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INTRACELLULAR PROTEIN CATABOLISM AND ITS CONTROL DURING NUTRIENT DEPRIVATION AND SUPPLY

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INTRODUCTION

Protein degradation in animal cells plays an essential role in a number of functions. In addition to its involvement in enzyme regulation and cell remodeling, for example, it serves as an important source of amino acids for gluconeogenesis and other systemic oxidative and biosynthetic reactions when exogenous substrate is not available. Because the amino acids are generated largely at the expense of proteins, which are rapidly regained with food intake, this function is in fact a form of fuel reserve. However, it differs from carbohydrate and fat storage in that normal intracellular proteins rather than specialized molecules are utilized as the amino acid source.

As to the nature of the tissues contributing to this response, early studies with rats have revealed that high-protein feeding increases protein concentration in a number of organs, particularly liver and kidney (1). After starvation, the largest protein loss appears in liver, with smaller decreases in kidney, intestine, heart, and skeletal muscle; none is lost in the eyes, testes, or adrenal glands (2). More recent investigations have shown that rats and mice lose 25–40% of their liver protein during 48 h of starvation (2, 70, 105, 164) without detectable alteration in the content of muscle protein (101, 102); beyond 48 h, though, protein degradation in muscle is accelerated and the content decreases (102). Because the mass of skeletal muscle is far greater than that of liver, the total amino acid contribution by muscle relative to that by liver cannot be determined with certainty. Nevertheless, one can conclude that tissues vary widely in response to the need for extra amino acids and that the rapid catabolism (of protein) seen in liver serves principally in the interval between feeding while the more delayed responses, like those of muscle, function mainly in prolonged starvation.

This review focuses primarily on the mechanism of intracellular proteolysis in animal cells and its physiological control during short-term nutrient deprivation. Although degradation is the dominant site of regulation (29, 50, 51), synthesis may be implicated, and reference to the latter is made where appropriate. Because of space limitations, the effects of long-term caloric deprivation on protein turnover and its modulation by thyroid hormone, glucocorticoids, and other physiological agents or conditions are not covered. The reader is referred elsewhere for reviews on these topics (13, 99, 172).

MAJOR FEATURES OF GENERAL PROTEIN DEGRADATION

Classes of Breakdown

Rates of protein degradation, based on the release of amino acids from labeled protein, fall into two readily distinguishable classes. The first is a rapidly

turning over or short-lived fraction; the second comprises the remaining output of label and is termed long-lived or resident protein degradation. As depicted in Figure 1, the short-lived release of [^{14}C]valine from liver protein after pulse-labeling has a half-life of about 10 min (70). The short- and long-lived components are readily separable, and no intermediate fractions are detectable (70). Although the half-life of the short-lived fraction is generally greater in isolated cells than in liver, in other respects the findings are similar (10, 44, 60, 79, 115, 133, 175).

It should be pointed out that because of the very rapid turnover of the short-lived fraction, its contribution to total protein synthesis, which could amount to as much as one third (70, 179), will be appreciably greater than its distribution in cellular protein (152). A reasonably close approximation of this value was obtained by labeling over a 3-day period (152). In these experiments, only 0.6% of the incorporated label was released by short-lived turnover while 99.4% remained in resident proteins. In addition to these distinctions, short-lived degradation is unresponsive to lysosomal inhibitors (115, 176) and less sensitive to effects of cooling (10, 115) than resident protein breakdown. Both groups, though, depend on metabolic energy for optimal function (61, 65, 133). The strong possibility that short-lived turnover is mediated by the ubiquitin/ATP-dependent proteolytic system has been suggested by studies with temperature-sensitive mutant cells (27, 28). However, recent data of Ahlberg et al (3) indicate that the lysosomal system may also degrade short-lived proteins.

Although the nature of the short-lived pool is not known, there is considerable evidence, summarized elsewhere (70), that the label is not extracted with liver protein and, for this reason, could exist largely as acid-soluble peptides (165). If so, they could have arisen from the rapid breakdown of unwanted protein fragments, possibly signal peptides or other early products of protein synthesis (179). This possibility is consistent with the observation that short-lived turnover is resistant to physiological regulation (10, 44, 70, 79, 115, 133, 176) and remains constant despite wide alterations in the rate of resident protein degradation (Figure 1).

Diversity of Turnover

One of the fundamental aspects of resident protein turnover is its heterogeneity, with individual half-lives ranging from minutes to days (for review, see 11, 53, 145, 178). Such diversity, though, is not observed when turnover is monitored from amino acid release (44, 60, 70, 79, 115, 133, 176). The reason for this is not fully understood, but it is probable that the degradation of some proteins is a sequential process, initiated by mechanisms that alter the function of specific proteins and mark them for a final stage of hydrolysis. While it is beyond the scope of the present chapter to discuss this question in

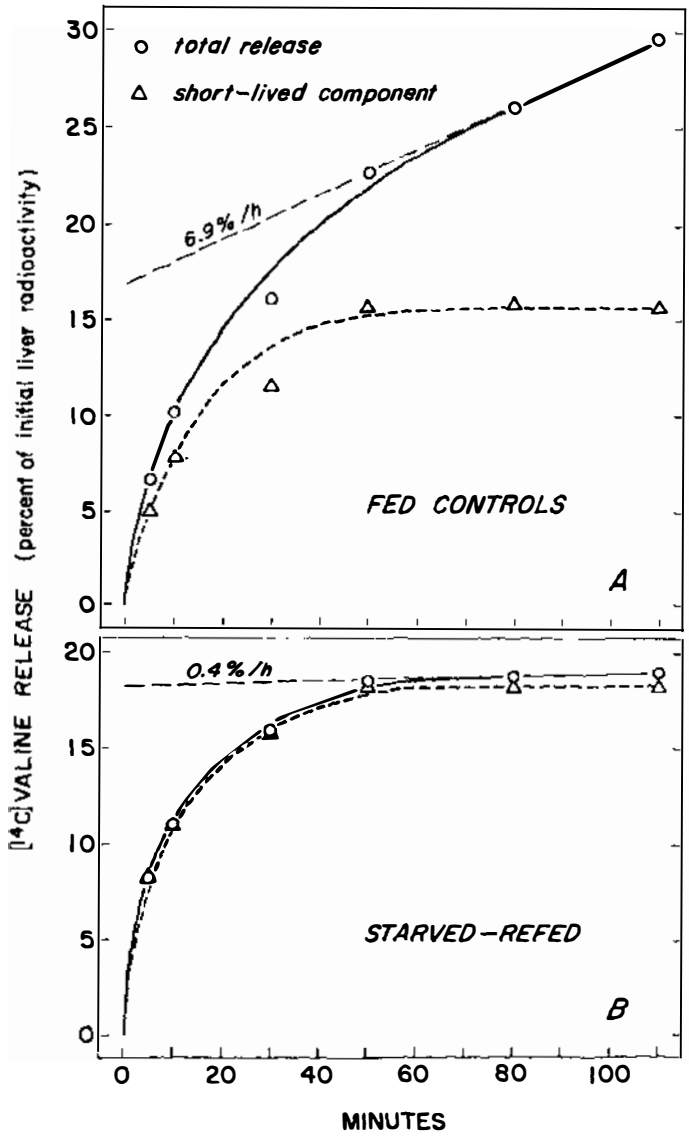

Figure 1 Release of [^{14}C]valine from protein in 10-min pulse-labeled livers of nonfasted and 48-h-starved–12-h-refed mice, cyclically perfused with a 15-mM valine chase. *Circles:* total release; *Triangles:* short-lived release, calculated as the difference between total and long-lived (resident) release. From Hutson & Mortimore (70).

detail, it is of interest to consider some aspects of it in a cell such as the hepatocyte where protein breakdown to the level of amino acids and oligopeptides (18) is largely an intralysosomal mechanism (see below).

The sequestration of intracellular protein and other macromolecules within hepatic macroautophagic vacuoles is an ongoing process in which discrete portions of cytoplasm are segregated by preexisting cellular membranes (see below). Under steady-state conditions, the overall rate of protein internalization will determine the rate of proteolysis. Using the thiol proteinase inhibitor leupeptin to retard digestion of the captured proteins, Kominami et al (80) found that intravacuolar concentrations of two inducible enzymes, tyrosine aminotransferase and aspartate aminotransferase, closely paralleled their respective values in cytosol; this suggests that their autophagic uptake was nonselective. A similar conclusion was reached by Henell & Glaumann (63) for lactate dehydrogenase and aldolase. Many other proteins, however, are attached to or in other ways associated with cytoplasmic membranes (145). Because the endoplasmic reticulum is a major target for sequestration (124, 152), differing rates of individual protein uptake and turnover would be expected, even at constant rates of cytoplasmic sequestration. One interesting example of this is the finding by Dice and coworkers (12, 12a, 42, 42a, 95a) that certain peptides microinjected into fibroblasts can be specifically targeted for macroautophagy (or their targeting prevented) on the basis of specific structural features that could affect their binding to membranes.

MECHANISMS OF PROTEIN DEGRADATION

Owing to the high rate of protein loss in liver, this tissue has been the subject of several investigations aimed at identifying specific morphologic and biochemical deficiencies after starvation. No specific alterations have been seen. Rather, the loss is associated with a diminution of most subcellular constituents (22, 47, 81, 124) and nearly proportional decreases in phospholipid and RNA (81). Because DNA and the number of nuclei remain relatively unchanged (82, 97), these deficits may be considered cytoplasmic in nature. It is of interest that the greatest decrease in cell volume, about 25%, occurs during the first 24 h of starvation (124). Most of this represents space that contains glycogen. However, substantial decrements of endoplasmic reticulum and ground substance also occur (22, 47, 124). Because glycogen is known to overlay, and thus obscure, much of the smooth endoplasmic reticulum (93, 134), the actual loss of the latter in the first 24 h is probably greater than is generally recognized.

Macroautophagy

Until the lysosomal-vacuolar system was discovered, no one could provide a satisfactory explanation for these cytoplasmic deficits. The realization that

cells can sequester and digest portions of their cytoplasm was eventually established (36), but the process was not initially perceived as having any physiological role in protein turnover. However, its relevance broadened after Ashford and Porter and others demonstrated that macroautophagy can be induced by glucagon in rat liver (7, 9, 38, 39, 146) in association with accelerated rates of protein degradation (71, 98). Later it was found that deprivation of amino acids, and insulin, or serum would evoke autophagic responses in the perfused rat liver (109, 113, 114) and heart (73) and in cultured cells (5, 104).

