c) Effect of Malnutrition on the Etiology and Progression of Dental Caries.

Dental caries is a multifactorial disease resulting from the interaction of race, climate, soil, water, culture, tradition and food habits. The condition is more prevalent in temperate than warmer climates, is not dependent on genetic differences and is not correlated with the socioeconomic conditions of the population (Bibby, 1968). Dramatic increases in the caries rate have been noted in the Eskimos and Maoris following a change in the dietary pattern that included the increased use of manufactured foods, such as refined sugar and flour products (Dunning, 1965). In Ghana and the Sudan (Jenkins, 1978), a rapid increase in caries occurred in those areas where refined sugars and wheat flours were ingested. Since the rural Cameroon population is basically subsisting on unrefined foods the caries problem would not be expected to be as severe as in the metropolitan areas of Younde and Douala. It is probable that as the dietary patterns become more westernized in the Cameroon the incidence and severity of dental decay will become more severe, and if the problem is to be alleviated it is important that the factors causing this disease be understood.

Of particular interest in the change of caries activity in underdeveloped areas, is the variation in dietary mineral intake. The dietary calcium-phosphorus ratio of caries inactive and caries active Eskimos have shown important differences (Kreitzman, 1976). In the latter group the ratio is around 0.5 while in the former it is closer to 1. Phosphate supplemented foods have been shown to result in caries reduction in both human and animal studies. This data with Eskimos seems to indicate that a difference in mineral intake is as
important an etiologic factor in caries as an increased intake of refined sugar. By investigating the role of diet in caries, methods can be found to predict those segments of a population which might develop the disease as a result of increased consumption of refined foods.

Minerals have been categorized as to their role in caries as follows (Navia, 1972): Fluoride and Phosphate are cariostatic; Molybdenum, Vanadium, Copper, Strontium, Boron, Lithium and Gold are mildly cariostatic, while Selenium, Magnesium, Cadmium, Platinum, Lead and Silicon are caries promoting. Studies of the trace element composition of plants in New Zealand (Cadell, 1964) showed a low caries rate in those areas of the country with high plant levels of Phosphate, Copper, Molybdenum, Calcium, Magnesium, Barium, Strontium and Aluminum. On the other hand, high caries was associated with increased levels of Iron, Manganese and Potassium. Studies of caries-immune naval recruits in the U.S.A. (Losee and Adkins, 1969) revealed their origin from areas with unusually high drinking water levels of Strontium, Molybdenum and Lithium compared to those individuals with a high caries rate. Barmes (1940), in a study of New Guinean natives, found Zirconium and Boron to be protective against caries, whereas Selenium, Beryllium, Barium and Lead bore a relationship to increased caries. A strikingly lower dental caries prevalence was found in Heliconia, Columbia S.A. compared to the neighboring town of Don Matias (Rothman, et al, 1972). The two communities were similar in population size, and dietary surveys revealed a higher consumption of dairy products in Don Matias while Heliconia had a higher consumption of sugar cane. Analyses of
fluoride drinking water levels revealed concentrations of less than 0.1 ppm for the two villages. There was, however, a difference in soil levels of certain trace elements, with Chromium, Copper, Nickel, Silver, Calcium, Magnesium and Strontium higher in Heliconia while Aluminum and Manganese were higher in Don Matias. The largest difference was for Copper with a median of 20 ppm in Heliconia and a median of 0 ppm in Don Matias. Drinking water samples revealed high levels of Calcium and Magnesium from Heliconia. This data is interesting because it points to an effect of many trace elements in the etiology of caries and explains differences in the incidence and severity of the disease in populations.

