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PROTEIN METABOLISM AND INJURY

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INTRODUCTION

A half century has passed since Cuthbertson made the observation that injury causes an increase in urinary nitrogen excretion. David Cuthbertson (27), a young clinical chemist at the Royal Infirmary in Glasgow, was urged by his surgical colleagues to study the metabolism of the fracture patient. They hoped

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to gain new insight into the reason why certain patients developed non-union after fractures of the lower third of the tibia. In the process of analyzing the urine of the fractured patients during the first two weeks after convalescence, Cuthbertson observed the large increase in the urinary excretion of nitrogen. These studies established negative nitrogen balance as the hallmark of injury. Subsequent studies revealed that the extent of the nitrogen loss was roughly proportional to the severity of the injury. Some of the most rapid and extreme losses of body nitrogen occur with cases of multiple injury, particularly if these are complicated by secondary infection. One may logically ask why these large losses of body nitrogen had not been reported many years before Cuthbertson's first studies. Previously, the concepts and chemical measurements required for nitrogen balance studies had been published in detail by Boussingault (64) in 1843, and the role of protein metabolism in nutrition was extensively studied by Voit, Rubner, and their colleagues during the last century (103). Therefore, the relatively late recognition of the loss of body nitrogen with injury was not due to lack of chemical methodology, or to lack of awareness of the importance of protein in nutrition. Rather, it seems probable that surgeons caring for the injured patient devoted their major attention to the initial shock, or hypotensive response to injury, until after World War II. In 1942, Cuthbertson published a paper (28) emphasizing the great importance of separating the metabolic and physiologic response to injury into two phases: the early "ebb," or hypotensive, phase, lasting 24–48 hr; and, the subsequent "flow," or catabolic phase, lasting days or even weeks. It is this later phase that is characterized by accentuated nitrogen loss, and that has received increasing attention during the past 30 years (74).

This review of protein metabolism and injury will start with a brief consideration of normal adult body composition and the various approaches for estimating body nitrogen content. The magnitude of weight loss will be discussed in relationship to the contribution of body protein. The interrelations between protein and energy metabolism will be discussed for healthy humans.

Injury is commonly associated with some degree of starvation. The changes in protein metabolism in starvation will be considered as part of the background for the changes that occur after injury. Measurements of whole body protein turnover, and the kinetics of protein in specific tissues and organs, will be discussed as they apply to injury. The influence of nutritional intake will then be reviewed, with special attention to the protein metabolism of the injured patient.

BODY COMPOSITION AND PROTEIN LOSS

Some of the most severe forms of weight loss occur following multiple injury. The rate of weight loss will generally change in parallel with the rate of nitrogen loss. The rate of weight loss after major injury is commonly between 400 and

800 g per day in previously healthy adult males, unless aggressive parenteral nutrition has been provided. This rate of weight loss approximates that seen in total starvation, even though severely injured patients commonly receive some nutritional intake, either by mouth or vein. Metabolic balance studies in surgical patients have shown that body protein contributes approximately 7–10% to the total weight loss during periods of relative diuresis after injury, but increases to comprise 10–14% during postdiuretic weight loss (72). Therefore, the physician who sees a patient with extensive weight loss who gives a reliable history of his normal weight prior to hospitalization, can assume that 10–15% of this weight loss will have been at the expense of normal protein stores.

The development of methods for estimating the major components of the body in vivo can provide important background information for understanding the influence of injury on protein metabolism. Unfortunately, methods for measuring human body composition have yielded much more information about body fat and body water than about body protein. Validation of indirect methods depends on data obtained from the direct analysis of the human body. Grande & Keys (58) summarized published values from the direct chemical analysis of three male cadavers, yielding an average of 62.6% water, 15.3% fat, and 16.4% protein. Early attempts to measure body fat involved underwater weighing of the subject, and calculations were based on the different densities of fat and lean tissue. Behnke (8) proposed a division of the body into fat and lean body mass. The latter was conceived as including approximately 10% of its weight as "essential fat." Other investigators have preferred to discuss the "fat-free" body, which is considered to be the sum of ether-extractable substances. If body fat is measured by the densitometric method, followed by the measurement of total body water by isotope dilution, one can calculate by difference from total body weight the dry fat-free substances which will be predominantly crude body protein and minerals in the skeleton. Moore and co-workers (92) did much to popularize the use of whole body potassium, measured by isotope dilution, to reflect the active lean tissue of the body. These authors introduced the term "body cell mass" to designate the homogenous, energy-exchanging, heat-producing portion of body tissue that could be represented by the measurement of total exchangeable potassium. Because the average intracellular potassium concentration is relatively constant, the total exchangeable potassium in milliequivalents could be multiplied by a coefficient (8.33 was proposed by Moore et al) to yield the body cell mass in grams. Studies by this group emphasized that the catabolic response of surgical patients resulted in a relatively greater protein loss in the body cell mass than in the extracellular supporting structures (90).

Over the last five years, a great deal of interest has developed around the use of neutron activation as a technique for measuring secondary radiation to indicate the body content of nitrogen, as well as that of potassium, sodium,

chloride, phosphorus, and calcium. Hill and co-workers (63), and McNeil and co-workers (85), have each used this technique, with particular attention to the body content of nitrogen in pathological conditions in an effort to determine whether nitrogen depletion could be improved with varying levels of nutritional intake. Future studies are needed to determine whether the nitrogen contained in the body cell mass can be separated from the nitrogen in extracellular supporting structures (dermis, collagen, and so forth) by the simultaneous measurement of total exchangeable potassium, or other ionic species, with a unique and well-defined distribution.

PROTEIN-ENERGY INTERRELATIONS

A curious parallel behavior appears to exist between the resting energy expenditure of an individual and the level of nitrogen excretion on a given level o nutritional intake. Factors that cause increases, or decreases, in energy expenditure are usually associated with a similar change in nitrogen excretion (73). The fundamental mechanisms underlying this relationship are poorly understood. Much of the current knowledge concerning the effects of energy intake and the endogenous supply of energy substrates on protein metabolism has been the result of nitrogen balance studies. The responses of whole body nitrogen metabolism to altered energy supplies appear to be regulated by the preexisting status of protein metabolism and the level of protein intake (104). This introduces complexity to the problem of separating the influence of energy balance and nitrogen balance in normal man and, particularly, in the presence of acute illness or injury.

Both the basal metabolic rate (76) and the protein synthesis rates are much higher in smaller species than in large species (144). Garlick (47) reported that the synthesis of whole body protein declines with increased body size when expressed per unit of body weight, but that the protein synthesis rate is relatively constant among species of widely differing body weight, when related to surface area or metabolic body size (per $kg^{0.75}$). Thus whole body protein synthesis shows an approximate correlation with body weight, as is found for basal energy metabolism, implying that the energy requirements associated with protein synthesis are presumably an important factor in variations found in whole body energy expenditure. Reeds et al (114) compared their estimates of protein synthesis with heat production in young pigs. Their findings indicated that the minimum proportion of energy expenditure, associated with protein synthesis, was approximately 15–25% of total body heat production. Young and co-workers (162) observed that in human subjects there appears to be a relatively constant relationship between whole body protein synthesis rates and resting energy metabolism from early infancy through adult life. These authors also observed that there is a broad similarity between the

contribution made by total body heat production by different organs and the contribution made by these organs to whole body protein synthesis. Young & Munro (161) estimated that muscle protein synthesis accounts for approximately 30% of whole body protein turnover in young, adult men. Assuming that total body protein turnover accounts for about 20% of basal energy metabolism, these authors estimate that about 6% of whole body energy metabolism is associated directly with the protein metabolism of muscle. These estimates are of interest in relation to injury, where Kien et al (70, 69) have reported that the increase in energy expenditure in burn injury is correlated with a rise in whole body protein turnover. Bilmazes et al (12) reported that the metabolic response to injury is accompanied by increased turnover of muscle protein.

