I. PATHWAYS OF INTRACELLULAR PROTEIN DEGRADATION

As stated by the organizers of this Congress, its theme and purpose is to encourage workers in basic medical and biological research to examine their own and related findings and other advances in their field from the point of view of determining what practical applications for the promotion of human health and well-being may be derived from it. I have attempted to meet this challenge in organizing my own modest contribution.

A major area of concentration in my laboratories in recent years has been on the mechanism and regulation of intracellular protein degradation. Fig. 1 illustrates features of this process in eukaryotic cells (as distinct from prokaryotic cells, which have no cell organelles, or postkaryotic cells, which once had subcellular organelles but no longer do, e.g., reticulocytes, erythrocytes, lens fiber cells; the inability of the last mentioned cell type to degrade oxidatively damaged lens protein has been proposed to be a major cause of cataract formation, which afflicts about 10% of the world's population [1]; ascorbic acid has been suggested as a possible preventative of this process [1]).

Most of the steps in Fig. 1 are self-explanatory, but a few comments may be useful. Step -1 indicates a premature degradation of precursors prior to conversion to mature forms, a process known to occur in several systems thus far [2-4]. A defective capacity of specific protein precursors to associate at a normal rate, thereby leading to excessive rates...
of breakdown of the precursor (step -1), might be the mechanism underlying certain congenital deficiencies of specific proteins. An inhibition of precursor breakdown in such cases might allow an increased formation of the active protein.

Step 2 represents proteolytic processing by removal of pre- and/or pro-pieces, which occurs in the maturation of a large proportion of newly synthesized proteins [5]. The processes represented in step -1 and step 2 give rise to most or all of the release of amino acids that occurs within minutes after their incorporation into peptide chains. This very early release of peptide-bound amino acids as a result of processing (step 2) or abortive synthesis (step -1) has almost universally and, in my opinion, erroneously been attributed to the degradation of "fast turnover proteins" of which few, if any, have been identified. To the extent that this is so, effects on early amino acid release have nothing to do with mechanisms and regulation of intracellular protein degradation.

This concept of the origin of the rapidly released amino acids also offers an explanation for the heretofore unexplained observation of an inverse correlation between protein half-lives and molecular size [7], since it is obvious that a greater preponderance of precursor forms will be present in the high molecular weight fractions and of mature forms in the low molecular weight end of the spectrum.

Step 3 represents secretion from the cell and is a process that contributes to the turnover rate of secreted proteins. Intracellular protein half-lives are not properly converted to degradation rates without correction for the secretion rate, if any, [8].

Step 12 is, in most cases, a facilitated diffusion event. A congenital defect in the transfer of cystine out of the lysosome is known in humans, which leads to the disease cystinosis and can be treated with cysteamine [9].

The balance of amino acids in the amino acid pool must be adjusted to that represented by the amino acid composition of the proteins currently being synthesized in the cell for the most efficient utilization of amino acids to occur. The interconversion of amino acids in the pool is represented by the arrows and the symbol "B-6" in the amino acid pool in Fig. 1. If a vitamin B-6 deficiency exists, one would predict that the dietary protein (amino acid) requirement would be increased to assure an adequate supply of amino acids in the pool.

II. FACTORS THAT ALTER PROTEIN TURNOVER

In Fig. 1, only pathways of amino acid utilization for protein synthesis and of amino acid production from protein breakdown are shown. Fig. 2 illustrates additional pathways of amino acid metabolism. The fraction of the total amino acid pool broken down in these degradative pathways varies with the nutritional state of the subject, ranging from as little as 10% in malnourished children (i.e., 90% of the pool is utilized for protein renewal) to progressively higher values as the dietary protein intake increases [10, 11]. It is also known that carbohydrate and energy intake have sparing effects on the dietary amino acid requirement, since regulation of the interconversion of amino acids and carbohydrates is

![Fig. 2. Pathways of amino acid supply and utilization.](attachment:fig2.png)

1 "Early" is defined arbitrarily and differently by different workers. In perfused mouse liver, Hutson and Mortimore found a half-time for early amino acid release of 10 min or less [6].
shifted toward carbohydrate formation in the absence of adequate dietary carbohydrate and the oxidation of amino acids is increased when other body sources of energy are depleted [12, 13]. In diabetes and other wasting diseases, as well as in starvation, there is a net breakdown of myofibrillar protein to provide amino acids to the pool for carbohydrate and energy production [13].

The absence of amino acids in tissue perfusates or cell cultures leads to an increase in protein degradation, as well as to the expected decrease in protein synthesis. In liver the increased degradation can be blocked by the addition of certain amino acids, while in muscle breakdown is inhibited by leucine alone (or more effectively by its keto acid analog, a-ketoisocaproate [14]).

The cycle of peptide bond formation and peptide hydrolysis not only has an energy cost for the maintenance of the steady state, but, it would appear, a material cost as well. Since a fraction of the amino acids released into the pool are withdrawn in pathways other than protein resynthesis, a depletion of the pool, either continuous or intermittent, depending upon the feeding habits of the organism, is obviously required. The quantity of new amino acids required depends, as noted above, not only on the quantity of amino acids catabolized, but on the nature of the amino acids catabolized and the nature of the amino acids in the diet as well. An acceleration of the turnover or replacement rate of body protein, as occurs in a variety of disease states, in nutritional and hormonal imbalances, and in development [10-16], would be predicted to entail an increase in the dietary protein requirement for the maintenance of tissue protein levels.

III. EFFECT OF DEVELOPMENT AND OF DYSTROPHY ON PROTEIN TURNOVER IN CHICKEN MUSCLE

Some of the effects mentioned above are illustrated by some recent results from my laboratory in collaboration with Dr. M. S. Hudecki of this Department. In order to define the temporal relationship between the onset of turnover changes and other symptoms of the disease, we turned to comparisons of protein turnover in muscle tissue from a period prior to onset of functional disability in the dystrophic animal to the time when overt disease symptoms appear. Since a unique aspect of the disease process in the dystrophic chicken is the preferential expression of symptoms in fast-twitch, glycolytic muscle, while slow tonic, oxidative muscle is little affected or entirely spared, we compared both the posterior latissimus dorsi (PLD) muscle as an example of the former and the anterior latissimus dorsi (ALD) muscle as an example of the latter. These muscles have been

![Fig. 3. Rates of \[^3\text{H}\]-tyrosine incorporation into PLD and ALD total protein (T), myofibrillar protein (M), and non-myofibrillar protein (NM) from normal (●) and dystrophic (○) chickens of various ages. Incubations were for 4 h in MEM containing 10 \(\mu\text{Ci}\) of \[^3\text{H}\]-tyrosine.](image)
extensively characterized in the chicken and are composed of homogeneous populations of fast-twitch or slow tonic fibers, respectively.

In order to determine how early in the disease process the abnormality in protein turnover appears, tyrosine incorporation rates were measured in muscle from animals of approximately 2 weeks, 1 month and 2 months of age. As may be seen in Fig. 3, significant increases in incorporation rates into PLD muscle appeared at about one month and were intensified in two month old animals. These differences were present in both myofibrillar and non-myofibrillar fractions. No augmented fractional incorporation rates were seen in ALD muscle.

Similar results were obtained in measurements of protein breakdown determined by tyrosine release. On the other hand, no changes in PLD muscle weight or protein content were found before 2 months ex ovo. Histological changes were extensive at 2 months and barely evident at 1 month. Thus, changes in protein turnover precede both gross anatomical and histological changes in the susceptible muscle.

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