The beneficial effects of fluoride in drinking water are well known. When the level of fluoride is 1 mg per liter (1 ppm) the caries incidence decreases approximately 50% compared to areas where only 0.2 mg per liter of fluoride is present (Dean, 1942). Further slight decreases in caries occurs with levels of 2 mg per liter but there also occurs an increase in mottled enamel. It is generally agreed that the greater benefit from fluoride is when ingestion occurs during tooth formation. Most natural waters contain some fluoride, the concentration depending on the fluoride containing minerals with which the water is in contact. The level of the trace element is generally greater in water from wells compared to lakes (Shaw, 1978). In the former, the content is dependent upon the depth of the well, length of contact between the water and soil, and the fluoride content of the soil and rock strata. Although a fluoride level of 1-2 ppm produces optimum caries reduction, in certain countries lower levels may prove just as beneficial. This is due to differences in dietary intake of fluorides, especially in societies ingesting large amounts of
Not only is fluoride important during the period of tooth development but deficiency of other trace elements and vitamins can lead to an increase in dental decay. Tooth formation starts during the sixth week of fetal life and continues through the teenage years at which time the third molars are formed. A defect in enamel of deciduous teeth known as linear hypoplasia is a common finding in malnourished populations. This defect usually coincides with the area of the tooth under development just at, or shortly after birth and has been implicated in the high caries rate of children. Enwonwu (1973) found a 21% incidence of hypoplastic defects in the deciduous tooth enamel of children living in village areas of Nigeria. Seventy-two percent of the hypoplasia was in areas of the tooth developed prenatally or just about the time of birth. Although all minerals and vitamins are necessary for the formation of teeth, studies with animals have shown a high correlation of linear hypoplasia with vitamin D deficiency. In Great Britain (Mellanby, 1936) the incidence of deciduous tooth enamel hypoplasia correlated with the economic status of the children and since vitamin status in such populations is marginal, this certainly implicates nutrition as an important factor in the etiology of this defect.

The eruption times of deciduous teeth have been used as a method to determine the age of children in underdeveloped countries. This can be calculated by simply counting the number of erupted teeth and adding six to the total, thereby yielding the child's age in months (Jelliffe and Jelliffe, 1973). Although a number of factors such as hormonal, sex differences and infection affect eruption times,
severely malnourished children usually have retarded eruption times for deciduous teeth. Enwonwu (1972) found 84% of a middle class group of urban Nigerian children to have erupted central incisors while only 15% of children in the same age group, but from outlying villages presented with erupted incisors. Studies conducted of Nigerian children admitted to hospitals in Lagos Nigeria also revealed a significant delay in eruption times compared to healthy children. Eruption times are important in surveys to determine age, and although the relationship to caries susceptibility is not known, it can be used as a method to assess malnutrition in children.

The formation and maturation of teeth is intrinsically related to maternal nutrient intake. Animal studies have shown delayed eruption, reduction in size of molars and incisors, missing cusps, small mandibles and increased caries susceptibility in the teeth of offspring born to pregnant and lactating animals with protein deficiency (Moller, 1967). The formation of teeth appears to be very sensitive to protein status and although this is not a problem in developed countries, it can be assumed that deficiency of this nutrient is a factor in the etiology of caries in underdeveloped countries. In addition to protein, the offspring of rats fed high sucrose diets during pregnancy and lactation have an increased caries susceptibility. Although comparable information from human studies is not available, a relationship between high sugar intake in the early years of life and caries of the deciduous dentition has been established (Sognnaes and Shaw, 1954). Therefore populations with marginal protein intake and high carbohydrate consumption may be more prone to dental caries as a result of a dentition with increased susceptibility to the effects of oral bacteria.
This proposal will study the effect of dietary trace elements, carbohydrates and protein on the incidence and severity of dental caries in different age groups. In addition, the effect of trace elements on the gross morphology, micromorphology and ultrastructure of teeth will be investigated by means of light and electron microscopy. This will add new information concerning the role of nutrition in dental caries and lead to improved oral health in the Cameroon.
d. Nutrition Background and Health Care System

Major food crops grown in various parts of Cameroon include millet, sorghum, corn, cassava, rice, sweet potatoes, yams, taro, plantains, beans, groundnuts and green leaves (May, 1968). In addition to these crops which are grown primarily for domestic consumption, cocoa, coffee, cotton, bananas and palm oil are grown as cash crops. Pigs and poultry are raised in the south while cattle, sheep, goats, horses and donkeys are raised in the central Adamaoua plateau. Fishing is carried out in the areas of Kribi and Daoula and fish consumption is not an important protein source. The internal transport system of the Cameroon is generally inadequate, making it difficult to transport foods from areas of high to low production (May, 1968).