The studies of Shulman et al (131) indicate that hyperglycemia, coupled with some increase in circulating insulin, may drive the synthesis of alanine nitrogen formation in tissues and its subsequent release into the bloodstream. If a significant amount of alanine is formed in skeletal muscle, then such a response might lead to a depletion of nitrogen in this tissue, particularly if the alanine nitrogen is transformed in the liver to urea. Studies by Young and co-workers (162) with both isotopic leucine and isotopic lysine, indicated that the rate of entry into the metabolic pool, via endogenous protein breakdown at each energy level, was significantly higher in the postabsorptive state than in the fed state. Diets containing a relatively higher carbohydrate content were associated with a significantly higher change in net leucine retention (95).

The influence of reducing physical activity was studied by Schoenheyder et al (130) who used ^{15}N-glycine to measure the protein synthesis rate in subjects who were immobilized for several days in a plaster cast. A negative nitrogen balance, as a result of this treatment, was found to be associated with a decrease in the rate of protein synthesis rather than a change in protein breakdown. Young and co-workers (159, 162) conclude that between the extremes of major exercise and total inactivity there is a pattern of physical activity that is consistent with, and necessary for, the maintenance of a satisfactory state of body protein metabolism.

PROTEIN METABOLISM IN STARVATION

Injury is commonly followed by a period of partial starvation that influences the metabolic response associated with injury. Therefore, the metabolic response to uncomplicated starvation should be considered as background information, starting with the postabsorptive state (38). During this time, certain tissues are oxidizing free fatty acids and the output of glucose from the liver continues at normal levels, although only 25% of this glucose is derived from gluconeogenesis. Nearly all amino acids are potentially glucogenic. However, the pattern of amino acid exchange between organs is such that alanine is the major

amino acid utilized for hepatic gluconeogenesis (39). After an overnight fast, muscle is in negative nitrogen balance, and the output of alanine and glutamine exceeds that of all other amino acids (37). The predominance of alanine in the outflow of amino nitrogen from muscle has been explained on the basis of its synthesis by transamination of glucose-derived pyruvate. It is generally thought that the branched-chain amino acids that are selectively catabolized in muscle provide the amino groups for alanine synthesis (100). Thus gluconeogenesis from alanine represents in part a recycling of carbon analogous to the Cori cycle for lactate. The overall regulation of gluconeogenesis depends upon substrate availability, hormonal setting, and enzyme activity (40). In the postabsorptive state, approximately 33% of the alanine delivered to the liver is extracted by hepatic cells. Consequently, gluconeogenesis can be increased or decreased by altering precursor availability or altering hepatic extraction. Early in starvation, gluconeogenesis is increased by means of increased extraction of substrates. However, in prolonged starvation, gluconeogenesis progessively declines as the result of decreased precursor availability.

The decline in the secretion of insulin, when there is cessation of ingested nutrients, appears to be the important metabolic signal for initiating the metabolic response to starvation. The overall effect of insulin may be characterized as stimulating a variety of processes designed to enhance body fuel storage. Amino acid mobilization from muscle is increased when there is a fall in insulin levels, as protein synthetic processes decline, at a time when catabolic reactions may increase in activity. While a primary site of insulin action is the regulation of gluconeogenesis in the liver, the most striking effects of insulin can be demonstrated on amino acid mobilization (105). A fall in circulating amino acids accompanies a rise in insulin secretion, and elevated levels of branched-chain amino acids occur with a fall in insulin levels. The responsiveness of branched-chain amino acid levels to insulin secretion may derive from their role as fuel in muscle, as well as their importance in regulating protein synthesis in muscle. Glucagon may play a role in early starvation, serving to maintain the breakdown of liver glycogen and to stimulate gluconeogenesis.

Stimulation of gluconeogenesis is required as starvation continues for up to a week with the depletion of glycogen stores and the continued need of the brain for glucose oxidation (19). During this week, there is a drop in plasma insulin and a rise in glucagon. There is an increase in the alanine extraction by the liver, despite a fall in plasma alanine levels. Gluconeogenesis increases two- to threefold above postabsorptive levels, which requires an increase in proteolysis and amino acid mobilization from muscle. This is associated with a negative nitrogen balance of 10–12 g/day, indicating a breakdown of 75–100g/day of protein.

During prolonged starvation, there is a progressive fall in urinary nitrogen excretion as starvation extends beyond one week. The rate of urinary nitrogen

loss can become as low as 3 g/day and the main component has changed from urea to ammonia (110). The marked decline in hepatic glucose production at this time is associated with diminished release of precursors, such as alanine from muscle, with an associated fall in circulating alanine levels (41). Although insulin and glucagon have received the most attention in the study of starvation, other hormone changes have been shown to occur: an increase in circulating growth hormone; a diminished secretion of cortisol; a fall in serum triiodothyronine in association with increased levels of catecholamines. The exact role of the changes in these hormones in starvation has not been defined.

PROTEIN METABOLISM IN INJURY

The source of nitrogen and/or other intracellular constituents that are lost following injury has never been completely defined. However, the nitrogen-sulphur and nitrogen-potassium ratios of the urinary losses suggest that the nitrogen loss is mainly from muscle (27). Moore & Ball (91) presented detailed balance studies of different forms of surgical injury, emphasizing the magnitude of the losses of body nitrogen and potassium that could occur. The cumulative nitrogen losses after uncomplicated elective operation are commonly between 40 and 80 g of nitrogen. Complications that delay the use of the gastrointestinal tract may result in nitrogen losses of 100–150 g. Severe episodes of visceral sepsis can result in the loss of over 200 g, and the major burn with complications can lose over 300 g. The magnitude of these losses can be appreciated more readily when one remembers that 1 g of nitrogen is the equivalent of approximately 30 g of hydrated lean tissue. Therefore, a loss of 300 g of nitrogen would be the equivalent of approximately 9000 g of lean tissue. The rapid weight loss accompanied by fatigue and weakness that occurs in acute surgical catabolism is associated with an increased nitrogen loss, consistent with a loss of muscle mass and muscle work capacity. Loss of body weight over a few days may be predominantly the loss of body water, but sustained weight loss over two to three weeks, or more, can be assumed to be made up of protein and fat as well as water. Recent studies of cumulative calorie and nitrogen balance in patients with major injury have shown that the protein contribution to the weight loss over a three-week period amounted to 10–13% of the weight loss (72). Of particular interest was a similar analysis of the data from the famous study by Benedict (9) that revealed that protein contributed 12% of the weight loss over the first three weeks of total starvation. This information suggests that body protein represents a surprisingly reproducible proportion of sustained weight loss after injury, and that this may be similar to that seen in other conditions, including starvation.

The metabolic integration of organs and tissues has been reviewed by Munro (101) for both physiologic circumstances and in various disease conditions. He

has emphasized that when considering amino acid metabolism this metabolic integration is particularly important among intestinal mucosa, liver, muscle, and kidney. Miller (88) has shown in studies of the perfused liver that seven of the ten essential amino acids for the growing rat undergo degradation in the liver, but not in the carcass. In contrast, the branched-chain amino acids are actively catabolized in the carcass and the nonessential amino acids are degraded in both locations. The absorbed essential amino acids normally pass to the liver where their fate is determined in relation to the needs of various tissues. Thus the majority of the essential amino acids will have the amount introduced into the systemic circulation regulated by the liver. This is in contrast to the branched-chain amino acids, which pass the liver to enter the systemic circulation in increased amounts relative to other amino acids. They are taken up by muscle and adipose tissue, which is stimulated by insulin. Muscle also releases large amounts of two amino acids, alanine and glutamine, the latter a source of ammonia for the kidney and also a precursor of alanine in intestinal mucosa. The alanine formed in the intestinal mucosa and that coming from muscle can be used by the liver for gluconeogenesis. The kidney consumes glutamine under conditions of acidosis. The movement of amino acids between organs and tissues is particularly under the influence of insulin, where there may be an insufficient supply (as in diabetes), or an excessive circulating level due to a lack of hepatic regulation (as in cirrhosis), or to insulin resistance of the tissues (which occurs in renal failure, injury, and sepsis). Insulin levels have been shown to be inappropriately low for the corresponding degree of hyperglycemia following elective operation or accidental injury (86). The use of a glucose pulse dose followed by intravenous diazooxide in injured patients indicated that insulin in injured patients had a shortened half-life (87).

