alanine and glutamine and urea is produced as a consequence of the reactions of urea cycle.[28] The increase in urea production has been reported in weight losing cancer patients[21] and in advanced gastrointestinal cancer patients.[29] Whereas the rate of urea production is a reflection of net protein catabolism, not all the urea produced is excreted in the urine. Some urea diffuses from the blood into the gut, where bacteria contain the enzyme urease. This enzyme enables the bacteria to digest urea to CO_2 and NH_3. The NH_3 can diffuse back into blood, where it is delivered to the liver by way of the portal circulation and can be incorporated into urea or amino acids. The rate of recycling of N into urea in humans varies from as little as 10% of production under normal conditions[30] to more than 60% in pathological conditions. For example, in burn patients, 40% of urea produced is not excreted.[11] Although the status of urea cycling has never been examined in patients with cancer, this mechanism could possibly be important for the understanding of protein balance in cancer patients.

Constant essential amino acid tracer infusion

Essential amino acids such as leucine and phenylalanine are the amino acids that unconditionally needed to maintain the integrity of the body, but are not synthesized in the body. However, certain concentration of these amino acids is maintained in plasma or intracellular space of the certain type of cells. These free essential amino acids found in either plasma or intracellular space are considered to be derived either from oral intake or from the breakdown of body protein. Thus, certain essential amino acid tracers labeled with stable isotope have been used to evaluate the in vivo rate of whole-body protein breakdown by measuring the rate of appearance of essential amino acid in plasma. For instance, leucine tracer is being used to calculate the rate of whole-body protein breakdown, in which a 2 hour primed-continuous infusion of stable isotopically labeled leucine tracer has been used. However, some of the branched-chain essential amino acid such as leucine is in part oxidized intracellularly producing alpha-keto acid. Therefore, the leucine appeared intracellularly as the result of protein breakdown is recycled in which leucine are reincorporated into protein before it appears in plasma. This leucine model enables to calculate the rate of whole-body protein synthesis by subtracting the rate of intracellular leucine oxidation from the rate of appearance of leucine, as well as the rate of protein breakdown. In contrast, because of the fact that phenylalanine that is also an essential amino acid is not oxidized in the cells, phenylalanine appeared in the cell and in plasma is considered to be generated solely as a result of protein breakdown in the circumstance where no phenylalanine is exogenously given.

Flooding dose technique

The rate of protein synthesis has been measured not only in the whole-body level but also in tissue level such as skeletal muscle. The method of choice for the measurement of the muscle protein synthesis rate most frequently used in humans has been a primed-constant infusion of an amino acid tracer.[31] To calculate the rate of protein synthesis, this method requires measurement of the rate of incorporation of the labeled amino acid into protein and the precursor pool enrichment, *i.e.*, the isotopic enrichment of the tracer at the site of protein synthesis.[32] However, a major drawback of this method is the inability to measure the enrichment of the true precursor pool, *i.e.*, the amino acyl-tRNA pool, because of the large tissue sample required and the tedious and elaborate procedure involved in its isolation and purification. To overcome this problem in human studies Garlick and co-workers[33] adopted a flooding dose technique that requires considerably shorter period of the isotope infusion than the constant infusion method (1.5 versus 4–8 hours) and permits an indirect estimate of the precursor pool enrichment by making measurements of the isotopic enrichment of the tracer amino acid in plasma. From the theoretical standpoint, the enrichment of the precursor pool can be accurately measured by analyzing plasma enrichment. Because in this flooding dose method, the labeled amino acid is given together with a large amount of unlabeled amino acid, it causes rapid equilibrium of the free extracellular amino acid pools, which enables to have the equal enrichment of the plasma as the enrichment of the precursor pool from which amino acids are used to charge tRNA for protein synthesis. However, the flooding dose method gives fractional muscle protein synthesis rates that are markedly greater than those obtained by the constant infusion method.[34] One possible explanation for this discrepancy is a direct stimulatory effect of the large dose of amino acid or that of the hormonal response it elicits on protein synthesis rate.[35]

A-V difference techniques

Measurement of tracer kinetics across a tissue bed has been used for many years in attempts to gain more information about the regional metabolism. Most commonly, metabolism of peripheral tissue is assessed by determining the balance of substrate and/or amino acids in and out from either the forearm or the leg. It is also possible to apply this model across the liver to determine the balance of substrate or amino acids in and out from the liver, although this model involves meth-