LITERATURE SURVEY

NUTRITIONAL ASSISTANT

The first step in the nutritional care process is the assessment of the patient's current status. Nutritional status expresses the degree to which physiologic needs for nutrients are being met (Mahen and Escott-Stump 1996). Studies indicate that upon admission to acute care facilities, 33 to 65% of all patients demonstrate some degree of malnutrition (Mahen and Escott-Stump 1996). Furthermore, in patients who are hospitalized for longer than 2 weeks, nutritional status deteriorates for reasons not identified (Mahen and Escott-Stump 1996). Current methods for nutritional status assessment include anthropometric, biochemical, dietary and clinical evaluation. At this time there is no single test that provides both an accurate and reliable measure of nutritional status. For the purpose of this research, the focus of discussion is on anthropometric and biochemical evaluation.

Anthropometry is the science dealing with measurement of size, weight and proportion of the human body. A variety of anthropometric methods have been developed to measure body composition. Anthropometric measurements can be used to estimate subcutaneous fat, which is representative of 50% of body fat stores, and skeletal muscle bulk, which represents 60% of the total body protein pool and is the primary source of amino acids in times of starvation and stress (Manning and Shenkin 1995). The body is influenced by changes in nutritional status and therefore, anthropometric measurements are important in identifying certain types of malnutrition that affect body size and

composition. However, in critically ill patients who have rapid changes in body composition, skinfold anthropometry is of little value. For example, a patient who initially had a skinfold measurement above the normal range, but at time of assessment was within the normal range, may be mistakenly classified as healthy although the patient has lost body fat and protein stores (Manning and Shenkin 1995). In addition, fluid retention or dehydration can influence anthropometric measurements, which puts the patient at risk for a misdiagnosis of malnutrition. Anthropometric measurements performed every several months may help to evaluate a patient's progress when compared to previously recorded measurements. There still is a need for more suitable measurement of body composition in critically ill patients who can undergo rapid body composition changes.

Body weight is assessed upon admission to the hospital and weight loss reflects the immediate inability to meet nutritional requirements, and therefore may indicate nutritional risk. Weight change is assessed on a regular basis. Generally, a five-pound weight change over a six-month period is an indication of a need for further investigation (Simko et al. 1984). The degree of weight loss can be an extremely important index of change in nutritional status. Inadequate caloric intake induces loss of protein from the body cell mass. During starvation, there is mobilization of proteins, carbohydrates and fatty acids, which leads to a rapid decrease in lean body mass (LBM). However, decreases in LBM can be overlooked when edema exists. Acute weight loss in critically ill patients may only reflect changes in fluid balance. Weight change and other anthropometric measures need to be used in conjunction with other methods

assessment.

Biochemical parameters are often measured to assess both macro- and micronutrient status. In broad terms, indicators of nutritional status can be classified as static or functional indices. Static indicators directly assess specific nutrient content of biological fluids or tissues. Functional indicators are indirect measures of a metabolic physiological function that is dependent upon a specific nutrient.

Immune parameters are a subset of functional indicators that are used to measure nutritional status. Malnutrition is the most common cause of secondary immunodeficiency (Puri and Chandra 1985), which has led to the incorporation of many cell-mediated immunity (CMI) indexes, such as total lymphocyte count (TLC) and delayed cutaneous hypersensitivity (DCH), both of which are used in the assessment of nutritional status in the hospital (Harvey et al. 1981; Miller 1978; Pretsch et al. 1977). These tests are altered during the early stages of undernutrition and therefore provide a functional measure of mild deviations from normal nutritional health (Puri and Chandra 1985) and offer a broadspectrum analysis of nutritional status that is appropriate since it is rare for a patient to be deficient in only one nutrient. However, the sensitivity and specificity of these assessments has not been well documented in acute nutrient deprivation.

T lymphocytes are involved in CMI. A progressive decline in immune function is indicated by a decrease in TLC and by a delayed or absent cutaneous hypersensitivity response to common skin test antigens (Twonmey et al. 1982).