Chronically starved man is dependent for survival on conserving body protein while providing a continuing fuel supply for vital organs. These opposing needs are met by reducing the overall energy needs of the body (thus conserving fat stores) and reducing the need for gluconeogenesis as ketoacids begin to substitute for glucose as a brain fuel (thus conserving amino acid precursors from muscle tissue). In the later stages of starvation, the utilization of glucogenic amino acids is reduced by at least 50% (111). This adaptation to starvation is characterized by a steady decrease in nitrogen excretion and a rise in circulating ketoacids. Sherwin et al (129) have shown that the infusion of beta-hydroxy-butyrate in similar subjects results in a reduced urinary nitrogen excretion. Wedge and colleagues (147) have shown the same relationship between hyperketonemia and nitrogen sparing in a heterogeneous group of normally nourished patients following accidental injury. The effects of semi-starvation prior to injury or operation are less clear, although poor nutritional intake is known to reduce the degree and length of the protein catabolic response to injury. Rich & Wright (117) divided 78 depleted surgical patients

into two groups, depending upon whether they exhibited normal or elevated levels of blood ketones. Those patients who underwent an operation tended to retain their preoperative hormonal and substrate profile. The patients with lower ketone levels excreted more nitrogen, had greater loss in arm muscle circumference and serum protein levels, and mortality was greater. These investigators propose that the patients who fail to become "starvation adapted" have some interference with the insulin-glucagon balance owing to disease or injury.

Counterregulatory hormones that oppose the action of insulin, namely glucocorticoids, glucagon, and catecholamines, are commonly elevated in association with injury and sepsis (75). In these conditions, blood sugar and insulin are both elevated, possibly associated with insulin resistance due to the counterregulatory hormones. The secretion of insulin accelerates the branched-chain amino acids entering muscle, while there is an increased release of alanine and glutamine from muscle. This allows for an increased rate of gluconeogenesis in the face of hyperglycemia associated with increased levels of glucagon (2).

Woolfson and co-workers (153) reported that severely injured patients show a considerable reduction in urea production when pharmacologic doses of insulin are given along with glucose and amino acids, whereas noncatabolic cases showed no substantial reduction in urea production rate when insulin was added. Munro (102) summarizes data from several investigators and suggests that the nitrogen loss after injury is due to decreased synthesis after moderate injury, but that severe injury also causes increased myofibrillar protein breakdown in muscle. Muscle protein synthesis and breakdown are sensitive to several hormones, notably insulin and corticosteroids. Munro cites studies from his laboratory indicating that corticosteroid levels in the plasma do not affect myofibrillar protein breakdown until the corticosteroid levels in the plasma are grossly elevated. This is seen in severe catabolic states, where the concomitant elevation of insulin is insufficient to offset the muscle protein catabolism due to the steroid.

Isotopic glucose studies have shown that the hepatic output of glucose is not only increased following injury, but cannot be inhibited by the usual infusion of exogenous glucose (80). One may speculate that there is an increased need for glucose for the manufacture of acute phase proteins, all of which are glycoproteins and all of which are manufactured in the liver. The metabolic response to injury may also be associated with an increased use of muscle amino acids for gluconeogenesis in order to guarantee a continuing supply of glucose for wound repair. Connective tissue requires carbohydrate for synthesis of glucosaminoglycans, and primitive fibroblasts depend upon glycolysis as a source of energy. Wilmore and colleagues (149) have emphasized the influence of the burn wound on local and systemic responses to injury, particularly the high uptake of glucose and output of lactate.

Thus it appears that the metabolic response to starvation differs from the metabolic response to injury, in regard to nitrogen excretion, gluconeogenesis, and resting energy expenditure. Starvation causes a decrease in each variable, and injury uniformly tends to cause an increase. It seems reasonable to regard the response to starvation as one of conserving tissue to achieve prolonged survival. The response to injury accentuates the loss of body tissue, as though the body places wound repair and host defense at a high priority and gambles that the critical period of convalescence will pass before the depletion of tissues becomes in itself a threat to survival. Moyer and colleagues (96, 97) have studied the patient with severe multiple injuries and documented changes in patterns of substrates and hormones, which characterize the progression to multiple systems organ failure and a fatal outcome in most cases, regardless of the efforts to provide specialized nutritional support. These investigators report an increased obligatory catabolism of amino acids in the nonsurviving patients. A peripheral energy fuel deficit has been proposed, with muscle release of an abnormal pattern of amino acids that compromise the function of the liver and the brain. This concept is consistent with such patients becoming comatose and deeply jaundiced at the time of death. Advances in our understanding of this tragic development of multiple systems organ failure will depend upon new ways to evaluate absolute loss of body protein, together with new understanding of the abnormal ways in which amino acids are utilized to synthesize adequate amounts of high priority protein to meet vital functional demands.

PLASMA AMINO ACIDS IN INJURY

Christensen (22) has presented a thoughtful review emphasizing the point that amino acid nutrition for an animal organism is not merely the random flow of amino acids from the alimentary tract to various cells. Rather, the diversification of cellular roles requires complex regulation of molecular traffic, particularly the amino acid nutrition that certain organs and tissues provide for other parts of the body. Abnormal plasma amino acid patterns offer one indication of how this interorgan traffic is disturbed in injury.

Studies by E. B. Man (82) examined the nitrogen metabolism of patients after severe injury and discovered that the concentration of alpha-amino acid nitrogen in the plasma was usually low, despite the fact that the nitrogen loss in these cases was often greater than normal. He felt that this fall of amino acid nitrogen was an intrinsic part of the reaction to injury. The depression of the plasma amino acids remained until convalescence was well advanced. The separation of individual amino acid concentrations into a normal pattern and the distorted patterns seen with disease and injury slowly became evident as new methodology became available over the next 30 years.

Woolf and co-workers (152) studied patients with serious postoperative complications and other patients with fractures of the femoral shaft. These findings were compared with uncomplicated postoperative convalescence on the first and third postoperative days. The fracture patients appeared to have normal plasma amino acids, except for decreases in ornithine, taurine, and aspartic acids. These findings were in contrast to patients with infection, where the phenylalanine and methionine were elevated, suggesting to the authors some impairment in liver metabolism. Dale and co-workers (29) studied 40 patients with varying severity of operation and varying nutritional intakes. These authors were unable to relate changes in amino acid levels to the severity of the operative procedure. However, increasing the postoperative glucose intake was associated with a higher plasma alanine and a lower methionine level, suggesting transient liver dysfunction. Groves and co-workers (59) compared plasma amino acids of burned patients with patients following uncomplicated abdominal operation. Phenylalanine remained high in the burned group, again suggesting abnormal liver metabolism.

Cerra and co-workers (20) followed the plasma hormone and substrate profile of postoperative, septic, and cirrhotic patients. All patients received similar nutritional support with glucose and amino acids in a ratio of 100 cal/g N. The level of plasma proline was found to be a good indicator of mortality in septic patients and showed a positive correlation with blood lactate levels. These authors proposed that the metabolic abnormality in the injured patient who develops sepsis is a progressive energy fuel deficit in peripheral tissues, possibly from a progressive inhibition of substrate entering the Krebs cycle.