DYNAMICS OF MACROAUTOPHAGY The stringent omission of amino acids in the perfused rat liver induces a strong macroautophagic response (109, 152) virtually indistinguishable from that observed with glucagon (151). Because the response is reversible (109), amino acids have been used as a probe to explore the regulation of macroautophagy and its relationship to deprivation-induced proteolysis. With the aid of quantitative electron microscopy, it has been possible to measure the course of autophagic induction and regression with time in perfused livers (152). Immediately after amino acid withdrawal, nascent macroautophagic vacuoles (autophagosomes or AVi) appear and, after a lag of 7–8 min, are transformed into digestive vacuoles (autolysosomes or AVd). The lag represents the time required for AVi to acquire acid hydrolases by fusion from secondary lysosomes and to initiate digestion (for review of autophagy, see 52, 59, 66).

A recent study (170) has demonstrated a direct relationship between sequestered protein and the quantity of enzyme acquired by fusion over a wide range of AVd size, which suggests that fusion is regulated by information derived from vacuolar volume. Because AVi formation in liver can be suppressed by several agents, including amino acids (109, 152), insulin (107, 114), cycloheximide (83), and 3-methyladenine (155), the turnover of macroautophagic vacuoles can be indirectly assessed from the exponential regression of total autophagic volume. Estimates of the half-life in liver, including one determination based on sensitivity to osmotic shock (114), range from 6 to 9 min (83, 114, 121, 125). Similar values have been reported for autophagic turnover in pancreatic acinar and seminal vesicle cells (83).

QUANTITATIVE RELATIONSHIP BETWEEN MACROAUTOPHAGY AND ACCELERATED PROTEIN DEGRADATION The aggregate volume of hepatocyte cytoplasm sequestered by AVi at amino acid levels between 0 and 10 times normal plasma concentrations correlates directly with the increase in resident protein degradation above the basal rate (152). Calculations of cytoplasmic turnover, based on an autophagic half-life of 8 min ($k = 0.087$ min^{-1}) and steady-state volumes of AVi or AVd, determined over the range

of amino acid concentration, give values that nearly equal the corresponding fractional turnover of resident protein (Table 1). Moreover, as shown in Figure 2, the quantities of sequestered protein predicted by these calculations are in agreement with direct measurements of degradable protein entrapped within lysosomal particles (70, 108, 110). These findings, together with the 3-methyladenine inhibitor studies of Seglen & Gordon (155), leave little doubt that macroautophagy can account for accelerated proteolysis in the hepatocyte. This conclusion very likely holds for other cells as well.

MACROAUTOPHAGY AND PROTEIN DEGRADATION IN MUSCLE In the myocyte, evidence is accumulating that macroautophagic activity of the type described in hepatocytes may be limited to nonmyofibrillar proteins. Although myofibrils comprise the largest volume fraction of the cell, they turn over slowly (14, 90, 163) and have not been observed within autophagic vacuoles in heart (32, 129) or skeletal muscle (149); images of mitochondria, sarcoplasmic reticulum, and glycogen, though, are commonly seen (32, 33). This localization of activity, of course, complicates any attempt to correlate protein degradation with autophagic sequestration as was done in the hepatocyte. More recent studies revealed striking differences in the effects of starvation and insulin on total and myofibrillar protein breakdown. The former, in contrast to the latter, appears to decrease in the perfused rat heart after starvation (163) and can be suppressed by insulin and amino acids in perfused heart and skeletal muscle (90, 92, 163). Since total protein degradation appears to be dominated by faster turning over components of the nonmyofibrillar compartment, it is likely that the effects of insulin and amino acids on protein turnover are mediated by suppression of macroautophagy. It is of interest that macroautophagy in heart (129), as in liver (105, 128, 129), is decreased by food intake in vivo.

Table 1 Values of accelerated fractional turnover for liver cytoplasm and resident proteins[a]

Amino acid level	AVi		AVd + DB		Protein	
	Volume (% liver)	Turnover (h^{-1})	Volume (% liver)	Turnover (h^{-1})	Proteolysis (μmol Val h^{-1})	Turnover (h^{-1})
0X	0.584	0.030	0.518	0.027	14.2	0.031
0.5X	0.354	0.018	0.341	0.018	8.9	0.019
1X	0.133	0.007	0.125	0.007	3.7	0.008
4X	0.013	0.001	0.025	0.001	0.2	0.000

[a] Calculations were based on an autophagic rate constant of 0.087 min^{-1} and increases above basal levels (ten times normal plasma amino acids) in the fractional liver volumes of AVi, AVd + DB (secondary lysosomes), and rates of valine release. Livers from nonfasted rats were perfused in the single-pass mode with multiples (X) of a normal plasma amino acid mixture. See text for further discussion. From Schworer et al (152).

Micro- or Basal Autophagy

Owing to the extreme heterogeneity of autophagic vacuoles, de Duve & Wattiaux (36) coined the term *microautophagy* to express the idea that the sequestered volume or "bite" could extend below the accepted limit for macroautophagy and into the molecular range. Although the process was not explicitly defined, it has since become clear that in most cells intracellular protein can be sequestered and degraded by lysosomal particles other than macroautophagic vacuoles (see below). In the perfused rat liver, macroautophagy is virtually eliminated by adding 4 to 10 times normal plasma levels of amino acids (109, 152). Proteolysis is maximally inhibited under these conditions and the residual or basal rate averages 1.5% of liver protein per hour, about one third of the maximal rate of protein breakdown obtained in the absence of amino acids (139, 152).

Degradable protein is demonstrable in lysosomal fractions under basal as well as accelerated states (70, 108, 110), and the overall relationship between this pool of protein and rates of intracellular protein degradation is depicted in Figure 2. The linearity of the slope and its regression through the zero-intercept strongly support the notion that resident protein degradation is mediated by the lysosomal system under accelerated as well as basal conditions (70, 110). It is of further interest that the value of the slope, 0.096 min^{-1}, is close to the rate constant of autophagic vacuole regression, 0.087

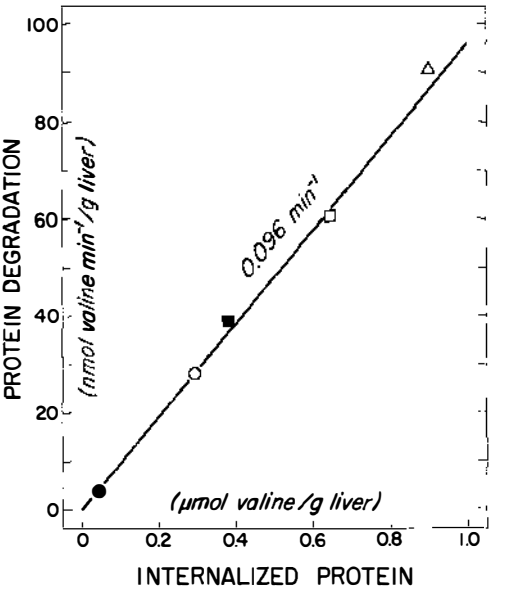

Figure 2 Relationship between lysosomal pools of degradable protein (internalized protein) and rates of breakdown of liver protein. From Hutson & Mortimore (70).

min^{-1} (114, 121, 152). Thus, it is likely that the turnover of degradable protein remains the same irrespective of the size of the degradable pool or the nature of the lysosomal-vacuolar particle that contains it. Utilizing a more direct approach to evaluate autophagic turnover under basal conditions, Henell & Glaumann (64) found that the aggregate volume of lysosomal-vacuolar components in the perfused rat livers increased at a rate of 1.34% h^{-1} when lysosomal proteolysis was inhibited with leupeptin. Although some uncertainties are evident, their major finding does agree remarkably well with the average rate of basal protein turnover and supports the notion that basal autophagy is an ongoing process.

Concerning the question of how proteins are taken up by lysosomes under basal conditions, two categories of possibilities may be considered. The first is transmembrane flow of protein molecules analogous to the transit of signal peptides across membranes (16). Although such a mechanism might offer selectivity, it would not provide intralysosomal protein pools that correlate so well with intracellular degradation (Figure 2) nor would it explain the presence of glycogen and membrane remnants in secondary lysosomes (109, 113, 150, 152). The second category includes the following: (*a*) invagination of the lysosomal membrane (34, 40, 41, 94, 105, 126, 148), (*b*) invagination of the smooth endoplasmic reticulum with movement of material through the intracisternal space to nascent lysosomes (116), (*c*) formation of single-walled primary vesicles followed by lysosomal fusion (113, 151), and (*d*) any combination of the above. But regardless of the mode of sequestration, the vesicles formed would be smaller than macroautophagic vacuoles and, because of higher surface-to-volume ratios, would capture a higher proportion of membrane-associated proteins. In principle, these geometric considerations would satisfy both the requirements of pool size and diversity of turnover to the extent that the latter is influenced by interactions between proteins and membranes. Consistent with this notion is the observation that correlations between hepatic protein degradation and subunit size and negative charge are abolished under conditions where macroautophagy is presumed to play a prominent role in protein catabolism (43).

Nonlysosomal Proteolysis

Although the lysosomal-vacuolar pathway is clearly the dominant process for resident protein turnover in the hepatocyte, nonlysosomal mechanisms may operate to varying degrees in many cells. This conclusion, though, is necessarily indirect since, with the exception of the ubiquitin/ATP-dependent cytosolic system (28), the lysosomal pathway represents the only defined process in eukaryotic cells for the complete breakdown of cytosolic proteins. Neutral proteases, such as those in the calcium-dependent group, have been extensively studied (37, 55, 112, 131), but their catalytic functions appear to be too specific for this requirement.