The absolute number of T lymphocytes is reduced in protein-energy malnutrition (PEM) and the reduction correlates with the degree of weight deficit (Chandra 1979; Ferguson et al. 1974). This departure from normal value is rapidly and completely reversed by nutritional repletion (Puri and Chandra 1985). When nutritional support was given to Crohn's disease patients, the number of T cells increased within 5-10 days, whereas serum albumin concentration took several weeks to become normal (Chandra 1986). Total lymphocyte count is sensitive to protein malnutrition and refeeding, however, it is not specific to protein status. Drug therapy, acute illness, stress, infection and neoplastic disease can influence TLC and can lead to an incorrect evaluation of nutritional status (Wilson 1996). Therfore, TLC needs to be used in conjuction with additional indices of nutritional status.

Delayed cutaneous hypersensitivity measures the cell-mediated response of various injected antigens and is used as an indicator of immune function. The standard method is to intradermally inject several antigens into the forearm, and then measure the amount of induration (hardening due to inflammation), in millimeters, after 48 hours (Zeman 1991). Many investigators have found depression of DCH responses during undernutrition (Puri and Chandra 1985). The absence of induration is associated with anergy. Correlations between the extent of anergy, severity of nutritional deficiency and prognosis in terms of sepsis and mortality have been reported (Christou et al. 1981; Griffeth et al. 1984; Johnson et al. 1979). It was determined in a study of 272 seriously ill, hospitalized patients, that an improvement in or maintenance of a positive DCH response was the most accurate predictor of a favorable outcome (as

compared to plasma proteins, TLC, and anthropometry). Ninety percent of patients whose DCH response was improved or remained positive were discharged (Harvey et al. 1981). However, undernutrition is not the only cause of decreased DCH. It is also affected by surgery, drugs, gastrointestinal hemorrhage, infection, liver disease, shock, burns, trauma, malignancy, myocardial infarction, renal failure and age (Blackburn and Thornton 1979). Delayed cutaneous hypersensitivity may be of value as a prognostic indicator, but is of little value as a marker of nutritional status (Twomey et al. 1982). It has been suggested that assessment of immunocompetence by currently available methods such as TLC and DCH in conjunction with other methods of nutritional assessment can identify individuals who are most in need of nutritional intervention, and thus provide crucial prognostic information in terms of risk of disease, duration of hospitalization, and chances of survival (Puri and Chandra 1985).

Acute Nutritional Deprivation and the Immune System

A cluster of differentiation (CD) groups leukocyte monoclonal antibodies that bind the same antigen, but may differ in epitope (specific site on antigen) specificity or isotype (gene variant present in all healthy members of a species) (Grindhem 1996). Mature CD4+ cells are called T helper (Th) cells because they help B cells to induce an immune response as well as help regulate CD8 maturation. CD4+ cells recognize their specific antigens in association with MHC class II molecules. This leads to T cell activation and the release of soluble protein mediators collectively called cytokines. Cytokines regulate cell growth, cell activation, inflammation, immunity, tissue repair, fibrosis and

morphogenesis (Roitt 1996). Mature CD8+ cells are called cytotoxic T lymphocytes (CTL) and their function is to kill cells infected with a virus, cancer, fungi or other infectious agent. Another division of CD8+ cells are called suppressor cells. Their function is to suppress immune reactions. Collectively CD4+ and CD8+ cells help to regulate CMI.

Changes in CMI in acute nutrient deprivation has not been well documented. However, in chronic malnutrition, histological studies have been performed to show atrophy in lymphoid tissues and depletion of lymphoid cells (Chandra and Kumari 1994). Chandra and Kumari (1994) examined several other effects of chronic malnutrition on the immune system such as: a reduction in the number of fully differentiated T lymphocytes, an increase in the number of null cells, which is a characteristic of lymphocyte immaturity, a decrease in the number of CD4+ cells and a less marked decrease in CD8+ cells, resulting in a reduction of the CD4/CD8 ratio. Lymphocyte proliferation and synthesis of DNA are also depressed and this may be due to a lack of essential dietary nutrients in the patient's plasma (Chandra and Kumari 1994). Many enzymes that play a key role in immune responses require the presence of specific micronutrients for optimal function; such as zinc for thymidine kinase and iron for ribonucletidyl transferase. This would suggest that malnutrition may alter immune responses.