Shenkin and co-workers (127) studied the influence of administering large amounts of amino acid nitrogen compared with giving no amino acid nitrogen during the first eight days following severe trauma when both groups received an isocaloric intake. The sum of plasma levels of the branched-chain amino acids and the other essential amino acids was increased to a greater extent in the high nitrogen group. In spite of the marked difference in nitrogen balance, 3–methylhistidine excretion was increased, but equal in the two nutritional groups. This suggests an increased rate of muscle protein breakdown in both groups, which appears not to be influenced by amino acid administration.

Snelling and co-workers (133) studied patients with severe burns to determine if the transamination of branched-chain amino acids was greater in burned patients without sepsis than in control subjects. This comparison was based on the use of phenylalanine release as an indicator of net catabolism of skeletal muscle protein. Arterial and femoral venous amino acid concentrations were measured. These authors concluded that burned patients had increased proteolysis, but transamination of branched-chain amino acids relative to net proteolysis was not occurring at a greater rate in burned patients. This finding differed from findings by this group in septic dogs and septic humans.

MUSCLE AND PLASMA AMINO ACIDS IN INJURY

Injury causes characteristic changes from normal values (11) of muscle and plasma amino acid concentrations. These are seen to varying extents in operative trauma (141, 142, 4, 5), severe accidental injury (3), and sepsis (3, 98). There are two- to threefold increases in muscle concentrations of the large neutral amino acids (LNAA), including leucine, isoleucine, valine, methionine, phenylalanine, and tyrosine, with much smaller or no changes in plasma concentrations. As a result, muscle:plasma concentration ratios rise as much as threefold. There is an increase in net protein breakdown in muscle and increased net transport of amino acids from muscle to liver. If a rate limiting step in this process is the capacity of the leucine preferring amino acid transport system (23) to transport LNAA, it would explain the increase in muscle:plasma ratios. The relatively normal values, in these conditions, for plasma concentrations of methionine and the aromatic amino acids indicate that liver function is adequate. However, in severely septic patients, who later die, plasma concentrations of methionine and phenylalanine increase markedly in response to amino acid infusions, suggesting some degree of liver failure (98). Concentrations of the small neutral amino acids, threonine, serine, alanine, and glycine, which are preferentially transported by the alanine preferring system (23), tend to increase in muscle and decrease in plasma as a result of injury. Again, muscle:plasma ratios are markedly increased. The decreased plasma values presumably reflect increased clearance by the liver. The basic amino acids tend to decrease in both muscle and plasma, but the changes are less marked and less consistent than for the neutral amino acids. There is even less change in concentrations of the dicarboxylic amino acids. The behavior of glutamine is exceptional in that muscle concentrations fall after injury to 50% or less of normal values. Plasma concentrations decrease, but to a lesser extent than muscle, and muscle:plasma ratios decrease. This decrease in muscle glutamine is also seen in fasting (33, 67) and in postoperative (67) or glucocorticoid treated dogs (99). Under all these conditions, net protein breakdown and transport of glutamine from muscle to visceral organs are increased. It seems probable, therefore, that there is a change in the "setting" of the muscle transport system for glutamine resulting in a lowered muscle:plasma concentration ratio despite increased net flow of glutamine from muscle to plasma. The decrease in plasma glutamine concentration presumably reflects marked clearance of glutamine by liver, gut, or kidney.

These changes seen in injury are largely independent of diet, although fasting, hypocaloric diets, or inactivity may influence them. Fasting (33, 67), but not hypocaloric intake (our unpublished observation), causes the marked decline in muscle glutamine; but postoperative patients on normal diets also show it (141). Fasted subjects have increases in branched-chain amino acids

that are proportionally as high in plasma as in muscle, but have no changes in phenylalanine concentration or in muscle:plasma ratios of the LNAA (33).

PROTEIN SYNTHESIS AND DEGRADATION

The rate of protein turnover in the adult human, as measured by whole body methods, is approximately 300 g/day (146, 163). Since incorporation of one amino acid into protein requires a minimum of four high-energy phosphate bonds, protein turnover accounts for 12% or more of resting energy expenditure. This energy consuming process provides for very flexible regulation of the concentrations and thereby the functional activities of individual proteins. With few exceptions, all proteins are synthesized and degraded more or less continuously, with half-lives ranging from a few minutes to several months. Protein degradation appears to be a first order process and therefore is the primary determinant of the turnover time or half-life of each protein (123). The concentration of a protein will be affected by changed rates of either synthesis or degradation, and there are multitudes of factors that affect these rates for any individual protein. There are also factors that have a general effect on rates of synthesis or degradation, but that appear to differentiate between individual proteins. Glucocorticoids appear to have a generally stimulating effect on protein synthesis in the liver. An injection of glucocorticoid, or normal diurnal increases in plasma concentrations, can cause two- to eightfold increases in tyrosine transaminase or tryptophan hydroxylase, which have turnover times of a few hours, but no apparent effect on arginase, which has a turnover time of several days (123). These differences occur although the proportional increases in the rates of synthesis are the same for all three proteins. Thus concentrations of proteins with short turnover times respond rapidly to changed stimuli, and those with long turnover times respond slowly. It is presumably no accident that rapid changes occur in the hepatic enzymes that regulate the plasma concentrations of tyrosine and tryptophan, and through them the brain concentrations of norepinephrine and serotonin that affect or regulate a variety of activities including sleep, appetite, and respiration (155); and it takes several days for significant changes in response to diet or hormone stimulation to occur in concentrations of arginase that regulate hepatic synthesis of urea (124).

In catabolic conditions such as injury or sepsis, there are increases in plasma concentrations of a number of proteins, termed acute phase reactants; there may be increased rates of synthesis of white blood cells to combat infection and promote wound healing; and there are increased rates of cell proliferation at the wound site. All of these require increased net synthesis of protein that occurs primarily in the liver and bone marrow. The major nondietary source of amino acids for this visceral protein synthesis, in the human, is the breakdown of muscle protein. In addition, in the absence of adequate dietary intake, brain

requirements for glucose must be met mainly from muscle protein. Major concerns of recent research in this area have been to determine: (*a*) whether these net changes in muscle protein breakdown and visceral protein synthesis are due to changes in unidirectional protein breakdown, or synthesis, or both together; (*b*) the nature of the metabolic and endocrine factors mediating these changes; and (*c*) the effects of nutrition on these processes.

Whole Body Protein Turnover

Techniques for measurement of whole body protein turnover have been developed almost exclusively for human studies because of the great difficulty or impossibility of applying the more invasive techniques used in animals. The basic method involves administration of a known amount of isotopic amino acid, measurement of the rate of excretion of isotope, and estimation of the isotope content of the precursor of protein synthesis from measurement of the specific activity of plasma amino acid, or urea and ammonia, pools. All the administered isotope not excreted is assumed to enter protein, and the unidirectional rate of protein synthesis is obtained by dividing the protein isotope incorporation by the specific activity of the precursor. The rate of degradation is obtained by subtracting net synthesis of protein, obtained by balance methods, from the unidirectional synthesis rate. The original method, introduced in 1949 (122, 134), used a single administration of ^{15}N labeled amino acid. A subsequent modification used a constant infusion (112), and later modifications have used ^{14}C, ^{13}C, or ^{3}H labeled amino acids. Many variations of the basic model have been used and have been reviewed elsewhere (145, 137, 47).

It is obvious that with such a simple model for such a complex process there are a host of assumptions, many of which have not been validated. A very important assumption is that the specific activity of precursor, estimated from plasma constituents, adequately represents the specific activity of precursor at the sites of protein synthesis; and that the ratio of these specific activities does not change markedly with changing physiologic state. Gan & Jeffay (46) infused ^{14}C labeled L-lysine into rats until an approximate plateau of specific activity was reached. The ratio of tissue to plasma specific activity in fed rats was 0.5 for both muscle and liver. Calculation of whole body protein synthesis from plasma specific activity would thus give 50% of the rate calculated from tissue specific activities. After one day of fasting, the tissue-to-plasma ratio dropped to 0.1 in the liver but was changed little in muscle. Use of plasma specific activities would underestimate rates of protein synthesis much more in liver than in muscle, and might indicate a decreased rate of protein synthesis when there was actually an increase. A further complication relates to amino acyl-tRNA, which is the immediate precursor of protein synthesis. The specific activity of amino acyl-tRNA differs from that of either the plasma or intracellular pool. In liver (1) and heart (84) it tends to fall between the two pools, a

finding that, if anything, improves the credibility of whole body methods. However, in muscle this may not always be true (21).