The first suggestion that cells contain more than one proteolytic pathway came from attempts to block lysosomal proteolysis by using weak bases and protease inhibitors. Although results and interpretations have differed, there is general agreement that accelerated rates of proteolysis can be suppressed by these agents (5, 35, 60, 68, 79, 115, 177). On the other hand, inhibition of basal turnover has been observed either consistently (35, 91, 115, 176, 177), weakly (5, 132), or not at all (61, 79). A major problem in the use of these agents as a means of distinguishing between lysosomal and nonlysosomal proteolysis is that they do not inhibit completely and are variable in their effectiveness (3). In the case of weak bases, for example, maximally inhibitory concentrations raise intralysosomal pH to values ranging from 5.9 to 6.2 (67, 118). Nevertheless, in general agreement with Knowles & Ballard (79), it is reasonable to conclude that some of the ineffectiveness of lysosomal inhibitors relates to the existence of nonlysosomal pathways in some cells. In myocytes, for example, lysosomotropic agents are not effective inhibitors of myofibrillar protein breakdown (92, 120, 184), and it is probable that the initial steps are nonlysosomal in nature (142), involving a calcium-activated disassembly of myofibrils into separate contractile proteins (161, 184). However, the possibility cannot be excluded that autophagy is utilized as a final step (74, 161).

The microinjection of labeled proteins and even subcellular organelles (45, 147) into cultured cells has provided an opportunity to investigate this question in more detail (for review, see 95). It is clear that the degradation of some labeled proteins in fibroblasts will change in parallel with endogenous protein breakdown when the latter is altered by nutritional shifts and cell growth (10, 12, 42, 62), results that are consistent with lysosomal processing. On the other hand, not all labeled proteins are associated with lysosomes during their degradation (15, 42, 167). While these findings have little bearing on general protein catabolism and its regulation, they do indicate that nonlysosomal sites of catabolism exist and, at the same time, underscore the complexity of problems related to the selection of individual proteins for degradation in different cells and under different conditions of growth.

REGULATION OF ACCELERATED PROTEIN DEGRADATION

Control by Amino Acids and Other Agents

In liver, amino acids may be regarded as the primary regulators of resident protein degradation since complete plasma mixtures can evoke responses over the full range of accelerated breakdown in the absence of hormonal agents such as insulin and glucagon (Figure 3*a*). Reproducible inhibitory effects of complete amino acid mixtures are readily elicited at levels as low as half the normal plasma levels in the perfused rat liver (135, 139, 152), whereas

otherwise comparable responses in preparations of isolated hepatocytes appear to require concentrations that are several-fold higher (157). It is also important to note that resident protein synthesis, which is not under acute physiological control by amino acids (123, 181), is in balance with degradation under basal conditions (139).

Despite the known cellular heterogeneity of liver, the total volume occupied by Kupffer and other nonhepatocytes is quite small (17), and as much as 99% of total cellular protein appears to reside in hepatocytes (105). Moreover, macroautophagic activity is evenly distributed between the periportal and pericentral hepatocytes (124, 137, 152). Thus from the standpoint of protein turnover, the perfused liver is a relatively homogeneous preparation of hepatocytes and, because of its responsiveness, is a useful in vitro model for simulating hepatic responses in vivo (135, 139).

REGULATORY AND NONREGULATORY AMINO ACIDS Several studies with the perfused rat liver (139, 181) and isolated hepatocytes (68, 156, 157, 166) have revealed that the number of amino acids that directly inhibit hepatic proteolysis is small, although it is larger than in skeletal and cardiac muscle,

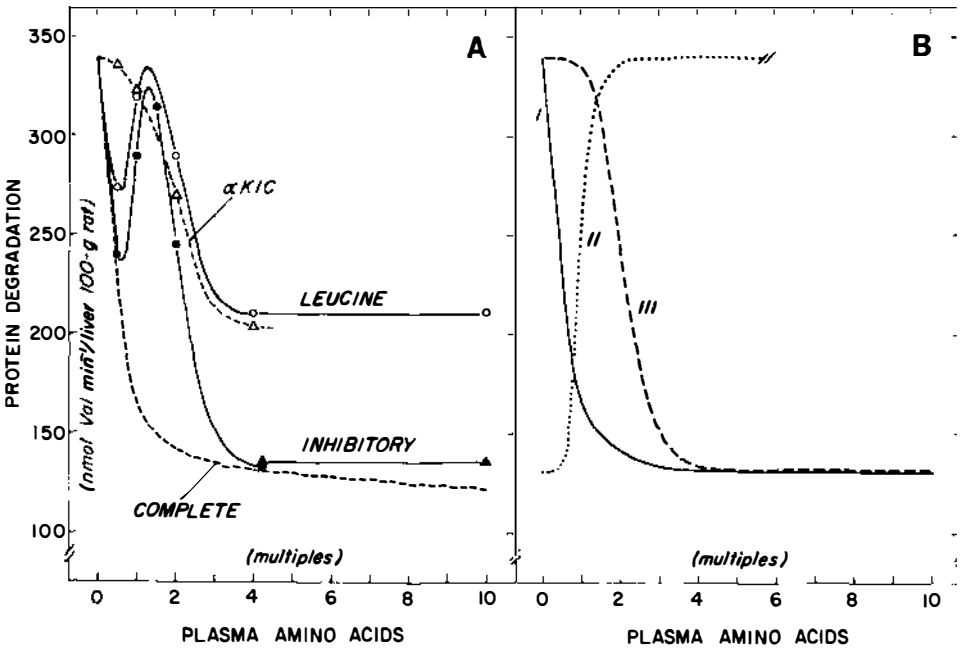

Figure 3 (*a*) Rates of resident protein degradation in rat livers perfused as in Figure 1. The effects of leucine and α-ketoisocaproate were taken from Pösö et al (139); the complete and regulatory amino acid dose responses were redrawn from Pösö & Mortimore (135). (*b*) Hypothetical components of the dose response of the regulatory mixture; see the section on Mechanism of Proteolytic Regulation by Amino Acids for explanation. From Pösö & Mortimore (136).

where only leucine is active (21, 25, 48). Detailed investigations with the perfused rat liver have shown that leucine, tyrosine, phenylalanine, glutamine, proline, histidine, tryptophan, and methionine are effective (139) and as a group can mimic the inhibition evoked by a complete plasma mixture at 0.5 and 4 times normal plasma concentrations (139). A somewhat smaller group (Leu, Phe, Asn, Gln, and His), each added at 10 mM, has been shown to suppress autophagy in electropermeabilized hepatocytes (156). In contrast to the perfused liver (139), histidine proved to be the strongest inhibitor, while little suppression was obtained with leucine (156).

Whether or not all of the eight amino acids are true inhibitors is not easily determined. In some instances individual activity is too low to measure accurately (139). With glutamine, though, difficulty arose from the finding that at concentrations higher than four times normal (3 mM), free ammonia was generated in amounts sufficient to inhibit lysosomal proteolysis directly (138, 139). Finally, because phenylalanine is rapidly hydroxylated to tyrosine (159, 160), activities of the two aromatic amino acids cannot be differentiated. However, since they inhibit to the same extent and their effects are not additive, it is likely that tyrosine rather than phenylalanine is the active regulator (139). Despite the possibility that the number of regulatory amino acids may be overestimated, it is clear that the 12 complementary amino acids as a group exhibit no direct inhibitory activity at physiological concentrations (139).

Leucine is by far the most effective inhibitor in the perfused rat liver (Figure 3*a*), suppressing the deprivation response by about 60% at 0.8 mM or 4 times normal (4X) concentrations (139). The other two branched-chain amino acids, valine and isoleucine, are devoid of inhibitory activity (139, 157, 158). When various combinations of the regulatory amino acids were examined at the 4X level, both leucine and proline appeared to be required for maximal inhibition, while glutamine, tyrosine, and phenylalanine could be deleted without affecting the overall response (139). Other additive and nonadditive combinations observed in isolated hepatocytes (157) and the perfused liver (139) indicate that leucine plays a dominant role in proteolytic regulation.

REGULATORY AMINO ACIDS AND THE PERMISSIVE ROLE OF ALANINE Although proteolytic responses of the perfused liver to complete and regulatory amino acid mixtures are identical at low (0.5X) and high (4X) plasma concentrations, as depicted in Figure 3*a* they differ sharply in the intermediate range owing to an unexpected loss of effectiveness of the regulatory group in the vicinity of normal levels (1X). Leucine exhibits a similar zonal loss (139), and one may presume that the remaining amino acids do also. The loss was traced to a lack of alanine since 0.5-mM additions to the 1X regulatory

mixture restored the inhibition (135). Except for one report (122), alanine possesses little direct inhibitory activity and is the only amino acid displaying this permissive effect. Pyruvate and lactate could be substituted for alanine, but the concentrations required were 20-fold higher (135). Analogous effects, requiring both leucine and alanine for the suppression of proteolysis in perifused hepatocytes, have recently been reported (86).

MECHANISM OF PROTEOLYTIC REGULATION BY AMINO ACIDS Little is known of the cellular locus or nature of the amino acid recognition that mediates the regulatory effects depicted in Figure 3*a*. There is some evidence to support the view that the site(s) are on or close to the plasma membrane. The unique shape of the regulatory amino acid dose response curve indicates that control of proteolysis is complex and involves both positive and negative modulation from separate sites. Figure 3*b* depicts hypothetical components that could be involved. In interpreting the primary inhibition by leucine (curve I), it should be noted that α-ketoisocaproate, the ketoacid of leucine, fails to suppress at 0.1 mM although it closely mimics effects of leucine at higher concentrations. Because rates of leucine transamination in liver are extremely low (30, 139), the initial proteolytic inhibition indicates that the amino acid itself is required for the effect. The mechanism is unknown, but it could involve binding of leucine as well as other regulatory amino acids to their cognate tRNAs, as described by Scornik et al in histidine-dependent mutants of CHO cells (153, 154).

Why regulatory amino acids lose their effectiveness at concentrations above 0.5X (Figure 3*b*, curve II) is equally unknown, but the fact that the loss occurs at amino acid concentrations approximating those in portal vein plasma (119) suggests that it has physiological significance. It is not a feature of regulation by leucine in muscle (25, 173), but in liver it could provide a way to accelerate proteolysis in response to a deficiency of glucogenic substrate. A second inhibitory site is necessary to explain the suppression that occurs at amino acid concentrations greater than 1X (curve III). Such a mechanism would be important as a safeguard against excessive rates of protein breakdown mediated by glucagon or alanine deprivation. Note that the curve parallels the dose response for α-ketoisocaproate in Figure 3*a*. Because the ketoacid is not transaminated rapidly enough in liver for the effect to be mediated by leucine, the ketoacid must be recognized directly. Since curve III also parallels the response to leucine in the upper range (Figure 3*a*), both leucine and α-ketoisocaproate could be recognized at the inhibitory site, quite possibly through specific structural features of the side chain (78, 136, 139).