The French Office de la Recherche Scientifique et Technique Outre-Mer carried out nutrition surveys in different parts of the country (Masseyeff, et al, 1959). In the Far Northern Sudano-Sahelian area the population is constantly threatened by drought. Although a wide variety of vegetables and animal foods are eaten, during some years the intake of nutrients can be deficient. Calcium, vitamin A and vitamin C were found to be quite low. Although animal protein intake is inadequate, the total protein intake is adequate throughout the year.

Batouri is located at the northern limit of the equatorial forest and is bordered by areas of the country with different diets. These are the savanna where manioc is the basic food, the intermediate zone where corn is important and the forest where the basic food is plantain (May, 1968). Animal protein is procured in the savanna and forest by means of hunting wild game. The diet was found to be very low in
calories and protein in addition to being deficient in all other nutrients except possibly vitamin C due to the consumption of leaves and vegetables (Masseyeff et al., 1958).

The southern forest zone includes Younede. This area which is warm and humid throughout the year receives an abundant supply of rain. The diet includes many different vegetables and fruits with the basic foods including many roots and tubers with heavy consumption of manioc, plantains and bananas. Animal protein is either acquired as beef in the marketplace or from goat's meat and pork from animals grown at home. The palm tree provides sap from which a popular wine is brewed, while condensed milk is a favorite beverage of upper class women and children. Although there are a great variety of different foods available for consumption, the people of this area have deficiency of all nutrients except niacin and ascorbic acid.

In 1977 a national nutrition survey was undertaken by the Cameroon government with the assistance of the University of California at Los Angeles and the United States Agency for International Development (National Nutrition Survey, 1978). The survey sample comprised children, mothers and households from five provinces. While the incidence of acute undernutrition was only 1.0%, chronic undernutrition was found to have an incidence of 22.1%. 38.1% of children aged 6-59 months were found to be anemic as defined by WHO criteria. Measles was widespread and a positive relationship between undernutrition and historical measles mortality of young siblings in the same family was found.

Analysis of food intake in various regions revealed a low availability of dark green leafy vegetables in all areas. With the exception of Younede and Daoula, availability of fruits, meat, fish and eggs was low. Beans, seeds and nuts were consumed by a high number of all
families except those living in the Northern area of the country. In addition to diet, other factors related to undernutrition were economic status, overcrowding, illiteracy, inadequate child care, illness and poor utilization of health services. Although there are many factors that should be considered it is evident that there exists the potential for deficit of several nutrients which would explain the high incidence of chronic undernutrition, anemia, and mortality from infectious disease.

Tubers, including cassava, macabo, taro, plantains and bananas are a major staple for all families except those living in the Northern province. The presence of salivary hydrocyanic acid levels was tested by the starch filter paper test. Highest positive values of 74.3% were found in the East province, followed by 57.9% in the Central South and 48.4% in the Littoral province. Lowest incidence of positive tests were found in Daoula. This test indicates the incidence of recent cassava consumption and reveals the presence of hydrocyanic acid in the oral cavity. Since it is a potent protoplastic poison, hydrocyanic acid could be a factor in the etiology of periodontal disease and dental caries.

