Progress report

The synthesis and degradation of liver-produced proteins

In mammalian protein metabolism the term 'turnover' is used to convey the concept of the combined synthesis and degradation of both intracellular and secreted proteins. Although the synthetic and degradative processes are independently sited and are probably controlled by separate regulatory mechanisms, there exists in steady state conditions a means for the maintenance of a balance between the two. Only in these circumstances is the term 'turnover rate' synonymous with both the rate of synthesis and degradation. In the case of liver-produced proteins it is more often the rate of degradation which is measured and the rate of synthesis inferred when evidence is available that protein pool sizes are constant. With the development of methods for the direct measurement of hepatic protein synthetic rates in vivo, there is little justification for the retention of imprecise terminology. The measured process should be clearly defined.

For the purposes of this review, however, the concept of 'turnover' is retained in order to emphasize the principle that proteins secreted by the cell for export as well as proteins retained by the cell for home consumption consist of labile molecules which are continuously being catabolized and resynthesized. This phenomenon is not confined to situations in which there is net transfer of amino acid nitrogen from one tissue to another, nor is it prevented by the supply of exogenous protein sufficient to keep the organism in nitrogen balance. One cannot do better than quote from Schoenheimer's classical work on the 'Dynamic state of body constituents' published in 1942 which gives support to 'the generalization that all the reactions which the organism is capable of performing with protein constituents are carried out continually, even when the total amount and constitution of the proteins stay constant'. The amino acid products of protein degradation may undergo obligatory participation in a variety of metabolic cycles with the formation of new metabolites. These processes, like that of protein degradation, cannot be prevented by the exogenous supply of identical substances.

Although the basic concept of the dynamic interaction of body nitrogen was first proposed by Borsook and Keighley in 1935, it was not until the stable isotope of nitrogen, $^{15}$N, became available for biological use that it was possible to monitor accurately the interaction of food nitrogen with that of plasma proteins. These pioneer studies gave convincing experimental support to the hypothesis of a cyclic process of release and uptake of nitrogen by the tissues to and from a circulating metabolic pool of amino acids. In the case of the liver, this process is not dependent upon the replacement of effete cells by new cells. The life span of the rat liver cell is at least four to five months in contrast to the half life of total hepatic protein of two to four days.

The earliest measurement of protein turnover rates was based upon isotopic tracer techniques. Schoenheimer and his coworkers at Columbia University obtained the first measurements of turnover rates of individual
human proteins by the use of isotopically labelled biological compounds. In the course of these studies it was observed that oral administration of $^{15}$N-labelled glycine to man was followed by an early rapid phase of incorporation of $^{15}$N into newly synthesized circulating plasma protein\textsuperscript{10}. The peak of incorporation was immediately succeeded by a decline in the enrichment of the circulating proteins (Fig. 1a). Following the rapid phase of distribution throughout body compartments, the isotopic concentration fell at a rate which approximated a first-order exponential function, representing degradation and progressive replacement of labelled protein molecules with newly synthesized but unlabelled ones. In spite of the pitfalls due to reutilization of labelled amino acid and the heterogeneity of the plasma protein population, the biological decay of $^{15}$N-labelled protein indicated an average half-life of between 18 and 21 days, a figure not far different from that obtained for plasma albumin by more recent, refined labelling techniques\textsuperscript{11}. The sequel of a first-order mode of decay on completion of incorporation signified that the catabolic process was indiscriminate in singling out for degradation protein molecules of differing longevities. It mattered not whether protein molecules had been present in the circulation for a considerable length of time or had been newly synthesized and released from the liver. They were equally likely to be subjected to degradation. Such a phenomenon is in direct contrast with that observed for the haemoglobin molecule, which when contained within the protective red cell envelope is subjected only to those breakdown mechanisms which are capable of distinguishing aging from young red cells. Only when red cells have reached the end of their life span is there a decline in isotope concentration in labelled haemoglobin. The pattern of decline at the end of the life span is dependent upon an age spectrum and a survival spectrum in the label-containing cells (Fig. 1b).