In contrast to liver, rates of leucine transamination in muscle are very rapid (20), and there is mounting evidence suggesting that α-ketoisocaproate rather than leucine is the active regulator of proteolysis (103, 173). Inhibition of

transamination by cycloserine, for example, has been shown to block the effect of leucine on protein degradation without affecting the response to α-ketoisocaproate (103, 173). The notion that α-ketoisocaproate acts directly rather than through an oxidation product was recently advanced by Chua and coworkers, who found that 20-mM pyruvate in the perfused rat heart strongly suppresses the decarboxylation of the ketoacid without diminishing its inhibitory effectiveness (78). It is of interest that dose responses to leucine in the rat diaphragm (173) and to the ketoacid in the perfused rat heart (78) and liver (139) correspond to curve III in having a distinct sigmoidal shape; responses at low leucine concentrations, as represented in curve I for liver, have not been observed in muscle (25, 173). Thus effects of α-ketoisocaproate in both muscle and liver may be mediated from a site in which structural features of the ketoacid are important for the expression of inhibition (78, 139). In adipose tissue, protein degradation appears to be insensitive to leucine (174, 175), although it is inhibited by insulin (175).

METABOLITES Some proteolytic inhibition has been observed with metabolites other than amino acids, but no clear pattern of response has yet emerged. In cardiac muscle, lactate, pyruvate, acetoacetate, acetate, and propionate have been reported to suppress proteolysis (23, 25); the effects, though, have not been consistent in all preparations (169). In skeletal muscle, fatty acids are without effect (48, 89). The same is true for liver (139) except that octanoate (135) is able to block the modal loss of effectiveness of the inhibitory amino acids at 1X (see Figure 3*a*). Glucose has no known regulatory role in liver or muscle (31, 89, 150). Finally, the suggestion has been made that the redox state of muscle correlates with proteolysis, increased reduction being related to decreased degradation (171, 172). However, despite attempts, the observation has not been confirmed (24, 46, 162).

CALCIUM Calcium accelerates proteolysis in cardiac and skeletal muscle by lysosomal and nonlysosomal mechanisms (76, 143, 144, 161, 168, 184) and reportedly plays a role in macroautophagy in liver (58). The proposal has been made that the lysosomal system is activated by a calcium-induced increase in prostaglandin biosynthesis (143, 144). However, the putative link between protein degradation and PGE_2 release has not been confirmed in muscle preparations from rats with burn injury (96, 117). Thus, while PGE_2 may be important in activating proteolysis, it does not appear to be involved in the maintenance of the effect (96, 117). As mentioned earlier, calcium has been implicated in the lysosomal degradation of some contractile elements in muscle (161). On the other hand, myofibrillar protein degradation is not inhibited by insulin or lysosomotropic agents (91, 120), which suggests that a nonlysosomal pathway is involved. This is further supported by the failure of

lysosomotropic agents and inhibitors of macroautophagy to block the calcium ionophore stimulation of protein degradation (184). While it is likely that calcium-dependent proteases are involved in these initial steps, this complex group of enzymes has not been fully defined (37, 55, 112, 131), and further research is needed to clarify their biochemical and physiological roles.

Hormonal Regulation

GLUCAGON AND β-AGONISTS In spite of the ability of amino acids to exert wide-ranging control in vitro, the actual regulation of proteolysis in vivo must involve cooperative effects with hormonal agents since plasma amino acid concentrations are held within comparatively narrow limits except after food intake. However, owing to the difficulty of measuring proteolysis in vivo, little information is available on the nature of these interactions. As mentioned above, glucagon is a potent inducer of hepatic macroautophagy (7, 9, 38, 146, 150) and protein degradation (69, 98, 151, 182), and at normal plasma amino acid levels it can elicit responses comparable in magnitude to those after stringent amino acid deletion (151). Similar effects have been observed with cyclic AMP (69, 146) and epinephrine (146, 182). How the enhancement of autophagy is ultimately achieved is not known, but it is clear that protein synthesis is not required (69, 182) and that it is not mediated by depletion of intracellular glucogenic amino acids as was once thought (138). It is important to point out that autophagic and proteolytic stimulation by glucagon is abolished at amino acid concentrations above twice normal plasma levels (151). The block appears to be selective since glycogenolysis is not affected (151). Although the reason for this effect is also obscure, it is possible that the inhibition depicted by curve III in Figure 3*b* in some way nullifies the hormonal effect.

Curiously, in muscle these agents suppress rather than stimulate proteolysis. In rat heart, for example, glucagon and the β-agonist isoproterenol inhibit macroautophagy and protein degradation (26, 32); similar effects of cyclic AMP and isoproterenol on proteolysis have been reported in skeletal muscle (49, 88).

INSULIN In contrast to the tissue-specific reversal in the direction of effects of glucagon and β-agonists, insulin as well as other growth-promoting factors are consistently inhibitory (13). In the mammal, insulin suppresses protein degradation in liver (69, 107, 125, 127), cardiac (31, 141, 169, 180) and skeletal muscle (48, 72), kidney (130), adipose tissue (175), and cultured cells (4, 5). However, because rates of protein degradation must be enhanced before insulin and amino acids can evoke inhibitory responses, the degree of suppression reflects the intrinsic catabolic activity of the tissue, which is specifically affected by caloric deprivation. In liver, for example, protein

breakdown in the absence of amino acids and insulin is highest at the start of the postabsorptive state, and decreases with starvation; accordingly, the magnitude of the inhibitory effects is largest in the early phase of deprivation (107, 139). By contrast, degradation in cardiac and skeletal muscle increases with starvation, and 24–48 h of deprivation may be required (57, 75, 87, 92, 140)—longer in older/larger rats (56, 57, 92, 100)—before consistent inhibitory effects are observed.

Virtually nothing is known of the mechanism by which insulin inhibits macroautophagy. However, recent studies in the perfused liver (111) have shown that insulin selectively blocks the sharp zonal loss of inhibitory effectiveness at normal plasma amino acid levels that is exhibited by curve II in Figure 3*b;* this suggests that it may be related closely to actions of the inhibitory amino acids. This finding illustrates the importance of characterizing the amino acid recognition before hormonal action at this site can be understood.

EFFECTS OF NUTRIENT INTAKE AND DEPRIVATION ON PROTEIN DEGRADATION IN VIVO

Alterations in Protein Degradation and Synthesis

The foregoing effects of caloric deprivation on proteolysis in isolated liver and muscle suggest that rates in muscle, in contrast to liver, increase during the first 24–48 h. However, it must be recognized that determinations in vitro, especially in the case of skeletal muscle, may not accurately reflect the proteolytic state in vivo. Inasmuch as passive stretch strongly inhibits protein breakdown in muscle (54, 99), rates may rise unpredictably after tissue isolation. Methods utilizing differences between rates of synthesis and net changes in muscle protein mass in vivo require few assumptions and, although somewhat cumbersome, do provide reasonably certain estimates (102). Results in rats have shown that protein synthesis and breakdown both fall slightly but remain in balance in the first 48 h of starvation; no demonstrable net loss of protein occurs during this time, although breakdown is accelerated after 48 h (99, 100, 102). Similar early decreases in muscle protein breakdown based on 3-methylhistidine excretion have been reported in protein-deficient rats and fasting man (183).

Rates of total hepatic protein synthesis and degradation also fall early in starvation in the mouse and rat (97, 105). But in contrast to muscle, protein breakdown in liver remains consistently greater than synthesis, and significant losses of protein occur early in the postabsorptive period (105). The ingestion of food after a period of deprivation decreases hepatic protein degradation dramatically (29, 70, 77, 99, 105) in association with the resynthesis of

intracellular protein and other constituents (Figure 1). This growth-associated response has also been observed in skeletal muscle (90) and kidney (19), and cultured human fibroblast (14a).

Degradative Mechanisms

MACROAUTOPHAGY The intensity of protein degradation in liver makes it possible to establish a quantitative correlation between proteolytic rates and alterations of the lysosomal-vacuolar system during starvation and subsequent refeeding (70). The net loss of protein can be attributed largely to ongoing macroautophagy (70, 124) and accelerated breakdown rates that are consistently higher than those of synthesis (70); the same mechanism could also explain the loss of RNA (8, 84, 85). Since the rate of protein synthesis per cell or liver is determined by the quantity of rRNA, the decline in synthesis during starvation will parallel the loss of protein (70). With refeeding, macroautophagy and the accelerated phase of breakdown are abruptly suppressed (70, 77, 128).

BASAL PROTEOLYSIS Basal protein degradation in rat liver, measured in the presence of ten times the normal level of plasma amino acids, decreases by 50–70% during 48 h of starvation (106). Because basal degradation accounts for a large fraction of total turnover under these conditions, the effect can explain most of the aforementioned decline in degradation in vivo. Since macroautophagic vacuoles are virtually nonexistent in the basal measurements, the decrease must reflect changes in a basal lysosomal or nonlysosomal mechanism. The first possibility seems probable since rates of degradation over the period of starvation correlated directly with pools of degradable intralysosomal protein (106).

As discussed earlier, the mechanism(s) of protein uptake by lysosomes under basal conditions remains an unsolved problem. Invaginations or flap-like extensions of the lysosomal membrane with the formation of internal vacuoles have been described by several investigators under the term *microautophagy* (40, 41, 70, 94, 126). But how well this process correlates with hepatic basal turnover is uncertain. In one report, microautophagy increased with caloric deprivation (40), and in another, the number of membrane deformations fell slightly (126). In cardiac muscle, microautophagy appears to increase with starvation (41). Another form of hepatic autophagy, denoted by the fusion of small, single-walled vesicles with dense bodies, has been described under the term *type A dense body* (105, 109, 152); similar profiles containing glycogen have been called glycogenosomes (6). While these particles are clearly autophagic in nature, they differ morphologically from the classic macroautophagic vacuole and also by the fact that they are

continuously generated under basal conditions (152). Evidence has been obtained that this population can be used as a marker for basal or microautophagy during starvation and refeeding in the mouse (70).