The training of medical personnel and various auxiliaries in the health professions is carried out at the University Center for Health Sciences (UCHS) at Younde. UCHS trains physicians and other health workers through a curriculum that includes the basic sciences, clinical sciences and public health (Monekosso, 1972). The objectives of such a system is to train medical personnel who would be able to deal with the health problems of rural areas. A survey of Dentists made in 1973 (Cameroon Health Sector Assessment, 1975) revealed eight (8) practitioners and plans to train ten (10) additional dentists per year.
The scarcity of Dentists, the high incidence and severity of periodontal disease and dental caries, the poor state of nutrition and the high proportion of the population living in rural areas indicates that the plans of the Cameroonian government to deal with oral health are inadequate. To effectively deal with this problem a comprehensive prevention program should be put into effect. This should be based upon knowledge concerning the incidence and severity of oral disease, the role of nutrition in these diseases and an evaluation of how different health workers could implement such a program.

Oral disease can be prevented by the control of local factors such as plaque and materia alba deposits on the teeth and by adequate intake of nutrients for proper oral tissue metabolism. Providing dental services in the form of reparative services would be entirely out of line with the limited resources of the Cameroonian government. Rather, paramedical personnel who regularly visit rural areas could be taught how to educate the population at the village level on the importance of nutrition to oral health. In addition, control of local factors through toothbrushing and flossing would be essential to such a program. Health workers could be trained concerning the better utilization of methods such as sticks currently in use by the population to clean their teeth. The use of such unconventional ways to remove dental plaque can be improved if the population knows where deposits frequently occur on the teeth and their frequency of occurrence. Improved oral health means a reduction in infection, better ability to masticate food and an improvement in the health of the population.
Cassava is one of many plants which yields hydrocyanic acid. This substance is found in linamarin, a cyanogenetic glucoside which on hydrolysis yields glucose, acetone and hydrocyanic acid (Oke, 1968). This can be liberated from the glucoside by the action of linamarase, an enzyme found in cassava, or by acidic conditions (Nemoto, 1945). The hydrocyanic acid content of the tuber has been found to vary depending upon whether the inner aspect or the rind has been measured (Oke, 1969). In addition, processing into flour, the duration of cooking, the time of harvest, and climactic conditions during the growing season have been found to affect the level of the glucoside.

The principal source of hydrocyanic acid in the Cameroon diet is cassava which accounts for the largest amount of carbohydrate ingested. The incidence of tuber consumption for children aged 6-11 months is 31% in the Central South province and 21% in the South West Littoral province and Younde/Daoula areas. A higher incidence of 66% and 61% was found in the East and South provinces respectively for individuals aged 12-23 (National Nutrition Survey, 1978). Using starch filter as an index of cassava consumption, positive results ranged from 22% of the mothers in Daoula and 74.3% of the mothers in the East province. This test indicates the presence of salivary hydrocyanic acid arising from the consumption of cassava or other foods with cyanogenetic glycosides.

In Nigeria where cassava is the principal dietary staple, the condition of ataxic neuropathy is common. These individuals have an optic atrophy, nerve deafness and sensory spinal ataxia. Raised
plasma thiocyanate and total serum vitamin B₁₂ levels are found (Monekosso and Wilson, 1966) and a clinical improvement is seen when non-cyanogenic foods are ingested. In addition, angular stomatitis, glossitis and other mucocutaneous lesions have been associated with this condition. (Osuntokun et al., 1969). These studies point to a role of cyanide intoxication from cassava as the principal factor in this disease.

The presence of hydrocyanic acid in the tuber could come about as a result of the action of the enzyme linamarase on the glycoside. This could occur during physical disruption bringing the enzyme into contact with its substrate (Montgomery, 1969) and result in the availability of hydrocyanic acid in the mouth when cassava is eaten. In addition, another source of hydrocyanic acid could be the hydrolysis of glucosides as a result of a lowered PH in the mouth. Free cyanide in the tissues is converted to thiocyanate by the enzyme rhodanase. Also, hydroxycobalamin takes up cyanide as cyanocobalamin and the thiocyanate radicle may be transferred in the plasma by cobalamin (Makene and Wilson, 1972). Although thiocyanate is present in the blood of most individuals, this is raised to as high as 11.9±1.3μm moles/1.0 ml in those with ataxic neuropathy (Osuntokun et al., 1969).