The very concept of a single, whole body rate of protein turnover has severe limitations. Although it can serve effectively to compare overall rates of protein synthesis in different species or at different ages (Table 1), it is not suitable for elucidation of the interorgan shifts of protein that appear to take place in injury and other forms of stress.

Despite the problems and reservations cited above, the considerable efforts that have been made to measure whole body protein turnover have contributed an important body of literature (146, 145, 137, 47).

Normal Values

Rates of whole body protein turnover decrease with increasing weight of different species but are roughly proportional to weight raised to the 0.75 power (Table 1). In human subjects (163), and in rats (47), protein turnover decreases with age whether expressed per kg or per $kg^{0.75}$. The difference between the values for adult man of 5.7 g/kg·day measured with ^{14}C-tyrosine (65), and 3.0 measured with ^{15}N-glycine (163), illustrates the variability of the method. In general, comparisons between different individuals or groups are valid only if the measurements are made using the same method, as performed by the same investigators.

Effects of Nutrition and Exercise

In normal adults or children, increasing nitrogen intake above maintenance requirements has no effect on whole body protein synthesis as measured with ^{15}N (55, 136), but reducing it below requirements markedly reduces protein

Table I Ratios of whole body protein turnover in adult animals and in human subjects of different ages

Species	Age	Weight (kg)	Protein turnover (g/kg·day)	Protein turnover ($g/kg^{0.75}$·day)
Mouse[a]	Adult	0.04	43	20
Rat[a]	Adult	0.51	21	17
Rabbit[a]	Adult	3.6	18	25
Dog[a]	Adult	10	12	22
Sheep[a]	Adult	67	5.3	16
Man[a]	Adult	77	5.7	17
Cow[a]	Adult	628	3.7	19
Man[b]	Newborn	1.9	17	20
	1 Year	9	6.9	12
	Adult	71	3.0	9
	Elderly	56	1.9	5

[a]Data of Garlick (23) obtained with ^{14}C- or ^{3}H-tyrosine.
[b]Data adapted from Young et al (13) obtained with ^{15}N-glycine.

synthesis within two days (49). In malnourished children, protein synthesis rates are below normal (56), but during nutritional therapy, they increase above normal values (112, 56). If nitrogen intake is adequate, protein synthesis rates increase with increasing energy intake (56), but are reported not to decrease with decreasing intake (151). Studies using leucine infusions in the first 36 hr after major abdominal surgery (108) are somewhat at variance with these findings. Compared with values during normal saline infusion, subsequent administration of amino acids markedly increased protein synthesis and slightly decreased protein breakdown rates, while infusion of glucose and insulin reduced both rates. However, the decreases were greater for breakdown. When glucose, insulin, and amino acids were given together, there appeared to be a reduction in degradation rate and an increase in synthesis rate. In normal adults on mixed diets, protein synthesis rates are 30–40% higher (48, 115), and degradation rates are 30% lower (48) during the absorptive than during the postabsorptive phase.

Immobilization of normal subjects in plaster casts was shown to decrease synthesis rates by 15–20% (130), and similar effects are seen in rats (57). During strenuous exercise, rates of whole body protein breakdown increase more than 50% while there is a decrease in rates of myofibrillar protein breakdown (116). For the five hours after exercise, both synthesis and degradation rates were above normal (116). In another study, whole protein synthesis rates decreased during exercise (164).

Effects of Injury on Whole Body Protein Turnover

In two studies of the effects of operation, decreases in rates of protein synthesis of 12% (109) and 23% (26) were observed with no apparent change in rates of degradation. In the first study, of the effects of abdominal surgery, patients were given normal diets preoperatively but were maintained on 5% dextrose postoperatively (109). The postoperative study was performed during the third postoperative day which is usually thought to be at the beginning of the flow phase of trauma (28). In this study, it is impossible to distinguish between surgical injury and dietary changes as the cause of the decrease in protein synthesis. In the second study, involving orthopedic patients, a normal diet was maintained postoperatively and the patients were studied the morning after surgery at a borderline time between the ebb and flow phases of trauma. Although diet was not a factor, it is possible that immobilization (see below) rather than surgical trauma was the cause of the decreased synthesis rates.

With more serious trauma, both synthesis and degradation rates appear to be markedly elevated. Birkhahn et al (13) used ^{14}C-leucine infusions to study patients with severe accidental trauma, maintained on 5% dextrose, 3–5 days after admission. The results were compared to values of normal subjects

maintained on 5% dextrose for 3 days. Protein synthesis rates were increased by 50% and protein breakdown by 79% in the trauma patients as compared to normals. Measurements of protein turnover were also made using ^{15}N-alanine infusions in a subset of these subjects (14). By this method, synthesis increased by 37% and breakdown by 79% in the injured patients. Muscle protein breakdown in these patients as measured by 3–methylhistidine excretion increased to nearly three times normal (78), proportionally much greater than for whole body protein breakdown.

Kien et al (70) studied the effects of burn injury on protein turnover in children (aged 4–12 years) using ^{15}N-glycine. Studies were performed within two weeks of injury and at successive times thereafter. Patients received adequate nutrients given orally or intravenously and were in positive nitrogen balance. The results were compared to values for metabolically normal children (4–18 years) admitted for reconstructive surgery one year or more after burn injury. Rates of both synthesis and breakdown remained twice normal in those children with burns on 60% or more of body surface area. Both synthesis and breakdown rates were correlated positively with burn size. Paired measurements made in the first 20 days, and in the ensuing 20 days, showed decreases in both rates with time. Increases in protein synthesis rates were positively correlated with increases in basal metabolic rate, and the authors estimate that 50% of the increase in energy expenditure can be accounted for by protein synthesis (69). The increases in rates of protein breakdown in muscle, measured with 3–methylhistidine, were proportional to whole body increases (12).

Long et al (79) used ^{15}N-alanine to study protein turnover in septic subjects maintained on 5% dextrose. Compared to normal subjects they had increases of 21% in both protein synthesis and breakdown rates.

The data currently available, as described above, indicate that severe mechanical injury, as well as burns and sepsis, increases rates of both protein synthesis and degradation. These changes occur regardless of nutritional state, although nutrition may modify the response. Thus the injured or septic adults, given hypocaloric glucose infusions, were in negative nitrogen balance and their rates of protein breakdown were greater than rates of synthesis. In the burned children with adequate nutrition who were in positive nitrogen balance, synthesis rates were greater than breakdown rates. In all injured and septic subjects, whole body synthesis and breakdown rates were increased above normal, and all had increased rates of muscle protein breakdown. Whether or not surgical trauma has an effect on protein synthesis or breakdown rates is less clear since the observed effects might be explained by changes in diet or by immobilization.

The metabolic, hormonal, or neural mechanisms by which injury increases in whole body protein turnover are not clear.

RATES OF PROTEIN SYNTHESIS AND DEGRADATION IN INDIVIDUAL ORGANS OR TISSUES

A great deal of work has been performed in the last 10–15 years concerning rates of protein synthesis and degradation, and factors affecting these rates, in individual organs and tissues. Although most of these studies have been in animals, a growing number of methods are becoming applicable to human studies. As yet there have been few studies of the effects of injury, in part, perhaps, because of the problems in developing suitable animal models for human trauma. A detailed review of this literature is outside the scope of the present article but is to some extent available elsewhere (145, 47, 126, 24, 54). Particularly important advances have been made in elucidating the mechanisms of protein degradation (126, 24), an area that has lagged far behind that of protein synthesis. Lysosomes appear to be mainly responsible for degradation of normal endogenous proteins, and the rate of entry of an individual protein into the lysosome may be the major determinant of its rate of degradation. An ATP-dependent protease system located in the cytosol seems to be responsible for rapid degradation of abnormal or foreign proteins but has little effect on normal proteins (52).