SUMMARY

The continuous turnover of intracellular protein and other macromolecules is a basic cellular process that serves, among other functions, to regulate cytoplasmic content and provide amino acids for ongoing oxidative and biosynthetic reactions during nutrient deprivation. The intensity of breakdown and pattern of regulation, though, vary widely among cells. Rat hepatocytes, for example, exhibit high absolute rates of proteolysis and regulatory effects that diminish during starvation, while corresponding responses in skeletal and cardiac muscle move in the opposite direction. It is also becoming apparent that effects of insulin and other acute regulatory agents on muscle breakdown are limited to nonmyofibrillar components. The latter may be sequestered and degraded within autophagic vacuoles, whereas myofibrillar proteins require an initial attack by calcium-dependent proteases in the cytosol. By contrast, most if not all of the breakdown of resident (long-lived) proteins as well as RNA in the hepatocyte can be explained by lysosomal mechanisms.

The uptake of cytoplasmic components by lysosomes can be divided into two major categories, macroautophagy and micro- or basal autophagy. The first is induced by amino acid or insulin/serum deprivation. In the hepatocyte, amino acids alone can regulate this process almost instantaneously over two thirds of the full range of proteolysis, 4.5% to 1.5% per hour. Glucagon, cyclic AMP, and β-agonists also stimulate macroautophagy in hepatocytes but have opposite effects in skeletal and cardiac myocytes. Basal autophagy differs from the macro type in that the cytoplasmic "bite" is smaller and sequestration is not acutely regulated. It is, however, adaptively decreased during starvation in parallel with absolute rates of basal turnover. Since endoplasmic reticulum comprises an appreciable fraction of the vacuolar content, volume sequestration would be compatible with the known heterogeneity of individual protein turnover if some proteins (or altered proteins) selectively bind to membranes.

The amino acid control of macroautophagy in the hepatocyte is accomplished by a small group of direct inhibitors (Leu, Tyr/Phe, Gln, Pro, Met, Trp, and His) and the permissive effect of alanine whereas only leucine is involved in myocytes and adipocytes. Of unusual interest is the fact that the inhibitory amino acid group alone evokes responses in perfused livers that are identical to those of a complete plasma mixture at 0.5 and 4 times normal plasma levels but loses effectiveness almost completely at normal concentrations. This loss is abolished by the addition of 0.5-mM alanine, which

is not directly inhibitory in the fed rat. These findings suggest a novel role for alanine that could be of importance in elucidating the link between energy demands and proteolysis in liver.

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Literature Cited

1. Addis, T., Lee, D. D., Lew, W., Poo, L. J. 1940. The protein content of the organs and tissues at different levels of protein consumption. *J. Nutr.* 19:199–205
2. Addis, T., Poo, L. J., Lew, W. 1936. The quantities of protein lost by the various organs and tissues of the body during a fast. *J. Biol. Chem.* 115:111–18
3. Ahlberg, J., Berkenstam, A., Henell, F., Glaumann, H. 1985. Degradation of short and long lived proteins in isolated rat liver lysosomes. Effects of pH, temperature, and proteolytic inhibitors. *J. Biol. Chem.* 260:5847–54
4. Amenta, J. S., Brocher, S. C. 1980. Mechanisms of protein turnover in cultured fibroblasts: Differential inhibition of two lysosomal mechanisms with insulin and NH_4Cl. *Exp. Cell Res.* 126:167–74
5. Amenta, J. S., Brocher, S. C. 1981. Mechanisms of protein turnover in cultured cells. *Life Sci.* 28:1195–1208
6. Amherdt, M., Harris, V., Renold, A. E., Orci, L., Unger, G. H. 1974. Hepatic autophagy in uncontrolled experimental diabetes and its relationship to insulin and glucagon. *J. Clin. Invest.* 54:188–93
7. Arstila, A. U., Trump, B. F. 1968. Studies on autophagocytosis: The formation of autophagic vacuoles in the liver following glucagon administration. *Am. J. Pathol.* 53:687–33
8. Ashford, A. J., Pain, V. M. 1986. Effect of diabetes on the rates of synthesis and degradation of ribosomes in rat muscle and liver in vivo. *J. Biol. Chem.* 261:4059–66
9. Ashford, T. P., Porter, K. R. 1962. Cytoplasmic components in hepatic cell lysosomes. *J. Cell Biol.* 12:198–202
10. Auteri, J. S., Okada, A., Bochaki, V., Dice, J. F. 1983. Regulation of intracellular protein degradation in IMR-90 human diploid fibroblasts. *J. Cell. Physiol.* 115:167–74
11. Bachmair, A., Finley, D., Varshavsky, A. 1986. In vivo half-life of a protein is a function of its amino-terminal residue. *Science* 234:179–86
12. Backer, J. M., Bourret, L., Dice, J. F. 1983. Regulation of catabolism of microinjected ribonuclease A requires the amino-terminal twenty amino acids. *Proc. Natl. Acad. Sci. USA* 80:2166–70

12a. Backer, J. M., Dice, J. F. 1986. Covalent linkage of ribonuclease S-peptide to microinjected proteins causes their intracellular degradation to be enhanced during serum withdrawal. *Proc. Natl. Acad. Sci. USA* 83:5830–34

13. Ballard, F. J., Gunn, J. M. 1982. Nutritional and hormonal effects on intracellular protein catabolism. *Nutr. Rev.* 40:33–42
14. Bates, P. C., Millward, D. J. 1983. Myofibrillar protein turnover. *Biochem. J.* 214:587–92

14a. Berger, J. J., Dice, J. F. 1986. Proteolysis in cultured cells during prolonged serum deprivation and replacement. *Am. J. Physiol.* 251:C748–53

15. Bigelow, S., Hough, R., Rechsteiner, M. 1981. The selective degradation of injected proteins occurs principally in the cytosol rather than the lysosomes. *Cell* 25:83–93
16. Blobel, G., Dobberstein, B. 1975. Transfer of proteins across membranes. I. Presence of proteolytically processed and unprocessed nascent immunoglobulin light chains on membrane-bound ribosomes of murine myeloma. *J. Cell Biol.* 67:835–51
17. Blouin, A., Bolender, R. P., Weibel, E. W. 1977. Distribution of organelles and

membranes between hepatocytes and nonhepatocytes in the rat liver parenchyma. *J. Cell Biol.* 72:441–55
18. Botbol, V., Scornik, O. A. 1983. Peptide intermediates in the degradation of cellular proteins. Bestatin permits their accumulation in mouse liver in vivo. *J. Biol. Chem.* 258:1942–49
19. Bur, J. A., Conde, R. D. 1982. Decreased rate of protein breakdown during nutritional recovery of mouse kidney. *Am. J. Physiol.* 243:E360–66
20. Buse, M. G., Biggers, J. F., Friderice, K. H., Buse, J. F. 1972. Oxidation of branched chain amino acids by isolated hearts and diaphragms of the rat. *J. Biol. Chem.* 247:8085–96
21. Buse, M. G., Reid, S. S. 1975. Leucine: A possible regulator of protein turnover in muscle. *J. Clin. Invest.* 56:1250–61
22. Cardell, R. R. Jr. 1977. Smooth endoplasmic reticulum in rat hepatocytes during glycogen deposition and depletion. *Int. Rev. Cytol.* 48:221–79
23. Chua, B. H. L., Kao, R. L., Rannels, D. E., Morgan, H. E. 1979. Inhibition of protein degradation by anoxia and ischemia in perfused rat hearts. *J. Biol. Chem.* 254:6617–23
24. Chua, B. H. L., Kleinhans, B. J. 1985. Effect of redox potential on protein degradation in perfused rat heart. *Am. J. Physiol.* 248:E726–31
25. Chua, B. H. L., Siehl, D. L., Morgan, H. E. 1979. Effect of leucine and metabolites of branched chain amino acids on protein turnover in heart. *J. Biol. Chem.* 254:8358–62
26. Chua, B. H. L., Watkins, C. A., Siehl, D. L., Morgan, H. E. 1978. Effects of epinephrine and glucagon on protein turnover in perfused rat heart. *Fed. Proc.* 37:1333A
27. Ciechanover, A., Finley, D., Varshavsky, A. 1984. Ubiquitin dependence of selective protein degradation demonstrated in the mammalian cell cycle mutant ts85. *Cell* 37:57–66
28. Ciechanover, A., Finley, D., Varshavsky, A. 1984. The ubiquitin-mediated proteolytic pathway and mechanisms of energy-dependent intracellular protein degradation. *J. Cell Biochem.* 24:27–53
29. Conde, R. D., Scornik, O. A. 1976. Role of protein degradation in the growth of livers after a nutritional shift. *Biochem. J.* 158:385–90
30. Crabb, D. W., Harris, R. A. 1978. Studies on the regulation of leucine catabolism in the liver. Stimulation by pyruvate and dichloroacetate. *J. Biol. Chem.* 253:1481–87
31. Curfman, G. D., O'Hara, D. S., Hopkins, B. E., Smith, T. W. 1980. Suppression of myocardial protein degradation in the rat during fasting. *Circ. Res.* 46:581–89
32. Dämmrich, J., Pfeifer, U. 1981. Acute effects of isoproterenol on cellular autophagy. Inhibition in myocardium but stimulation in liver parenchyma. *Virchows Arch. (Cell Pathol.)* 38:209–18
33. Dämmrich, J., Pfeifer, U. 1983. Cardiac hypertrophy in rats after supravalvular aortic constriction. II. Inhibition of cellular autophagy in hypertrophying cardiomyocytes. *Virchows Arch. (Cell Pathol.)* 43:287–307
34. Dean, R. T. 1977. Lysosomes and membrane recycling. A hypothesis. *Biochem. J.* 168:603–5
35. Dean, R. T. 1979. Macrophage protein turnover: Evidence for lysosomal participation in basal proteolysis. *Biochem. J.* 180:339–45
36. de Duve, C., Wattiaux, R. 1966. Functions of lysosomes. *Ann. Rev. Physiol.* 28:435–92
37. DeMartino, G. N., Croall, D. E. 1985. Calcium-dependent proteases from liver and heart. In *Intracellular Protein Catabolism*, ed. E. A. Khairallah, J. S. Bond, J. W. C. Bird, pp. 117–26. New York: Liss. 702 pp.
38. Deter, R. L., Baudhuin, P., de Duve, C. 1967. Participation of lysosomes in cellular autophagy induced in rat liver by glucagon. *J. Cell Biol.* 35:c11–16
39. Deter, R. L., de Duve, C. 1967. Influence of glucagon, an inducer of cellular autophagy, on some physical properties of rat liver lysosomes. *J. Cell Biol.* 33:437–49
40. de Waal, E. J., Vreeling-Sindelárová, H., Schellens, J. P. M., Houtkooper, J. M., James, J. 1986. Quantitative changes in the lysosomal vacuolar system of rat hepatocytes during short-term starvation. A morphometric analysis with special reference to macro- and microautophagy. *Cell Tissue Res.* 243:641–48
41. de Waal, E. J., Vreeling-Sindelárová, H., Schellens, J. P. M., James, J. 1986. Starvation-induced microautophagic vacuoles in rat myocardial cells. *Cell Biol. Int. Rep.* 10:527–33
42. Dice, J. F., Backer, J. M., Miao, P., Bourret, L., McElliogott, M. A. 1985. Regulation of catabolism of ribonuclease A microinjected into human fibroblasts. See Ref. 37, pp. 385–94