The role of hydrocyanic acid in the etiology of periodontal disease has not been investigated. Based upon the highly toxic nature of this substance, its presence in the oral cavity following cassava ingestion and the high degree of gingival tissue permeability it could adversely affect oral tissue metabolism and be a causative factor in the etiology of periodontal disease. This will be
investigated by measuring plasma cyanide and salivary hydrocyanic acid levels by the method of Feldstein and Klendshoj, 1957. Levels of cyanide will be correlated with incidence of tuber consumption and the incidence and severity of periodontal disease and dental caries.
III. OBJECTIVES:

The research described in this proposal will provide basic information concerning the incidence of periodontal disease and dental caries in the Cameroon and the role of nutrition in the etiology of these diseases. By assessing the systemic nutritional status new data will be added concerning vitamin and mineral deficiencies in the population, while studies of cyanide status will deepen our understanding of the role of toxicity in oral disease. This proposal will provide the Cameroon with new information on how to deal with oral disease and contribute to a reduction in mortality.

The specific studies in this research program can be divided as follows:

a) Measure the dietary intake and serum levels of vitamins A, D, C, B₁₂, folic acid, and the mineral iron and correlate these with the Gingival Index (Loe and Silness, 1967) and Inflammation Index of gingival tissues and the Diseased, Missing and Filled Score (DMF) of teeth.

b) Measure the salivary hydrocyanic acid and blood thiocyanate levels and correlate these with the Gingival Index, Inflammation Index and DMF Score and dietary cassava intake.

c) Measure the enamel levels of trace elements and correlate these with the ultrastructure and micromorphology of teeth and the DMF Score.

d) On the basis of the survey data, provide a oral health prevention program which can be administered through paramedical personnel to the Cameroon people in both rural and urban areas of the country.
IV. METHODS OF PROCEDURE

a) Patient Recruitment

Patients will be recruited from the Central South and Littoral provinces. From each province three villages and the urban areas of Yaounde and Daoula will be studied. Rural areas will be chosen following a preliminary visit to the country and will be representative of the dietary intake, medical health and dental conditions of the province. In order to carry out analysis of nutrients rural areas must have facilities to conduct blood analysis or be within a few hours transportation time to such an area. Subjects from urban areas of Yaounde and Daoula will be from poor and high socio-economic status. Participants will be classified into age groups of five year intervals according to World Health Organization criteria (1977) as follows: 1-5, 6-10, 11-15, 16-20, 21-25, 26-30, 31-35, 36-40, 41-45, 46-50, 51-55, 56-60, 60+. Twenty individuals will comprise each age group for a total of 780 rural participants and 260 urban participants per province.

All participants will be recruited on the basis of a need for diagnostic services or dental treatment. Oral exams, dietary histories, blood samples and a gingival biopsy will be taken before dental treatment. The objectives of the study will be explained to the patient as well as the results of the oral exam.

b) Oral Examination and Dietary Surveys

Oral health status will be measured using the combined oral health and treatment assessment form of the World Health Organization (1977). This allows a measure of the utilization of existing dental services,
consequences of tooth loss with respect to the need for full and partial dentures, the periodontal and caries status of teeth.

Dietary assessment will be carried out using methods similar to those used by the National Nutrition Survey of the Cameroon (1978). Patients will be questioned as to the ingestion of a particular type of food the day before the interview. Data will be quantified as to percentage of individuals eating a specific food group per site. This data will be correlated with the blood analysis to determine relationships of nutrition to systemic nutrient status.

c) Assessment of the Role of Nutrition in Caries

From each site extractions will be carried out with local anesthesia from individuals who have pain as a result of caries or periodontal disease. Following cleaning, teeth will be stored and along with samples of drinking water and soil where crops are grown, will be analyzed for trace elements by spark source mass spectrometry using organic bovine femur shaft as a reference standard (Curzon and Losee, 1977). This will be carried out at the Louisiana State University Dental School to investigate the enamel levels of the following trace elements: lithium, beryllium, boron, aluminum, fluorine, sulfur, potassium, titanium, vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, selenium, bromine, rubidium, strontium, niobium, molybdenum, cadmium, zirconium, silver, tin, antimony, iodine, barium and lead. Only teeth from the maxillary arch will be used and a total of at least ten teeth from each age group per site will be measured. Levels of trace elements will be correlated with the DMF scores, basic morphologic cusp pattern, micromorphology
and ultrastructure as studied with the light and electromicroscope. This will provide new information relating trace element concentration in human enamel, soil and water to caries incidence.