Both in vivo and in vitro techniques are used for measurement of protein synthesis rates. Isotopes may be administered as a pulse or by constant infusion. Isotope incorporation into protein and some estimate of precursor specific activity must be measured for each tissue. As with whole body methods, uncertainty with respect to the specific activity of amino acyl-tRNA at the site of protein synthesis is often a problem. Nevertheless, much more accurate rates can be obtained than by whole body methods. Degradation rates may be obtained together with synthesis rates by estimating net rates of protein synthesis from (*a*) changes in protein content, or (*b*) rates of net release or uptake of amino acids. Degradation rates are usually not measured by in vivo studies. Degradation rates may also be measured, independently, from the rate of tissue release of amino acid when synthesis is inhibited, or, in the case of muscle, from the rate of urinary excretion of 3–methylhistidine (106, 161), although recent studies indicate that other tissues, particularly the intestine, may contribute substantially to urinary 3–methylhistidine excretion (143).

Rates of protein synthesis, taken from Garlick (47), are shown for various tissues of the rat in Table 2. Fractional renewal rates are highest for intestinal mucosa and other visceral tissues, and lowest for muscle. Nevertheless, because of its great mass, muscle is a major contributor to total body protein synthesis. The discrepancy between whole body synthesis and the sum of individual organs, 46% of whole body, illustrates the approximate nature of these measurements, and that different techniques were used for whole body and individual organ measurements. Skin, in the rat, was found to have a

Table II Ratios of protein synthesis in tissues of the young rat [Adapted from Garlick (23)].

Tissue	Synthesis rate		Percentage of whole body
	Fractional (%/day)	Absolute (g/100·day)	
Small intestine mucosa	136	0.41	9.8
Liver	87	0.55	13.0
Spleen	76	0.06	1.3
Kidneys	48	0.07	1.6
Lung	33	0.04	0.8
Heart	17	0.01	0.3
Brain	17	0.02	0.6
Muscle	13	0.79	18.7
Whole body		4.22	100

fractional renewal rate of 28% and to synthesize 0.5 g protein/100 g·day, roughly equal to muscle, liver, or gut (21).

Liver

Khairallah (68) and colleagues measured rates of protein synthesis and breakdown in vivo in livers of rats just before and immediately after a three hr meal. The effect of the meal was to increase synthesis by 20% and decrease breakdown by 80%. The dramatic effect on breakdown was attributed largely to suppression of the number and size of liver lysosomes and associated autophagic vacuoles. Both insulin and amino acid mixtures suppress lysosome size and number as well as protein degradation in liver, while glucagon acts oppositely (94). The much smaller changes seen in protein synthesis may result from the dietary protein. Administration of amino acids to rats increases the rate of liver protein synthesis in vivo (154) and in the perfused organ (44). In vivo, the effect is specifically due to tryptophan and is associated with an increase in the ratio of polyribosomes to mono- and diribosomes (154). Insulin has little or no effect on liver protein synthesis (93). In the liver, net protein synthesis or breakdown is regulated primarily by changes in rates of degradation rather than by synthesis. This is true for extended fasting (46, 50) and growth (125) as well as for diurnal variations (68, 50).

Studies comparing rates of albumin (108, 132, 120) or globulin (108) synthesis in postoperative patients show increased rates (5–10%) with hypocaloric amino acid infusions, and decreased rates with glucose as compared to saline infusions. When glucose and amino acids were infused together, rates of synthesis lay between the two extremes (108).

Muscle

In vitro studies show that glucose, insulin, and leucine act independently to both increase rates of synthesis and decrease rates of breakdown of muscle

protein in rats (18, 45, 54, 66). Similar results were obtained with human muscle biopsies, except that insulin had no effect on degradation (81). The effects of leucine on protein breakdown are due to its transamination product, α–ketoisocaproic acid (53). Prior fasting decreases synthesis and increases degradation rates in muscle, in vitro (77, 77a). In the rat, in vivo, fasting decreases protein synthesis (50). In humans, (35, 160, 17), fasting either decreases or effects no change in the rate of muscle protein degradation measured by 3–methylhistidine excretion. Since there is a net loss of muscle protein during fasting, synthesis rates must decrease. The decreases in glucose and insulin concentrations in fasting might account for the decrease in synthesis rates, but apparently do not stimulate protein breakdown, as occurs in rat muscle in vitro. Leucine concentrations rise in both muscle and plasma during fasting (33, 41) but do not prevent, although they may limit, the decrease in protein synthesis. It is possible that the increased muscle leucine concentration may prevent increased protein breakdown that might otherwise occur, owing to the decrease in insulin and glucose concentrations.

In young men fed a mixed diet hourly for 12 hr, or fasted for 15 hr, rates of muscle protein synthesis measured in vivo were twice as high during feeding as during fasting (115). Muscle protein synthesis of 168 g/day accounted for 50% of whole body synthesis as compared to 20% in the rat (Table 2).

Effects of Injury on Protein Turnover in Individual Organs

In studies of protein synthesis in rats on maintenance diets after fracture of the femur, increases of 15–50% were observed in liver, kidney, heart, and skeletal muscle (138). These changes were very sensitive to nutritional state, and most of these increases occurred only if the pretrauma diet contained amino acids. On diets containing only glucose, the effect of trauma was to decrease the rate of protein synthesis in skeletal muscle. In other studies (161), femur fracture had no effect on the rate of muscle protein degradation as measured with 3–methylhistidine.

Williamson et al (148) found no increase in rates of 3–methylhistidine excretion in patients after skin graft or orthopedic surgery, or who had sustained relatively mild accidental injury as indicated by hyperketonemia at admission and relatively small loss of nitrogen. However, in patients with more severe accidental injury who were normoketonemic and in markedly negative nitrogen balance, they observed a doubling of the 3–methylhistidine excretion. As noted above, in other studies of accidental injury in adults (78) and burns in children (12), increases in 3–methylhistidine excretion were approximately 200% and 100%, respectively. Shenkin et al (127) observed increases of 100–200% in excretion of 3–methylhistidine in patients after severe accidental injury. Intravenous administration of large amounts of amino acids with glucose and fat markedly reduced the negative nitrogen balance in these patients compared to glucose and fat alone, but had no effect on 3–methylhistidine

excretion. Although it is impossible to know accurately the extent to which muscle, gut, or other tissues contribute to increases in 3–methylhistidine (143), the large size of these increases with severe stress, and indirect evidence from other sources, makes it seem likely that a major part must come from increased breakdown of muscle protein.

Thus it appears that severe accidental trauma or burns can effect two- to threefold increases in muscle protein breakdown which are relatively insensitive to nutrition. The extent of increase in these conditions is greater than the increases in net whole body protein breakdown, measured by nitrogen excretion, indicating simultaneous increases in protein synthesis. Since nitrogen balance, but not muscle protein breakdown, is affected by diet, the effects of diet must be mediated by changing rates of protein synthesis in muscle, or of synthesis or degradation in other tissues. By contrast, moderate trauma has little effect on rates of protein degradation in muscle, at least as indicated by the relatively slow measurement of 3–methylhistidine excretion. Acute measurements during intraabdominal surgery show intraoperative and postoperative increases in leg output of amino acids, suggesting an immediate effect on net muscle protein degradation that could be explained by either a decrease in synthesis or an increase in degradation rate (139). In the rat, after moderate trauma, increases or decreases in muscle protein synthesis occur that are dependent on nutritional factors.