Fig. 1 Turnover of human proteins as revealed by biosynthetic labelling. $^{15}$N concentration in (a) serum proteins and (b) haemin extracted from red cell haemoglobin after feeding $^{15}$N-labelled glycine for two days. (Reprinted from London, I.M., 1950\textsuperscript{10})
Returning to the Schoenheimer quotation, it is necessary to emphasize his statement that turnover is carried on continually, even when the total amount and composition of the proteins remains constant. It is in these latter circumstances that we talk of a ‘steady-state’ condition—a situation which must imply a balance between anabolic processes on the one hand and catabolic processes on the other, and intrinsically therefore also implies the maintenance of normal body pool sizes. Any perturbation in the balance between synthesis and catabolism will produce a change in pool masses. In the absence of a compensatory response from the opposite side of the metabolic equation, the perturbation will ultimately lead to the destruction of the system. It is to the nature of these disturbing forces in the metabolism of liver-produced proteins and the compensatory responses induced by them that I should like to devote attention. Although they are finely balanced, the two sides of the turnover equation may for didactic purposes be considered separately. Physiologically they are very distinct biological processes. In terms of feedback control, however, they may have a common denominator, namely, the protein pool size and the concentration of the proteins in the subdivisions of the body compartments.

Protein Catabolism

The half-life of a protein in the plasma, or for that matter an intrahepatic enzyme, is inversely proportional to the fractional catabolic rate expressed as a percentage of the relevant pool mass per unit time. This value can be the primary determinant of the relative pool sizes of individual proteins. This point may be illustrated more clearly by the following example. Two proteins may have identical turnover rates when expressed in absolute terms, i.e., g per hour, but widely differing catabolic rates when expressed in terms of half-life or fractional rates of degradation. Their differing half lives reflect the relative turnover times of differing pool masses. Since protein degradation appears to be an obligatory physiological process obeying first-order kinetics, it follows that it is the fractional rate of degradation rather than the rate of synthesis expressed in absolute terms which may be responsible for the intrinsic differences in pool masses of various proteins and which determines the change in pool mass in response to a change in synthesis. Furthermore, in certain non-steady state conditions, modulation of catabolism alone is potentially capable of restoring the status quo. This is illustrated in Figure 2. A protein pool size of 100 g may be maintained in balance by an input by synthesis of 10 g and an output by degradation of a similar quantity. A reduction in the mass of protein in the pool by the removal of 50 g would require only that the fractional rate of breakdown of 10% of the pool per day be maintained in order to restore the pool size to normal. Synthesis would exceed catabolism in absolute terms and the pool would enlarge (Fig. 2a). Likewise, expansion of the pool by the infusion of 50 g of protein would also be counterbalanced by maintenance of the normal fractional catabolic rate. In these circumstances the absolute catabolic rate would exceed the synthetic rate and the pool size would fall to normal (Fig. 2b). In contrast, a primary defect in synthesis would result in a progressive fall in the pool mass with an accompanying reduction in the mass of protein degraded. Only when the pool has fallen to a level where the absolute rate of degradation is equal to the rate
Fig. 2  Metabolic responses mediated solely by the maintenance of a constant fractional rate of catabolism. The effects of (a) a reduction in pool size; (b) an increase in pool size; and (c) a reduction in the rate of synthesis. $S =$ absolute rate of synthesis; $C =$ absolute rate of catabolism.

of synthesis will it stop shrinking (Fig. 2c). In the latter situation a response which is geared to the maintenance of a constant fractional rate of degradation is capable only of re-establishing steady state conditions but is not in itself able to restore the pool size to normal.