d) Assessment of Nutritional Status

To assess the relationship of nutrition to oral health 10cc of blood will be drawn by venipuncture from 20 subjects of different age groups. This will be analyzed for serum levels of retinol, vitamin B₁₂, folic acid, vitamin D₃, white blood cell ascorbic acid, total iron binding capacity, serum iron and cyanide. The results will be correlated with the incidence of periodontal disease and dental caries as defined by W.H.O. criteria (1977).

Two cubic centimeters of whole blood will be taken for analysis of serum retinol and vitamin D₃. After clotting, the sample is centrifuged and to .10 ml of serum, .05 ml ethyl alcohol and .30 ml diethyl ether are added (McLaren et al., 1969). This is vortexed for 1 minute, centrifuged and the supernatant transferred to another test tube. Following evaporation under nitrogen at room temperature, the residue is dissolved in .20 ml methanol and .10 ul volumes injected into the chromatograph (Waters Associates ALC/GPC-204 equipped with a Model 6000A solvent delivery system and fixed ultraviolet monitor) for measurement of serum retinol at 313nm and vitamin D₃ at 263nm. This system uses a uBondapak C₁₈ (4mm i.d. x 30 cm) column with methanol as solvent in reverse phase high pressure liquid chromatography and a flow rate of 0.9 ml per minute. Standards are all trans-retinol and cholecalciferol made up in methanol.

White blood cell ascorbic acid is determined on 1 ml of blood using heparin as an anticoagulant. To the sample is added 0.1 ml isotonic methylcellulose (2%) which is allowed to set for 20 minutes
(Marchand and Pelletier, 1977). The supernatant containing the leukocytes is transferred to another test tube and 2.0 ml of 0.9% NaCl is added. Following gentle inversion of the test tube a 0.5 ml aliquot is taken for counting leukocytes by a Coulter Counter (Model S, Coulter Electronics). Following centrifuging (International Centrifuge, Model UV, with a no. 240 head) for 10 minutes the pellet is homogenized for two minutes in 0.6 ml 40% trichloracetic acid. This is transferred to a microcuvette and 0.04 ml orthophosphoric acid (85%), 0.32 ml α,α-Dipyridyl (1%) and 0.04 ml ferric chloride (3%) are added according to Zannoni et al., 1974. Following vortexing the sample is read at 525 nm in a spectrophotometer (Gilford, Model 300-N, equipped with a model 530 sampler and a model 4008 data lister).

Total vitamin B₁₂ and folic acid will be measured simultaneously using Radioassay (Simultrac, Becton Dickinson, Co.). Specimens are collected in a 5.0 ml B-D Vacutainer Brand evacuated glass tube and centrifuged at an RCF of 850-1000 xg. Following addition of reagents to standards and samples, tubes are incubated in a water bath. After cooling these are measured on a two channel gamma counter to determine nanogram levels of serum folate and picogram levels of total vitamin B₁₂. Radioassay (Simultrac, Becton Dickinson) will also be used to assess iron status. Samples will be measured on a two channel gamma counter to determine serum iron and total iron binding capacity.