Factors Mediating the Effect of Injury on Muscle Protein Degradation

Increased production of corticosteroids may play a role in these effects of severe injury since very high levels cause increases in muscle protein degradation in rats (107, 140).

Recent studies by Goldberg, Dinarello, and colleagues indicate that human leukocytic pyrogen (LP) mediates the effects of fever, sepsis, and injury to increase the rate of muscle protein breakdown (7, 51, 118, 119). Incubation with LP, in vitro at 36°C, increases protein degradation of rat muscle by 62–118%, but has no effect on synthesis. Concentration of prostaglandin E_2 increases fourfold. Indomethacin blocks both effects. Incubation of muscle at 39°C, without LP, increases protein degradation without increasing prostaglandin E_2. When the temperature is increased with added LP, the effects are more than additive.

THE INFLUENCE OF NUTRITION ON PROTEIN METABOLISM

Many surgical conditions are associated with diminished food intake, particularly those involving the gastrointestinal tract, biliary tract, or pancreas (62, 71). Malabsorption may occur as a result of surgical removal of absorptive

surface in the intestinal tract, deficiency of absorptive secretions and enzymes, or the metabolic changes that occur secondary to the bacterial overgrowth when a blind loop is created in a surgical operation. Such a blind loop may result in steatorrhea, diarrhea, malnutrition, and megaloblastic anemia. Some investigators have explained the nitrogen loss after injury on the basis of inadequate nitrogen intake and alterations in amino acid metabolism that result in increased synthesis and excretion of urea. Other investigators have emphasized the lack of adequate energy intake at a time of increased energy expenditure, and an increase in the oxidation of branched-chain amino acids in muscle. Preexisting malnutrition in a patient requiring a surgical operation usually involves deficiencies related to both energy and protein metabolism.

During the decade of the 1930s, nutrition for the patient undergoing operation was traditionally limited to two or three 1–liter bottles of 5% dextrose in water. Elman (31) emphasized the fact that injury was associated with increased nitrogen loss, and that the intravenous administration of protein hydrolysates was an important addition to postoperative nutrition. Because of the expense of hydrolysate solutions, and the fact that they contained a certain proportion of their nitrogen as small peptides rather than free amino acids, such solutions were not widely used in surgical care.

The development of a safe, intravenous fat emulsion by Wretlind and co-workers in 1961 (156) provided the opportunity for giving a normally balanced food intake to patients unable to take adequate nutrition by the intestinal tract. In 1968, Dudrick and co-workers (30) introduced a form of total parenteral nutrition known as "hyperalimentation." This approach utilized amino acids with glucose in sufficient amounts to substitute for the lack of intravenous fat. In contrast to the Scandinavian workers, others felt that hyperalimentation should provide a positive calorie balance of 50%, or more, beyond the level of resting energy expenditure. Many reports appeared showing dramatic clinical responses in depleted patients, who were unable to take adequate nutrition by mouth because of gastrointestinal disease, intestinal fistulae, short-bowel syndrome, and so forth.

Infusion of dextrose-free amino acid solution has been proposed by Blackburn and co-workers (15) as a method for sparing body protein in patients after major operation, or injury, with fewer technical and metabolic problems than were associated with total parenteral nutrition. The underlying concept of administering amino acids alone was to cause low levels of glucose and insulin in the plasma that would then allow mobilization of endogenous fat stores and promote ketone body synthesis.

A period of preoperative nutrition by either an enteral or intravenous route should make the patient less vulnerable to postoperative complications. Unfortunately, there is no agreement concerning the minimal length of time that must be devoted to preoperative nutrition in order to demonstrate fewer postopera-

tive complications. Various measurements have been proposed for the assessment of nutritional status, and presumably a period of preoperative nutritional preparation should demonstrate an improvement in one, or more, of the measurements used for a nutritional assessment. There has been general agreement that the primary objective of special preoperative nutrition was the restoration of diminished body protein. It has generally been believed that a minimum of 10–14 days of a positive nitrogen balance was required in order to justify delaying a surgical operation. Some investigators have demonstrated that the malnourished preoperative patient who was anergic might become responsive with one to two weeks of nutritional therapy, and if that occurred the incidence of postoperative complications would be less than if the patient were operated on in the anergic state.

Several investigators have attempted to simplify the number of preoperative measurements that could be used to predict the chance of postoperative complication. It has seemed reasonable to follow the concentration of certain specialized proteins in the plasma, such as transferrin, or retinal binding protein, as being proteins with a short half-life that were responsive to nutritional status. However, it is of interest that serum albumin, despite its relatively long half-life, has been found to have a strongly positive correlation with nutritional status. The most obvious explanation for a rise in serum albumin levels after a period of nutritional therapy was that albumin synthesis in the liver was increased because of a better supply of amino acids and energy. Starker and co-workers (135) have shown that malnourished patients started on total parenteral nutrition fall into two major groups. Patients who respond well develop a positive nitrogen balance while continuing to lose weight because of a spontaneous diuresis. This diuresis is apparently related to reducing an overexpanded extracellular fluid volume; therefore the serum albumin values show a significant rise during the first week of therapy at a time when there has been minimal improvement in total body stores. Preliminary evidence indicates that some of the patients who do not respond promptly will do so following several more weeks of therapy and that this correction of an overexpanded extracellular fluid volume, with a rise in serum albumin, may be an important indicator of when the patient is ready for an elective operation.

Collins and co-workers (25) studied postoperative patients receiving amino acids alone compared with lipid-free total parenteral nutrition. Postoperative therapy with amino acids alone improved the negative nitrogen balance, but was thought to have no other clinical benefits, while the levels of amino acids and protein in the plasma of postoperative patients remained more nearly normal when receiving total parenteral nutrition. Patients who were undergoing major colon or rectal operations received postoperative nutrition as amino acids alone or with hyperalimentation to study changes in body composition and plasma amino acids. Nitrogen balance studies were improved with infusion of

amino acids alone, but the branched-chain amino acids, as well as phenylalanine, methionine, and proline were elevated above normal values. In contrast, hyperalimentation spared more body protein and fat and restored the plasma amino acids to normal, thus providing a more favorable clinical outcome (158).

A group of acutely ill malnourished surgical patients were divided into two comparable groups and fed over a two-week period with an enteral diet, where the nonprotein energy source was 67% carbohydrate and 33% fat, while the other group received a course of intravenous nutrition with the same amount of nitrogen and calories. However, the nonprotein calorie source was entirely carbohydrate. The patients fed with the enteral diet had no significant change in body weight, fat, protein, or plasma protein. The patients fed intravenously had the same favorable response, except for an average gain of 3.2 kg of body weight that was mainly extracellular water.

Glucose intake has long been recognized to have a nitrogen sparing effect. Amino acid administration also has a nitrogen sparing effect that appears to be different. Moldawer and co-workers (89) studied an animal model in which a healthy rat was given either amino acids or glucose by vein after a femoral fracture. Whole body rates of amino acid turnover and release from protein, as well as the fractional synthetic rates of mixed muscle, liver, and plasma protein were measured using an infusion of ^{14}C-leucine. Both glucose and amino acids improved the nitrogen balance over that of fasting after injury. However, the protein-free glucose infusion was thought to impair the normal response to injury, which was aimed at increasing visceral protein synthesis and maintaining normal concentrations of essential amino acids in the plasma. McNeill and co-workers (85) used neutron activation for the measurement of total body potassium and total body nitrogen in patients receiving total parenteral nutrition for periods of over two months. Changes in lean body mass, as judged by anthropometry, were correlated with total body potassium, but changes in body nitrogen were not correlated with either of these variables. Therefore, it seems clear that measurements of body potassium cannot be used during nutritional rehabilitation as an index of the restoration of body protein.