42a. Dice, J. F., Chiang, H.-L., Spencer, E. P., Backer, J. M. 1986. Regulation of catabolism of microinjected ribonuclease A. Identification of residues 7–11 as the essential pentapeptide. *J. Biol. Chem.* 261:6853–59
43. Dice, J. F., Walker, C. D., Byrne, B., Cardiel, A. 1978. General characteristics of protein degradation in diabetes and starvation. *Proc. Natl. Acad. Sci. USA* 75:2093–97
44. Epstein, D., Elias-Bishko, S., Hershko, A. 1975. Requirement for protein synthesis in the regulation of protein breakdown in cultured hepatoma cells. *Biochemistry* 14:5199–5204
45. Evans, P. J., Mayer, R. J. 1983. Degradation of transplanted mitochondrial proteins by hepatocyte monolayers. *Biochem. J.* 216:151–61
46. Fagan, J. M., Goldberg, A. L. 1985. The rate of protein degradation in isolated skeletal muscle does not correlate with reduction-oxidation status. *Biochem. J.* 227:689–94
47. Fawcett, D. W. 1955. Observations on the cytology and electron microscopy of hepatic cells. *J. Natl. Cancer Inst.* 15:1475–1503
48. Fulks, R. M., Li, J. B., Goldberg, A. L. 1975. Effects of insulin, glucose, and amino acids on protein turnover in rat diaphragm. *J. Biol. Chem.* 250:290–98
49. Garber, A. J., Karl, I. E., Kipnis, D. M. 1976. Alanine and glutamine synthesis and release from skeletal muscle. *J. Biol. Chem.* 251:851–57
50. Garlick, P. J., Millward, D. J., James, W. P. T. 1973. The diurnal response of muscle and liver protein synthesis in vivo in meal-fed rats. *Biochem. J.* 136:935–45
51. Garlick, P. J., Millward, D. J., James, W. P. T., Waterlow, J. C. 1975. The effect of protein deprivation and starvation on the rate of protein synthesis in tissues of the rat. *Biochim. Biophys. Acta* 414:71–84
52. Glaumann, H., Ericsson, J. L. E., Marzella, L. 1981. Mechanisms of intralysosomal degradation with special reference to autophagocytosis and heterophagocytosis of cell organelles. *Int. Rev. Cytol.* 73:149–82
53. Goldberg, A. L., St. John, A. C. 1976. Intracellular protein degradation in mammalian and bacterial cells: Part II. *Ann. Rev. Biochem.* 45:747–803
54. Goldspink, D. F. 1976. The effects of denervation on protein turnover in rat skeletal muscle. *Biochem. J.* 156:71–80
55. Goll, D. E., Edmunds, T., Kleese, W. C., Sathe, S. K., Shannon, J. D. 1985. Some properties of the Ca^{2+}-dependent proteinase. See Ref. 37, pp. 151–64
56. Goodman, M. N., Larsen, P. R., Kaplan, M. M., Aoki, T. T., Young, V. R., Ruderman, N. B. 1980. Starvation in the rat. II. Effect of age and obesity on protein sparing and fuel metabolism. *Am. J. Physiol.* 239:E277–86
57. Goodman, M. N., McElany, M. A., Ruderman, N. B. 1981. Adaptation to prolonged starvation in the rat: Curtailment of skeletal muscle proteolysis. *Am. J. Physiol.* 241:E321–27
58. Grinde, B. 1983. Role of Ca^{2+} for protein turnover in isolated rat hepatocytes. *Biochem. J.* 216:529–36
59. Grinde, B. 1985. Autophagy and lysosomal proteolysis in the liver. *Experientia* 41:1089–95
60. Grinde, B., Seglen, P. O. 1980. Differential effects of proteinase inhibitors and amines on the lysosomal and nonlysosomal pathways of protein degradation in isolated rat hepatocytes. *Biochim. Biophys. Acta* 632:73–86
61. Gronostajki, R. M., Pardee, A. B., Goldberg, A. L. 1985. The ATP dependence of degradation of short-lived and long-lived proteins in growing fibroblasts. *J. Biol. Chem.* 260:3344–49
62. Hendil, K. B. 1980. Intracellular degradation of hemoglobin transferred into fibroblasts by fusion with red blood cells. *J. Cell. Physiol.* 105:449–60
63. Henell, F., Glaumann, H. 1984. Effect of leupeptin on the autophagic vacuolar system of rat hepatocytes. *Lab. Invest.* 51:46–56
64. Henell, F., Glaumann, H. 1985. Participation of lysosomes in basal proteolysis in perfused rat liver. *Exp. Cell Res.* 158:257–62
65. Hershko, A., Tomkins, G. M. 1971. Studies on the degradation of tyrosine aminotransferase in hepatoma cells in culture. *J. Biol. Chem.* 246:710–14
66. Hirsimäki, P., Arstila, A. U., Trump, B. F., Marzella, L. 1983. Autophagocytosis. In *Pathobiology of Cell Membranes,* ed. A. U. Arstila, B. F. Trump, 3:201–35. New York/London: Academic. 282 pp.
67. Hollemans, M., Elferinke, R. O., de Groot, P., Strijland, A., Tager, J. M. 1981. Accumulation of weak bases in relation to intralysosomal pH in cultured human skin fibroblasts. *Biochim. Biophys. Acta* 643:140–51
68. Hopgood, M. F., Clark, M. G., Ballard, F. J. 1977. Inhibition of protein degra-

dation in isolated rat hepatocytes. *Biochem. J.* 164:399–407
69. Hopgood, M. F., Clark, M. G., Ballard, F. J. 1980. Protein degradation in hepatocyte monolayers. Effects of glucagon, adenosine 3':5'-cyclic monophosphate and insulin. *Biochem. J.* 186:71–79
70. Hutson, N. J., Mortimore, G. E. 1982. Suppression of cytoplasmic protein uptake by lysosomes as the mechanism of protein regain in livers of starved-refed mice. *J. Biol. Chem.* 257:9548–54
71. Izzo, J. L., Glasser, S. R. 1961. Comparative effects of glucagon, hydrocortisone, and epinephrine on the protein metabolism of the fasting rat. *Endocrinology* 68:189–98
72. Jefferson, L. S., Li, J. B., Rannels, S. R. 1977. Regulation by insulin of amino acid release and protein turnover in the perfused rat hemicorpus. *J. Biol. Chem.* 252:1476–83
73. Jefferson, L. S., Rannels, D. E., Munger, B. L., Morgan, H. E. 1974. Insulin in the regulation of protein turnover in heart and skeletal muscle. *Fed. Proc.* 33:1098–1104
74. Jones, T. L., Ogunro, E. A., Samarel, A. M., Ferguson, A. G., Lesch, M. 1983. Susceptibilities of cardiac myofibrillar proteins to cathepsin D-catalyzed degradation. *Am. J. Physiol.* 245:H294–99
75. Kadowaki, M., Nagasawa, T., Hirata, T., Noguchi, T., Naito, H. 1985. Effects of insulin, amino acids and fasting on myofibrillar protein degradation in perfused hindquarters of rats. *J. Nutr. Sci. Vitaminol. (Tokyo)* 31:431–40
76. Kameyama, T., Etlinger, J. D. 1979. Calcium-dependent regulation of protein synthesis and degradation in muscle. *Nature* 279:344–46
77. Khairallah, E. A. 1978. In vivo determination of rates of protein degradation in livers of meal-fed rats: Implications of amino acid compartmentation. In *Protein Turnover and Lysosome Function,* ed. H. L. Segal, D. J. Doyle, 6:89–104. New York/London: Academic. 790 pp.
78. Kleinhans, B. J., Chua, B. H. L., Lautensack-Belser, N. 1985. Is leucine's effect on protein degradation mediated by α-ketoisocaproate in rat heart? *Fed. Proc.* 44:1091A
79. Knowles, S. E., Ballard, F. J. 1976. Selective control of the degradation of normal and aberrant proteins in Reuber H35 hepatoma cells. *Biochem. J.* 156:609–17
80. Kominami, E., Hashida, S., Khairallah, E. A., Katunuma, N. 1983. Sequestration of cytoplasmic enzymes in an autophagic vacuole-lysosomal system induced by injection of leupeptin. *J. Biol. Chem.* 258:6093–6100
81. Kosterlitz, H. W. 1944. Effect of dietary protein on liver cytoplasm. *Nature* 154:207–9
82. Kosterlitz, H. W., Cramb, I. D. 1943. The effect of fasting on the protein content of the liver. *J. Physiol.* 102:18P
83. Kovács, J., Fellinger, E., Kárpáti, P. A., Kovács, A. L., László, L. 1986. The turnover of autophagic vacuoles: Evaluation by quantitative electron microscopy. *Biomed. Biochim. Acta.* 45:1543–47
84. Lardeux, B. R., Heydrick, S. J., Mortimore, G. E. 1987. RNA degradation in perfused rat liver as determined from the release of [^{14}C]cytidine. *J. Biol. Chem.* Submitted
85. Lardeux, B. R., Mortimore, G. E. 1987. Amino acid and hormonal control of macromolecular turnover in the perfused rat liver: Evidence for two autophagic pathways. *J. Biol. Chem.* Submitted
86. Leverve, X. M., Caro, L. H. P., Plomp, P. J. A. M., Meijer, A. J. 1986. Control of proteolysis in isolated rat hepatocytes. *Biochem. Soc. Trans.* (Abstr.). 14:1062–63
87. Li, J. B., Goldberg, A. L. 1976. Effects of food deprivation on protein synthesis and degradation in rat skeletal muscles. *Am. J. Physiol.* 231:441–48
88. Li, J. B., Jefferson, L. S. 1977. Effect of isoproterenol on amino acid levels and protein turnover in skeletal muscle. *Am. J. Physiol.* 232:E243–49
89. Li, J. B., Jefferson, L. S. 1978. Influence of amino acid availability on protein turnover in perfused skeletal muscle. *Biochim. Biophys. Acta* 544:351–59
90. Li, J. B., Wassner, S. J. 1978. Effects of food deprivation and refeeding on actomyosin degradation. *Am. J. Physiol.* 246:E32–37
91. Libby, P., Goldberg, A. L. 1978. Leupeptin, a protease inhibitor, decreases protein degradation in normal and diseased muscles. *Science* 199:534–36
92. Lowell, B. B., Ruderman, N. B., Goodman, M. N. 1986. Evidence that lysosomes are not involved in the degradation of myofibrillar proteins in rat skeletal muscle. *Biochem. J.* 234:237–40
93. Luck, D. J. L. 1961. Glycogen synthesis from uridine diphosphate glucose. The