Few plasma proteins only conform to this pattern of behaviour (Fig. 3). One example is fibrinogen. Regardless of the plasma concentration or pool size, the fractional rate of breakdown remains constant. In this way, the mass degraded falls as the pool size falls (assuming a constant plasma volume). This example of first-order control could be based on the clearance of a constant volume of plasma by the degradation process. Loss of plasma protein into the gastrointestinal tract is one means of catabolism which conforms to the clearance principle. The fractional rate of degradation which is solely the result of leakage of protein into the gut is equal for all plasma proteins, implying 'bulk loss' of proteins in the same proportions as they exist in plasma. Regulation is intrinsically built into this mechanism of catabolism; a fall in plasma concentration is automatically accompanied by degradation of less protein.

In contrast, transferrin and haptoglobin are said to show an inverse relationship between fractional catabolic rate and plasma concentration. An increase in fractional catabolic rate as the plasma concentration and pool
The synthesis and degradation of liver-produced proteins

Fig. 3 The relationship between the fractional rate of catabolism and the plasma concentration of five plasma proteins. (Reprinted from Freeman, T., 1965[13])

size falls results in a constant absolute rate of breakdown, signifying first a complete lack of compensatory buffering in response to any change in synthesis or abnormal loss, and secondly a catabolic mechanism which consumes protein at a fixed rate, perhaps in the course of a metabolic process in which the protein engages[18]. This would seem to be a logical feature of haptoglobin metabolism fitting its role as a transport protein for haemoglobin released from effete red cells and which may be destined itself for simultaneous degradation by the reticuloendothelial system. Transferrin, on the other hand, must be reutilized many times after donating iron to erythroid precursors, since the turnover of plasma iron is many times faster than the turnover of transferrin. The transferrin molecule is not consumed during its transport function[17], and therefore cannot be considered as a 'suicidal' protein in contradistinction to haptoglobin[18]. Transferrin levels are increased classically in iron deficiency, as a result in part at least of increased hepatic synthesis[19]. Since we know nothing about the relative rates of degradation of iron-loaded and iron-free transferrin, it may be that in iron deficiency the constant rate of catabolism should be seen not against the expanded total plasma transferrin pool but rather against the reduced pool of saturated transferrin. Looked at in this way there is an increased fractional degradation rate which may reflect the increased turnover rate of iron which occurs in iron deficiency[20, 21].

Albumin and immunoglobulin G, in contrast, show a fractional rate of degradation which is directly proportional to their plasma concentration. This means in effect that they are capable of changing their plasma half-lives in response to alterations in pool size and represents the ultimate in catabolic compensation[18]. This example of a second order regulatory process may be based initially upon the clearance of a constant volume of plasma into a degradation pool. The presence of binding sites within the pool could offer
protection against proteolytic activity up to a saturation limit. A falling concentration of protein molecules in the cleared plasma would lead to relatively more complete binding and leave fewer available for degradation. A direct relationship between the plasma protein concentration and the fractional rate of degradation could thereby follow.

Examples of the protective function of the albumin degradation process are provided by the response to defects in synthesis. Patients with hepatic cirrhosis may have diminished synthetic rates, resulting in a low plasma albumin concentration and low intravascular pool sizes. The survival of $^{131}I$-labelled albumin in the circulation is greatly prolonged and both the fractional and absolute rates of degradation are much reduced. Hypoalbuminaemia due to malnutrition is likewise accompanied by a reduction in the fractional catabolic rate of albumin. In both these examples the primary disturbance is in the synthetic process. Secondarily, the concentration and pool size of the protein shrinks and the reduction in degradation follows. This sequence of metabolic alterations has been demonstrated by Kirsch and his coworkers in the rat subjected to experimental dietary protein depletion. There was an early reduction in albumin synthesis (measured directly by the $[14C]$carbonate method) and a correspondingly early fall in plasma albumin concentration. A reduction in catabolism followed only after a latent period. On repleting the animal with protein there was a rapid synthetic response with an overshoot. The normal synthetic rate was exceeded for a short time; the plasma albumin concentration increased and the fractional catabolic rate rose in a more gradual fashion as the original steady state was restored.