Skillman and co-workers (132) studied the rate of albumin synthesis in postoperative patients undergoing elective gastrointestinal operation. Five patients who received amino acids alone for four days were shown to have a higher rate of albumin synthesis than comparable patients receiving only 5% glucose. Such studies suggest that the administration of glucose, with or without the stimulation of endogenous insulin, may produce nitrogen rentention by stimulating the uptake of amino acids into insulin sensitive tissues, such as muscle, while amino acids given alone may have greater utilization for visceral protein synthesis.

Hill and co-workers (63) studied the effect of total parenteral nutrition on the body composition of 25 critically ill patients, before and after 14 days of therapy. The composition of muscle was studied over this same period. The

total body content of nitrogen, potassium, sodium, chloride, phosphorus, and calcium all showed average increases of 2–9%, but only the increase in potassium was of statistical significance. Analysis of muscle indicated an intracellular potassium depletion that was corrected over the study period. The results of the total body measurements indicated an average gain of 1.25 liters of extracellular fluid and an 0.5 liter increase in intracellular fluid.

Elwyn and co-workers (34) studied a group of ten depleted, surgical patients who were placed on a constant nitrogen intake by vein, together with three different levels of carbohydrate intake given sequentially for four days each. Analysis of the data indicated that during positive energy balance, resting energy expenditure increased by 1 kcal for each 5 kcal of intake. One interpretation could be that increasing energy intake restores mainly the portion of lean body mass associated with fat deposition and that rapid restoration of lean body mass requires high nitrogen intakes. At zero energy balance, nitrogen balance was only slightly positive, at an intake of 173 mg N/kg, which is about twice the intake of nitrogen required to maintain zero nitrogen balance in normal adults. This data have been combined with that from Bozetti (16), Rudman et al (121), and Plough et al (113) to construct a grid to represent the theoretical relations between nitrogen intake, nitrogen balance, and energy balance in depleted patients, as presented in Figure 1. Figure 1 emphasizes that a positive nitrogen balance can be achieved in two different ways when a nutritionally depleted individual is at nitrogen equilibrium and zero energy balance. Increasing the nitrogen intake at the same level of calorie intake should allow the synthesis of new protein, while increasing the calorie intake although the nitrogen intake remains unchanged should result in increasing body stores of both protein and fat (32). It should be emphasized that in this respect the nutritionally depleted patient resembles a growing organism rather than a normal adult, since the latter will have only a transient increase in N balance due to any increases in N intake.

Fischer et al (43) reported that the administration of amino acid solutions enriched with branched-chain amino acids produced normalization of the amino acid pattern in plasma and clinical improvement in hepatic encephalopathy. Sherwin (128) & Hagenfeld et al (61) showed that leucine administered alone caused lowering of the plasma aromatic amino acids and methionine. Eriksson et al (36) demonstrated that the administration of the three branched-chain amino acids to normal man gave similar results to the infusion of leucine alone, and that valine and isoleucine given alone were essentially ineffective in lowering the levels of aromatic amino acids and methionine.

Freund and co-workers (44 a, b) have shown that the administration of amino acid solutions enriched with the branched-chain amino acids could normalize the plasma amino acid pattern and improve nitrogen balance in septic patients or in uncomplicated postoperative patients. These investigators postulate that muscle proteolysis is accelerated in such conditions to provide energy from the

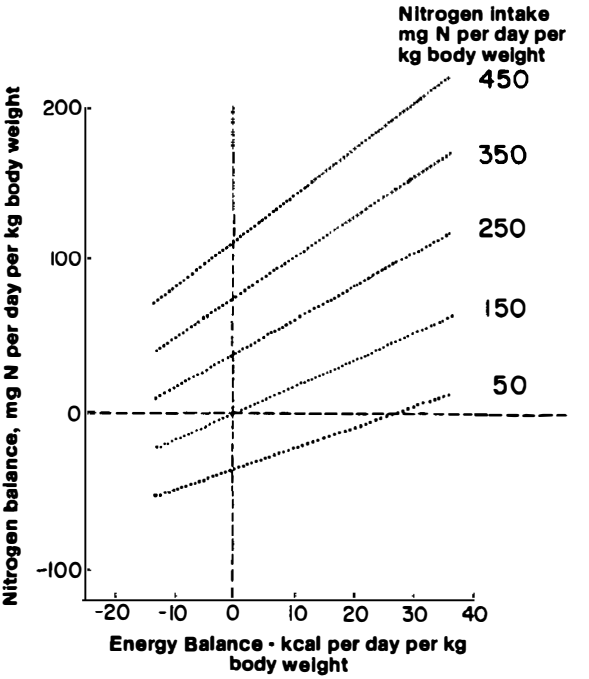

Figure 1 Schematic relations between nitrogen balance, energy balance, and nitrogen intake in nutritionally depleted adult patients. Based on data of Elwyn et al (34), Rudman et al (121), Plough et al (113), and Bozetti (16). [Reproduced with permission from Elwyn (32)].

oxidation of branched-chain amino acids at a time of insulin resistance and limited glucose and fat for oxidation in muscle. Such patients would benefit by the administration of glucose, insulin, and branched-chain amino acids. Blackburn and co-workers (15a) studied a septic-fracture rat model to determine the nitrogen retaining properties of branched-chain amino acids. ^{14}C-tyrosine was added to the diet to estimate protein synthesis in individual tissues. These workers reported that an amino acid diet containing 50% branched-chain amino acids resulted in the greatest preservation of total liver nitrogen and reduced the level of nitrogen excretion. Sapir et al (122a, b), have demonstrated prolonged improvement in nitrogen conservation by the administration of the alpha-keto analogs of the branched-chain amino acids during fasting, but comparable studies have yet to be performed in the injured patient.

FUTURE STUDIES OF THE EFFECTS OF INJURY ON PROTEIN METABOLISM

Although a variety of methods are available, particularly in animals, for studies of protein synthesis and breakdown, applications to the study of injury have been sparse. This may be due, in large part, to the difficulty of developing

suitable animal models for human injury. Indeed, most of the studies of injury to date have been in man, using either whole body turnover or 3–methylhistidine as a marker. Other methods are just becoming practicable for human studies and should become useful in studying the impact of injury on protein metabolism.

An important breakthrough is the recent study of Rennie et al (115) on changes in muscle, and whole body protein synthesis and degradation. These investigators infused L-leucine, labeled in the carboxyl position with the stable isotope ^{13}C, and sampled blood and expired CO_2 at frequent intervals. Needle muscle biopsies were taken at 2.5 and 7.5 hr when the label in free leucine of blood and muscle had reached plateau values. Isotope enrichment in free leucine and ketoleucine isolated from blood and muscle were measured by gas chromatography–mass spectrometry, which can utilize very small samples, but requires high isotope content. Isotope enrichment in expired CO_2 and in CO_2 of muscle protein leucine was measured by isotope ratio mass spectrometry which is very sensitive to isotope content but requires a larger sample. This technique, with possible modifications, should be generally applicable to human studies involving tissues that may be easily biopsied, such as muscle, skin, or adipose tissue. In particular, the needle biopsy technique first introduced by Bergstrom (10) makes this approach applicable to a variety of conditions.

A second method that may be useful is in vitro measurement of protein synthesis and degradation in muscle biopsies. Lundholm and colleagues (81) have effectively used muscle bundles, teased from open biopsies of human skeletal muscle, for in vitro studies of protein synthesis and degradation. The difficulties of obtaining open biopsies limit the applicability of this method. Recent, unpublished studies of Paul Greig and Jaime Rozovski in the authors' laboratory indicate that needle biopsies of muscle may also be suitable for such studies.

Regional studies of the leg, arm, brain, and splanchnic region (6, 22, 41, 42, 60, 83, 150) have contributed greatly to our knowledge of interorgan movements of substrates in normal man and selected disease states. This technique offers many advantages for future studies of the effects of injury on protein metabolism.

The availability of these newer techniques, used separately or in combination, holds great promise for integrating cellular and organ dynamics of protein metabolism with our classical observations of injury in man.

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