distribution of the enzyme in liver cell fractions. *J. Cell Biol.* 10:195–209
94. Marzella, L., Ahlberg, J., Glaumann, H. 1980. In vitro uptake of particles by lysosomes. *Exp. Cell Res.* 129:460–65
95. Mayer, J., Doherty, F. 1986. Intracellular protein catabolism: State of the art. *FEBS Lett.* 198:181–93
95a. McElligott, M. A., Miao, P., Dice, J. F. 1985. Lysosomal degradation of ribonuclease A and ribonuclease S-protein microinjected into the cytosol of human fibroblasts. *J. Biol. Chem.* 260:11985–93
96. McKinley, C. J., Turinsky, J. 1986. Protaglandin E_2 and muscle proteolysis: Effect of burn injury and cycloheximide. *Am. J. Physiol.* 250:R207–10
97. McNurlan, M. A., Tomkins, A. M., Garlick, P. J., 1979. The effect of starvation on the rate of protein synthesis in the rat liver and small intestine. *Biochem. J.* 178:373–79
98. Miller, L. L. 1960. Glucagon: A protein catabolic hormone in the isolated perfused rat liver. *Nature* 185:248
99. Millward, D. J. 1980. Protein degradation in muscle and liver. In *Comprehensive Biochemistry,* ed. M. Florkin, E. H. Stotz, 19B(1):153–232. Amsterdam: Elsevier/North-Holland. 528 pp.
100. Millward, D. J., Garlick, P. J., Nnanyelugo, D. O., Waterlow, J. C. 1976. The relative importance of muscle protein synthesis and breakdown in the regulation of muscle mass. *Biochem. J.* 156:185–88
101. Millward, D. J., Nnanyelugo, D. O., James, W. P. T., Garlick, P. J. 1974. Protein metabolism in skeletal muscle: The effect of feeding and fasting on muscle RNA, free amino acids and plasma insulin concentrations. *Br. J. Nutr.* 32:127–42
102. Millward, D. J., Waterlow, J. C. 1978. Effect of nutrition on protein turnover in skeletal muscle. *Fed. Proc.* 37:2283–89
103. Mitch, W. E., Clark, A. S. 1984. Specificity of the effects of leucine and its matabolites on protein degradation in skeletal muscle. *Biochem. J.* 222:579–86
104. Mitchener, J. S., Shelburne, J. D., Bradford, W. D., Hawkins, H. K. 1976. Cellular autophagocytosis induced by deprivation of serum and amino acids in HeLa cells. *Am. J. Pathol.* 83:485–98
105. Mortimore, G. E., Hutson, N. J., Surmacz, C. A. 1983. Quantitative correlation between proteolysis and macro- and microautophagy in mouse hepatocytes during starvation and refeeding. *Proc. Natl. Acad. Sci. USA* 80:2179–83
106. Mortimore, G. E., Lardeux, B. R., Wert, J. J. Jr., Adams, C. E. 1987. Adaptive regulation of basal autophagy and protein turnover in rat liver: Effects of short-term starvation. *J. Biol. Chem.* Submitted
107. Mortimore, G. E., Mondon, C. E. 1970. Inhibition by insulin of valine turnover in liver. *J. Biol. Chem.* 245:2375–83
108. Mortimore, G. E., Neely, A. N., Cox, J. R., Guinivan, R. A. 1973. Proteolysis in homogenates of perfused rat liver; Responses to insulin, glucagon, and amino acids. *Biochem. Biophys. Res. Commun.* 54:89–95
109. Mortimore, G. E., Schworer, C. M. 1977. Induction of autophagy by amino acid deprivation in perfused rat liver. *Nature* 270:174–76
110. Mortimore, G. E., Ward, W. F. 1981. Internalization of cytoplasmic protein by hepatic lysosomes in basal and deprivation-induced proteolytic states. *J. Biol. Chem.* 256:7659–65
111. Mortimore, G. E., Pösö, A. R., Kadowaki, M., Wert, J. J. Jr. 1987. Multiphasic control of hepatic protein degradation by regulatory amino acids: General features and modulation by insulin and glucagon. *J. Biol. Chem.* Submitted
112. Murachi, T., Yoshimura, N. 1985. Intracellular localization of low and high calcium-requiring forms of calpain. See Ref. 37, pp. 165–74
113. Neely, A. N., Cox, J. R., Fortney, J. A., Schworer, C. M., Mortimore, G. E. 1977. Alterations of lysosomal size and density during rat liver perfusion: Suppression by amino acids and insulin. *J. Biol. Chem.* 252:6948–54
114. Neely, A. N., Nelson, P. B., Mortimore, G. E., 1974. Osmotic alterations of the lysosomal system during rat liver perfusion: Reversible suppression by insulin and amino acids. *Biochim. Biophys. Acta* 338:458–72
115. Neff, N. T., DeMartino, G. N., Goldberg, A. L. 1979. The effect of protease inhibitors and decreased temperature on the degradation of different classes of proteins in cultured hepatocytes. *J. Cell. Physiol.* 101:439–58
116. Novikoff, A. B., Shin, W.-Y. 1978. Endoplasmic reticulum and autophagy in rat hepatocytes. *Proc. Natl. Acad. Sci. USA* 75:5039–42
117. Odessey, R. 1985. Effects of inhibitors of proteolysis and arachidonic acid metabolism on burn-induced protein breakdown. *Metabolism* 34:616–20

118. Ohkuma, S., Poole, B. 1978. Fluorescence probe measurement of the intralysosomal pH in living cells and the perturbation of pH by various agents. *Proc. Natl. Acad. Sci. USA* 75:3327–31
119. Ojamaa, K. M. 1985. *Roles of muscle contraction and availability of initiator methionyl transfer RNA in the translational control of protein synthesis.* PhD thesis. Penn. State Univ., Hershey. 135 pp.
120. Ord, J. M., Wakeland, J. R., Crie, J. S., Wildenthal, K. 1983. Mechanisms of degradation of myofibrillar and nonmyofibrillar protein in heart. *Adv. Myocardiol.* 4:198–99
121. Papadopoulos, T., Pfeifer, U. 1986. Regression of rat liver autophagic vacuoles by locally applied cycloheximide. *Lab. Invest.* 54:100–7
122. Parrilla, R., Goodman, M. N. 1974. Nitrogen metabolism in the isolated perfused rat liver. Nitrogen balance, redox state and rates of proteolysis. *Biochem. J.* 138:341–48
123. Peavy, D. E., Hansen, R. J. 1976. Lack of effect of amino acid concentration on protein synthesis in the perfused rat liver. *Biochem. J.* 160:797–801
124. Pfeifer, U. 1973. Cellular autophagy and cell atrophy in the rat liver during long-term starvation. *Virchows Arch. B* 12:195–211
125. Pfeifer, U. 1978. Inhibition by insulin of the formation of autophagic vacuoles in rat liver. *J. Cell Biol.* 78:152–67
126. Pfeifer, U. 1981. Morphological aspects of intracellular protein degradation: Autophagy. *Acta Biol. Med. Ger.* 40:1619–24
127. Pfeifer, U. 1984. Methods in laboratory investigation. Application of test substances to the surface of rat liver in situ: Opposite effects of insulin and isoproterenol on cellular autophagy. *Lab. Invest.* 50:348–54
128. Pfeifer, U., Bertling, J. 1977. A morphometric study of the inhibition of autophagic degradation during restorative growth of liver cells in rats re-fed after starvation. *Virchows Arch. B (Cell Pathol.)* 24:109–20
129. Pfeifer, U., Strauss, P. 1981. Autophagic vacuoles in heart muscle and liver. A comparative morphometric study including circadian variations in meal-fed rats. *J. Mol. Cell. Cardiol.* 13:37–49
130. Pfeifer, U., Warmuth-Metz, M. 1983. Inhibition by insulin of cellular autophagy in proximal tubular cells of rat kidney. *Am. J. Physiol.* 244:E109–14
131. Pontremoli, S., Melloni, E. 1985. Human erythrocyte Ca^{2+}-dependent neutral proteinase. New approaches to the understanding of the physiological function. See Ref. 37, pp. 127–40
132. Poole, B., Ohkuma, S., Warburton, M. 1978. Some aspects of the intracellular breakdown of exogenous and endogenous proteins. See Ref. 77, pp. 43–58
133. Poole, B., Wibo, M. 1973. Protein degradation in cultured cells. *J. Biol. Chem.* 248:6221–26
134. Porter, K. R., Bruni, C. 1959. An electron microscope study of the early effects of 3'-Me-DAB on rat liver cells. *Cancer Res.* 19:997–1009
135. Pösö, A. R., Mortimore, G. E. 1984. Requirement for alanine in the amino acid control of deprivation-induced protein degradation in liver. *Proc. Natl. Acad. Sci. USA* 81:4270–74
136. Pösö, A. R., Mortimore, G. E. 1985. Amino acid control of hepatic protein degradation: Novel requirement for alanine. See Ref. 37, pp. 427–36
137. Pösö, A. R., Pentillä, K. E., Suolinna, E.-M., Lindros, K. O. 1986. Urea synthesis in freshly isolated and in cultured periportal and perivenous hepatocytes. *Biochem. J.* 239:263–67
138. Pösö, A. R., Schworer, C. M., Mortimore, G. E. 1982. Acceleration of proteolysis in perfused rat liver by deletion of glucogenic amino acids: Regulatory role of glutamine. *Biochem. Biophys. Res. Commun.* 107:1433–39
139. Pösö, A. R., Wert, J. J. Jr., Mortimore, G. E. 1982. Multi-functional control by amino acids of deprivation-induced proteolysis in liver: Role of leucine. *J. Biol. Chem.* 257:12114–20
140. Preedy, V. R., Smith, D. M., Kearney, N. F., Sugden, P. H. 1984. Rates of protein turnover in vivo and in vitro in ventricular muscle of hearts from fed and starved rats. *Biochem. J.* 222:395–400
141. Rannels, D. E., Kao, R., Morgan, H. E. 1975. Effect of insulin on protein turnover in heart muscle. *J. Biol. Chem.* 250:1694–1701
142. Reddy, M. K., Etlinger, J. D., Rabinowitz, M., Fischman, D. A. 1975. Removal of Z-lines and α-actinin from isolated myofibrils by a calcium-activated neutral protease. *J. Biol. Chem.* 257: 1632–38
143. Rodemann, H. P., Goldberg, A. L. 1982. Arachidonic acid, prostaglandin E2 and F2 influence rates of protein turnover in skeletal and cardiac muscle. *J. Biol. Chem.* 257:1632–38
144. Rodemann, H. P., Waxman, L., Goldberg, A. L. 1982. The stimulation of