The liver-produced plasma proteins are distinctive in that they are for the most part catabolized at extrahepatic sites. It is unlikely, for example, that more than 10% of the overall breakdown of albumin can be accounted for by intrahepatic degradation. Most hepatic enzymes on the other hand are both synthesized and degraded exclusively within the hepatocyte. However, as Schimke has pointed out, both the proteins made for export and those retained within the cell demonstrate two features in common: first, the level of the protein (in the cell or plasma) may be modulated by changing either the rate of synthesis or the rate of degradation. Secondly, there is a heterogeneity of degradation rates of specific proteins. It had initially been assumed that ‘induction’ of enzyme activity by a suitable inducer, be it a factor in the diet, alterations of substrate level, or the administration of a drug or hormone, was solely the result of a change in protein synthesis. More recent evidence has demonstrated that the levels of certain enzyme proteins may be controlled by alterations in degradation, depending upon the nature of the inducer. The characteristics of turnover during and after the induction process which are necessary to ascertain the mechanism of induction have been defined. These are illustrated in Figure 4.

The administration of an ‘inducing’ agent will produce an increase in the enzyme level (from $E_{ss}^0$ to $E_{ss}^+$) until a new steady state level is reached where regardless of the mechanism of ‘induction’, synthesis again equals catabolism in absolute terms. Cessation of treatment is followed by a fall in enzyme concentration to the initial resting level with restoration of the original steady-state conditions. If the rate of fall in enzyme concentration is equal to the rate of rise during the induction process (B) then the increase was due to a higher rate of synthesis. If the rate of fall is more rapid (A) then in part at
The synthesis and degradation of liver-produced proteins

Theoretical time course for changes in steady state enzyme level during and after treatment. $E_{SS}^0 =$ enzyme level in the basal state. $E_{SS}^1 =$ new enzyme level produced by appropriate inducing agent. The only factor determining the time course of approach to the new steady state is the rate constant of degradation. The actual level achieved is determined by the ratio of the rate constant of synthesis to the rate constant of degradation. (Reprinted from Schimke, R. T., 1970)

least the induction process has involved a reduction in enzyme degradation. This method of analysis makes a number of assumptions. (1) When synthesis is altered, the rate of synthesis increases to the new rate abruptly on administration of the inducing agent, is maintained at a constant rate during treatment, and returns immediately to the basal rate on withdrawal of treatment. Moreover, the high level resulting from an increase in synthesis should not be accompanied by any secondary increase in the fractional rate of degradation. (2) When degradation is altered, withdrawal of treatment is immediately followed by a return of the rate of degradation to the basal level, and the half-life of decay of the elevated enzyme pool is similar to the half-life in the basal state. On the basis of this type of analysis, Schimke, Sweeney, and Berlin have shown that hydrocortisone produces a four- to fivefold increase in the synthesis of tryptophan pyrrolase without altering degradation, whereas administration of L-tryptophan retards the rate of degradation without affecting synthesis. Other examples of the primary role of degradation in the regulation of specific protein levels include the prevention by thymidine of inactivation of thymidylate kinase and the decrease in ferritin degradation as its iron content rises with iron administration.