Plasma cyanide status will be determined according to the method of Feldstein and Klendshoj (1957) using Conway microdiffusion dishes. Standards and samples are measured spectrophotometrically (Gilford, Model 300-N) at 580 nm and results are expressed per milliliter of
blood (Sunshine, 1972). Salivary samples will be taken by aspiration of the parotid duct and the level of cyanide measured according to Feldstein and Klendshoj (1957). This data will be correlated with food intake and oral health status.

e. Histology

Gingival biopsies will be placed in formalin, sectioned and stained with hematoxylin and eosin at the University of Detroit Dental School. From each section the state of inflammation will be measured by calculating the ratio of inflamed to noninflamed tissue by means of a planimeter.

f. Statistics

Correlation coefficients will be determined for all blood nutrient determinations and the incidence and severity of periodontal disease and dental caries. Correlation coefficients for trace element levels and dental caries will also be carried out. Critical values for these will be determined in addition to student "t" tests and analysis of variance.
V. FUNDING:

The costs of instruments are contingent upon the presence of these within the country and their availability for use in this proposal.

The following instruments will be required:

(1) High Pressure Liquid Chromatograph - (Waters Asso. Model ALC/GPC-204, with Model 6000A solvent delivery system and ultraviolet monitor) - $13,000

(1) Gilford microsample spectrophotometer, Model 300 N, equipped with a model 530 sampler and model 4008 data lister - $3500.00

(1) Solid crystal scintillation counter suitable for measuring two samples simultaneously - $10,000

(1) Portable centrifuge - $300.00
(1) Vortex mixer - $95.00
(2) Automatic pipettors - $198.00
(2) Automatic stirrers - $150.00
(2) Hamilton syringes - $20.00
(2) Sets of microcuvettes - $600.00

Assorted glassware including test tubes - $700.00

The following reagents will be needed:

6 gm All-trans retinol - $330.00
5 gm Vitamin D₃ - $97.50
50 gm Ascorbic acid - $6.00
50 liters Methanol (HPLC grade) - $77.50
4 liters Diethyl ether - $45.00
The following reagents will be needed (cont'd):

4 liters Ethanol - $20.00
10 liters Distilled water - $10.00
30 gm α-Dipyridyl - $65.00
100 gm Ferric chloride - $8.00
500 gm Trichloracetic acid - $20.00
1 liter o-Phosphoric acid - $14.00
20 Radioassay kits (Becton Dickinson Co.) for analysis of vitamin B₁₂, folic acid, iron - $2000.00

Dental instruments and supplies include:

(2) Sets of extraction forceps - $300.00
(4) Dental syringes - $40.00
(4) Needle holders - $30.00
(4) Instrument trays - $40.00
(4) Elevators - $30.00
(2) Sterilizing tubs - $20.00
(4) Bib chains - $6.00
(4) Boxes dental napkins - $40.00
(6) Boxes 2x2 gauze - $75.00
(10) Boxes silk sutures - $50.00
(2) Boxes disposable needles - $20.00
(8) Containers local anesthetic - $35.00
(15) Liters cold sterilizing solution - $150.00

Travel, room and board expenses for two dentists and 2 technicians for 3 months = $8000.00

Total projected cost = $40,092.00
VI. REFERENCES


Cameroon Health Sector Assessment, Africa Bureau, State Department, 1975.


Simultrac Radioassay Kit Vitamin B12 (57Co) and Folate (125I), Becton Dickinson Co., 1979.


DATE OF BIRTH: December 28, 1947
MARITAL STATUS: Married

EDUCATION:
1965-1969 St. Anselm's College, B.A. Degree
1969-1973 Tufts University School of Dental Medicine, D.M.D. Degree
1973-1975 Tufts University School of Graduate Dentistry, Certificate in Periodontology

EXPERIENCE:
1972 Dental Intern, State School For Retarded Children, Laconia, New Hampshire. Treated patients, conducted plaque control program and toothpaste abrasivity study.
1972 Member, Oral Cancer Screening Program, Tufts University School of Dental Medicine. Carried out head and neck examinations of elderly and underprivileged groups at various neighborhood dental clinics, old age homes.
1974-1975 Clinical Instructor in Periodontology, Tufts University School of Dental Medicine.
1974-1975 Assisted in experimentation involving gingival connective tissues and effects of diabetes on progression of gingivitis at Tufts University School of Dental Medicine.
1978 Teaching Assistant in Department of Nutrition and Food Science, Massachusetts Institute of Technology in course entitled "Physiological and Nutritional Biochemistry."
1978 Assistant Professor and Head of Research, Department of Periodontology, University of Detroit School of Dentistry.
CONTINUING EDUCATION COURSES:


SOCIETY MEMBERSHIPS:

American Dental Association; International Association for Dental Research; American Academy of Periodontology (Board-eligible, have completed Pt. I of qualification examination for board certification); American Association of Dental Schools.