Mitogenic stimulation of lymphocytes *in vitro* is believed to mimic *in vivo* antigen stimulation of T cells. Concanavalin-A (Con-A) is a mitogen known to stimulate T cells. Mitogens have been shown to stimulate T cells to produce cytokines and cytokine receptors which drive T lymphocytes through their life

cycle (proliferation). There is limited research on the effect acute starvation has on lymphocyte proliferation. Neuvonin and Salo (1984) stimulated whole blood lymphocytes with Con-A and reported a decreasing trend, but there was no significant change in lymphocyte proliferative response during a 3 day period of starvation in humans. During a very-low-energy all-protein reducing diet, proliferative responses to Con-A decreased significantly after 4 weeks and returned to base value after 2 weeks of refeeding in obese humans (Field et al. 1991). Although these studies indicate a relationship between dietary restriction and lymphocyte proliferation there is a need for further investigation in the area of lymphocyte response to stimulation during periods of acute nutritional deprivation. The pathogenesis of depressed CMI during nutrient depletion is not fully understood at this time. Cell mediated immunity may be impaired by a general depression in protein synthesis coupled with multiple nutritional deficiencies (Neuman et al. 1975) which may be responsible for reduced lymphocyte proliferation.

Some variation between normal feline peripheral blood lymphocyte distribution has been reported. According to Lin (1992) phenotypic expression of feline peripheral blood lymphocytes tends to be 20-25% CD4 and 6-18% CD8 under normal, healthy circumstances and Dean (1991) reported the mean lymphocyte subset percentages in healthy feline peripheral blood to be 33.9% CD4, 19.1% CD8 and the mean CD4/CD8 ratio to be 1.9 (range 1.2-2.6). We will consider values of 20-33.9% CD4, 6-20% CD8 and a 1.2-2.6 CD4/CD8 ratio to be normal for this study.

Phenotypic markers such as CD4 and CD8, can be identified by monoclonal antibodies that specifically bind to molecules on the cell surface membrane. Monoclonal antibodies are the primary probes used for detection of cellular antigens. Each monoclonal antibody is epitope specific within an antigen molecule (Grindem 1996).

There are few studies reporting the effects of acute nutrient deprivation on lymphocyte subsets and the results have been somewhat conflicting. Komaki et al. (1997) found significant decreases in TLC and total number of CD4 cells in 10 human subjects following a 10 day fast. Although there was a decreasing trend, Komaki and coworkers (1997) found no significant changes in CD8 cells. Gallagher and Daly (1993) reviewed several studies and concluded that during an undefined period of malnutrition in humans, there is a decrease in absolute T-cell count, CD4+:CD8+ ratio, and generation of cytotoxic (CD8) T cells. The effects of short-term (3 days) starvation on the functions and subpopulations of lymphocytes were studied in 10 healthy humans (Nuevonin and Salo 1984). Whereas several other studies (Ogawa et al. 1993; Wing et al. 1987; Komaki et al. 1997) have reported significant changes in TLC and lymphocyte subsets, Nuevonin and Salo (1984) observed no significant changes in TLC or in CD4:CD8 ratio. However, they did observe a decreasing trend in TLC and CD4:CD8 ratio and a slight increase in percentage of T-lymphocytes in white blood cells (WBC). Researchers (Neuvonin and Salo 1984; Ogawa et al. 1993) have reported significant increases in CD8+ cells during malnutrition and acute starvation of 4 day duration in mice. In contrast, Komaki et al. (1997) did not observe significant changes in CD8+ cells in humans during a 10 day fast.

Ogawa et al. (1993) observed a decrease in CD4:CD8 ratio in mice following a 4 day acute starvation. The decrease in ratio was attributed to the reduction in the proportion of CD4+ cells and increase in CD8+ cells.

In summary, the most commonly reported response in periods of acute starvation is a significant decrease in TLC, CD4+ and CD4:CD8 ratio and an increase in CD8+ cells (Ogawa et al. 1993; Wing et al. 1987; Gallagher and Daly 1993). Conflicting results between studies may be due to differences in duration and degree of nutritional deprivation when defined as acute nutritional deprivation in healthy subjects. It is therefore important to investigate the relationship between the length of time food is withheld, alterations in CD4 and CD8 differentiation and lymphocyte proliferation in healthy subjects. This information can be then used to accurately identify those patients who are malnourished and ultimately help to reduce disease states and accelerate recovery.

Cell Membrane Fluidity

Membrane associated functions are influenced by the fluidity and physical state of the cell membrane (Eze 1992). The cell membrane is primarily a polar lipid bilayer in which membrane proteins float (Singer et al. 1972). This lipid-protein matrix provides the basic structure of the membrane and serves as a relatively impermeable barrier to most water-soluble molecules. The protein molecules in the lipid bilayer mediate most functions of the membrane such as transport of specific molecules or catalyzing membraneassociated reactions, such as ATP synthesis (Alberts et al. 1994). The protein molecules constitute

about 50% of the mass of most animal cell membranes, nearly all the remainder being lipid.