protein degradation in muscle by Ca^{2+} is mediated by prostaglandin E2 and does not require the calcium-activated protease. *J. Biol. Chem.* 257:8716–23

145. Rogers, S. W., Rechsteiner, M. C. 1985. Degradation rates and intracellular distributions of structurally characterized proteins injected into HeLa cells. See Ref. 37, pp 405–16
146. Rosa, F. 1971. Ultrastructural changes produced by glucagon, cyclic 3':5'-AMP and epinephrine on perfused livers. *J. Ultrastruct. Res.* 34:205–13
147. Russell, S. M., Mayer, R. J. 1983. Degradation of transplanted rat liver mitochondrial-outer-membrane proteins in hepatoma cells. *Biochem. J.* 216:163–75
148. Saito, T., Ogawa, K. 1974. Lysosomal changes in rat hepatic parenchymal cells after glucagon administration. *Acta Histochem. Cytochem.* 7:1–18
149. Schiaffiano, S. S., Hanzilkova, V. 1972. Studies on the effect of denervation in developing muscle. II. The lysosomal system. *J. Ultrastruct. Res.* 39:1–14
150. Schworer, C. M., Cox, J. R., Mortimore, G. E. 1979. Alterations of lysosomal density by sequestered glycogen during deprivation-induced autophagy in rat liver. *Biochem. Biophys. Res. Commun.* 87:163–70
151. Schworer, C. M., Mortimore, G. E. 1979. Glucagon-induced autophagy and proteolysis in rat liver: Mediation by selective deprivation of intracellular amino acids. *Proc. Natl. Acad. Sci. USA* 76:3169–73
152. Schworer, C. M., Shiffer, K. A., Mortimore, G. E. 1981. Quantitative relationship between autophagy and proteolysis during graded amino acid deprivation in perfused rat liver. *J. Biol. Chem.* 256:7652–58
153. Scornik, O. A. 1983. Faster protein degradation in response to decreased steady state levels of amino acylation of $tRNA^{his}$ in Chinese hamster ovary cells. *J. Biol. Chem.* 258:882–86
154. Scornik, O. A., Ledbetter, M. L. S., Malter, J. S. 1980. Role of aminoacylation of histidyl-tRNA in the regulation of protein degradation in Chinese hamster ovary cells. *J. Biol. Chem.* 255:6322–29
155. Seglen, P. O., Gordon, P. B. 1982. 3-Methyladenine: Specific inhibitor of autophagic/lysosomal protein degradation in isolated rat hepatocytes. *Proc. Natl. Acad. Sci. USA* 79:1889–92
156. Seglen, P. O., Gordon, P. B. 1984. Amino acid control of autophagic sequestration and protein degradation in isolated rat hepatocytes. *J. Cell Biol.* 99:435–44
157. Seglen, P. O., Gordon, P. B., Poli, A. 1980. Amino acid inhibition of the autophagic/lysosomal pathway of protein degradation in isolated rat hepatocytes. *Biochim. Biophys. Acta* 630:103–18
158. Seglen, P. O., Solheim, A. E. 1978. Valine uptake and incorporation into protein in isolated rat hepatocytes. *Eur. J. Biochem.* 85:15–25
159. Shiman, R., Gray, D. W. 1980. Substrate activation of phenylalanine hydroxylase. *J. Biol. Chem.* 255:4793–4800
160. Shiman, R., Mortimore, G. E., Schworer, C. M., Gray, D. W. 1982. Regulation of phenylalanine hydroxylase activity by phenylalanine in vivo, in vitro, and in perfused rat liver. *J. Biol. Chem.* 257:11213–16
161. Silver, G., Etlinger, J. D. 1985. Regulation of myofibrillar protein accumulation in chick muscle cultures: Evidence for the involvement of calcium and lysosomes in non-uniform turnover of contractile proteins. *J. Cell Biol.* 101:2382–91
162. Smith, D. M., Sugden, P. H. 1983. Effect of insulin and lack of effect on workload and hypoxia on protein degradation in the perfused working rat heart. *Biochem. J.* 210:55–61
163. Smith, D. M., Sugden, P. H. 1986. Contrasting response of protein degradation to starvation and insulin as measured by release of N^{τ}-methylhistidine or phenylalanine from the perfused rat heart. *Biochem. J.* 237:391–95
164. Soberón, G., Sánchez, Q. E. 1961. Changes in effective enzyme concentrations in the growing rat liver. *J. Biol. Chem.* 236:1602–6
165. Solheim, A. E., Seglen, P. O. 1980. Subcellular distribution of proteolytically generated valine in isolated rat hepatocytes. *Eur. J. Biochem.* 107: 587–96
166. Sommercorn, J. M., Swick, R. W. 1981. Protein degradation in primary monolayer cultures of adult rat hepatocytes. *J. Biol. Chem.* 256:4816–21
167. Stacey, D. W., Allfrey, V. G. 1977. Evidence for the autophagy of microinjected proteins in HeLa cells. *J. Cell Biol.* 75:807–17
168. Sugden, P. H. 1980. Effects of calcium ions, ionophore A23187 and inhibition of energy metabolism in the rat diaphragm and epitrochlearis muscles in vitro. *Biochem. J.* 190:593–603
169. Sugden, P. H., Smith, D. M. 1982. The

effects of glucose, acetate, lactate and insulin on protein degradation in the perfused rat heart. *Biochem. J.* 206:467–72
170. Surmacz, C. A., Pösö, A. R., Mortimore, G. E. 1987. Regulation of lysosomal fusion during deprivation-induced autophagy in perfused rat liver. *Biochem. J.* 242:453–58
171. Tischler, M. E. 1980. Is regulation of proteolysis associated with redox-state changes in rat skeletal muscle? *Biochem. J.* 192:963–66
172. Tischler, M. E. 1981. Minireview: Hormonal regulation of protein degradation in skeletal and cardiac muscle. *Life Sci.* 28:2569–76
173. Tischler, M. E., Desautels, M., Goldberg, A. L. 1982. Does leucine, leucyl-tRNA, or some metabolite of leucine regulate protein synthesis and degradation in rat skeletal and cardiac muscle? *J. Biol. Chem.* 257:1613–21
174. Tischler, M. E., Goldberg, A. L. 1980. Leucine degradation and release of glutamine and alanine by adipose tissue. *J. Biol. Chem.* 255:8074–81
175. Tischler, M. E., Ost, A. H., Spina, B., Cook, P. H., Coffman, J. 1984. Regulation of protein turnover by glucose, insulin, and amino acids in adipose tissue. *Am. J. Physiol.* 247:C228–33
176. Vandenburgh, H., Kaufman, S. 1980. Protein degradation in embryonic skeletal muscle. Effects of medium, cell type, inhibitors, and passive stretch. *J. Biol. Chem.* 255:5826–33
177. Ward, W. F., Chua, B. L., Li, J. B., Morgan, H. E., Mortimore, G. E. 1979. Inhibition of basal and deprivation-induced proteolysis by leupeptin and pepstatin in perfused rat liver and heart. *Biochem. Biophys. Res. Commun.* 87: 92–98
178. Waterlow, J. C., Garlick, P. J., Millward, D. J. 1978. *Protein Turnover in Mammalian Tissues and in the Whole Body,* pp. 489–96. Amsterdam: Elsevier/North-Holland. 804 pp.
179. Wheatley, D. N. 1984. Intracellular protein degradation: Basis of a self-regulating mechanism for the proteolysis of endogenous proteins. *J. Theor. Biol.* 107:127–49
180. Wildenthal, K., Griffen, E. E., Ingwall, J. S. 1976. Hormonal control of cardiac protein and amino acid balance. *Circ. Res.* 38(Suppl.):138–42
181. Woodside, K. H., Mortimore, G. E. 1972. Suppression of protein turnover by amino acids in the perfused rat liver. *J. Biol. Chem.* 247:6474–81
182. Woodside, K. H., Ward, W. F., Mortimore, G. E. 1974. Effects of glucagon on general protein degradation and synthesis in perfused rat liver. *J. Biol. Chem.* 249:5458–63
183. Young, V. R., Munro, H. N. 1978. N^{τ}-methylhistidine (3-methyl-histidine) and muscle protein turnover: An overview. *Fed. Proc.* 37:2291–2300
184. Zeman, R. J., Kameyama, T., Matsumoto, K., Bernstein, P., Etlinger, J. D. 1985. Regulation of protein degradation in muscle by calcium. *J. Biol. Chem.* 260:13619–24