PUBLICATIONS:


BOOKS:

**BIOGRAPHICAL SKETCH**

(Give the following information for all professional personnel listed on page 3, beginning with the Principal Investigator.
Use continuation pages and follow the same general format for each person.)

**NAME**

Tetsuo Nakamoto

**TITLE**

Assistant Prof. of Physiology

**BIRTHDATE (Mo, Da, Yr.)**


**PLACE OF BIRTH (City, State, Country)**


**PRESENT NATIONALITY (If non-U.S. citizen, indicate kind of visa and expiration date)**

Japanese (Permanent Visa)

**SEX**

Male

**EDUCATION (Begin with baccalaureate training and include postdoctoral)**

<table>
<thead>
<tr>
<th>INSTITUTION AND LOCATION</th>
<th>DEGREE</th>
<th>YEAR CONFERRED</th>
<th>SCIENTIFIC FIELD</th>
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<tr>
<td>Nihon University, Tokyo, Japan</td>
<td>D.B.S.</td>
<td>1964</td>
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<tr>
<td>Nihon University School of Dentistry</td>
<td></td>
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<td>University of Michigan, Ann Arbor, MI</td>
<td>M.S.</td>
<td>1966</td>
<td>Prosthodontics</td>
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<tr>
<td>University of N. Dakota, Grand Forks, ND</td>
<td>M.S.</td>
<td>1969</td>
<td>Physiology</td>
</tr>
<tr>
<td>University of Michigan, Ann Arbor, MI</td>
<td>M.S.</td>
<td>1971</td>
<td>Physiology</td>
</tr>
</tbody>
</table>

**HONORS**

Merk, Sharp and Dohme Fellowship, Summer, 1967
Lilly Research Laboratories Fellowship, Summer, 1968
National Institute of Dental Research Post-Doctoral Fellowship, 1969-1978

**MAJOR RESEARCH INTEREST**

Growth and development of bones and tooth germs, neonatal malnutrition, protein-deficiency, collagen synthesis

**ROLE IN PROPOSED PROJECT**

Co-investigator

**RESEARCH SUPPORT (See instructions)**

**RESEARCH AND/OR PROFESSIONAL EXPERIENCE (Starting with present position, list training and experience relevant to area of project. List all or most representative publications. Do not exceed 3 pages for each individual.)**

9/67 - 5/68 Teaching Assistant in physiology, University of N. Dakota, Grand Forks, ND
9/68 - 2/69 Research Assistant in physiology, University of N. Dakota
9/69 - 6/72 National Institute of Dental Research Post-Doctoral Fellowship, U. of Michigan
1/71 - 6/72 Teaching Fellow in physiology, University of Michigan, Ann Arbor, MI
9/72 - 6/78 National Institute of Dental Research, Post Doct. Fellowship, MIT, Cambridge
1/76 - 5/76 Teaching Assistant in Nutritional Biochemistry and Metabolism, MIT, Cambridge
10/78 - present: Asst. Prof. of Physiology, School of Medicine, Louisiana State University Medical Center, New Orleans, Louisiana

**Professional Affiliations and Memberships**

International Association for Dental Research
American Association for Dental Research
Sigma Xi
Society for Nutrition Education
American Association for the Advancement of Science
American Association of Dental Schools

**Medical Certification**

Japanese National Dental Board Examination, 1964
National Board Dental Examiners, Part I and II, 1977

**Publications**

(please see attached list)