The lipid composition of the bilayer is primarily composed of phospholipids, cholesterol and glycolipids: cholesterol enhances the permeability-barrier properties of the lipid bilayer, phospholipids are involved in cell signaling within cell membranes and glycolipids are believed to protect the membrane from changes in pH and degradative enzymes. Charged glycolipids may be important for their ability to alter the electrical field around the membrane and alter concentrations of various ions, such as Ca^{2+}, at its external surface (Alberts et al. 1994). The fluidity of a lipid bilayer depends on its composition and therefore it is important to consider the fatty acid composition of a cell membrane when investigating membrane fluidity. The fluidity and physical state of the membrane may profoundly affect membrane-associated reactions such as transport (Eze 1990).

Cellular membrane function can be measured by determining the intracellular flux of calcium $[Ca^{2+}]_i$. Currently there is no existing information on membrane function in acutely nutrient deprived subjects. Burke and coworkers (1994) measured alterations in Ca^{2+} signal transduction in critically ill surgical patients. Monocytes from the critically ill patients exhibited a decreased Ca^{2+} flux as compared to normal, healthy patient monocytes. This reduction was attributed to disruption of early signal transduction.

Although no studies have determined a relationship between malnutrition and decreased membrane function, there is evidence that a relationship may exist.

Nutritionally deprived patients have decreased cellular immunity and patients with decreased cellular immunity have decreased membrane function (Burke et al. 1994).

Cytoplasmic free calcium (Ca^{2+}) is a key intracellular messenger of lymphocytes. An enzyme imperative in the facilitation of CMI cascades is protein kinase C (PKC), a calcium/phospholipid dependent and diacylglycerol (DAG) triggered enzyme (Nishizuka, 1984; Schuber, 1989). Both calcium and PKC are needed for the stimulation of lymphocyte proliferation. Protein kinase C phosphorylates surface molecules (CD 28 and TCR) leading to release of specific cytokines such as IL-2. T lymphocyte proliferation is regulated by IL-2 (Cantrell and Smith 1984), and IL-2 expression depends on the magnitude of $[Ca^{2+}]_i$ increase (Negulescu et al. 1994). The point of action of the increased $[Ca^{2+}]_i$ may be at the regulation level of IL-2 (Verheugen 1997). Therefore, an accumulation of $[Ca^{2+}]_i$ is indicative of a cell's activation state (Verheugan 1997).

The ability of T lymphocytes to increase $[Ca^{2+}]_i$ in response to stimuli is a measure of early signal transduction and thus cellular function. Increases in $[Ca^{2+}]_i$ have been correlated with the first cellular changes associated with cellular activation such as surface receptor expression, membrane antigen expression, protein turnover and phosphorylation (Ostergaard and Clark 1987). In a study to investigate T-cell receptor mediated signaling, Morford et al. (1997) quantitated the free $[Ca^{2+}]_i$ after ionomycin treatment of T cells from patients with intracranial tumors. The researchers determined that the T cells from these patients mobilized less calcium than the T cells obtained from normal

subjects, suggesting defects in the early transmembrane signaling events associated with T cell stimulation.

In mature peripheral blood lymphocytes, proliferation and differentiation into CD4+ and CD8+ cells occurs upon activation through the stimulation of the T cell receptor (TCR) (Crabtree et al. 1997). Verheugen and colleagues (1997) studied the enhancement of calcium signaling and proliferation responses in activated T lymphocytes. One of the early responses to TCR stimulation was a rise in $[Ca^{2+}]_i$. Calcium response in T lymphocytes is critically dependent on Ca^{2+} influx across the plasma membrane (Verheugen et al. 1997) and therefore an increase in $[Ca^{2+}]_i$ is indicative of increased activity of T cells for differentiation and proliferation.

The current findings in research indicate that intracellular calcium plays a vital role in the regulation of immunity. It is therefore important to investigate the relationship between acute nutrient deprivation (in our study, it was defined as 7 days), intracellular calcium concentration and lymphocyte differentiation and proliferation. This may prove to be a useful functional indicator of immune status to assess undernutrition.