Certain phenomena are common to all mechanisms of degradation: (1) it is an inevitable and seemingly ubiquitous process; (2) first-order kinetics imply a random process of degradation; (3) it is almost certainly an intracellular phenomenon. However the fact that (a) rates of degradation vary markedly between different proteins (cf the half life in the rat of ALA synthetase of
67 to 72 minutes\textsuperscript{37} with that of plasma albumin of three-and-a-half days\textsuperscript{38}), and (b) the rate of degradation of individual proteins can be altered by specific agents, as illustrated in the foregoing examples, would seem to imply the existence of a variety of mechanisms of degradation. Schimke\textsuperscript{12} has summarized the possible determinant factors which might decide when a given protein molecule is subjected to degradation. First the protein molecule itself may undergo conformational or other structural changes (possibly resulting from interactions with ligands) which render it more suitable or less suitable as a substrate for degradation. The stabilization of the apoenzyme tryptophan pyrrolase by conjugation with haematin in the presence of S. Tavill pyrrolase by conjugation with haematin in the presence of L-tryptophan is one such example\textsuperscript{39}. Similarly, recent evidence has demonstrated that a variety of plasma glycoproteins may undergo rapid degradation on removal of sialic acid by the enzyme neuraminidase followed by binding to the hepatocyte plasma membranes and subsequent intracellular uptake\textsuperscript{40,41,42}. The survival of such proteins is restored to normal either by restoration of the sialic acid residue or by further removal of the next residue of the carbohydrate moiety, namely, galactose\textsuperscript{43}. Likewise, ferritin with a high iron content is less susceptible to the action of proteolytic enzymes\textsuperscript{44} which might explain the decreased rate of degradation of ferritin synthesized under conditions of high iron load\textsuperscript{45}. Although these are elegant demonstrations of the effects of limited alterations in primary structure of a protein, no conclusive evidence has yet been offered to suggest that this is the physiological mechanism of degradation in vivo. Secondly, the degrading system itself may be altered in activity. This could take the form of a non-specific intracellular protease-peptidase system contained within lysosomal membranes. Control could be exercised by regulation of the level of the hydrolytic enzyme system, eg, inhibition of protein synthesis in rat liver inhibits the degradation of tyrosine transaminase\textsuperscript{45,46,47}. Such a model might be implicated in the changes in catabolism which occur in a response to protein deprivation. Alternatively, regulation of lysosomal activity might be mediated through stabilization or labilization of the lysosome membrane, eg, the increased rate of hepatic protein degradation which appears to occur following administration of glucagon to the perfused rat liver\textsuperscript{48,49}.

Hypoproteinaemic states in man may arise from a primary disorder of catabolism. When these situations are the consequence of an exaggeration of a normal but usually minor degradative pathway, as occurs in the protein-losing enteropathies, methods are available for specific localization of the route of loss\textsuperscript{50}. Similarly, in the nephrotic syndrome it is possible to attribute to the kidney an exaggerated role in plasma protein degradation above and beyond the protein overtly leaked in the urine. However, since no single organ or tissue has been identified as the primary site of degradation of the plasma proteins, other situations arise in which one is unable to pinpoint the basic mechanism of hypercatabolism. Such is the case in infectious and toxic states\textsuperscript{51} and in certain patients with malignant disease\textsuperscript{52}. Experimental work has suggested that pooling of plasma protein may develop in and around tumours\textsuperscript{53} which raises the possibility of subsequent catabolism of the protein as a source of amino acids for growth of the cancer cell. Finally, evidence has recently been obtained to suggest that reticuloendothelial hyperplasia may be associated with abnormal degradation of plasma proteins\textsuperscript{54}, although no proof could be offered that this represented expansion of a normal catabolic pathway.
Protein Synthesis

I should like to discuss now the possible mechanisms of the synthetic response, either to the direct effect of hormones, drugs, or the level of enzyme substrate, or to the indirect effects of changes in catabolism.

In the simplified schema of protein synthesis depicted in Fig. 5, the two levels at which synthesis may be controlled are distinguished, namely, transcription and translation. At the transcriptional level control of the rate of protein synthesis may be regulated by the rate of formation of mRNA from the DNA template by RNA polymerase, by the rate at which mRNA is transported from the nucleus to the cytoplasm or by the rate at which mRNA is degraded. Alternatively or additionally, the rate of protein synthesis could be regulated at a ribosomal level by the rate of translation of polypeptide chains, a process involving the activation of amino acids, their binding to tRNA and the ribosome, the attachment of the ribosome-tRNA-activated amino acid complex to the start of the mRNA strand, their relative movement along the message with sequential propagation of the polypeptide chain and their ultimate release with completion of the polypeptide chain at the end of the mRNA.

Control at the level of transcription would involve a Jacob-Monod model of gene expression. In this model the primary effect of an inducer of protein synthesis would be to act as a derepressor of the operator gene upon which the structural gene coding for that protein relies for its activation. Conversely, inhibitory agents could act as corepressors, undergoing allosteric interaction with the aporepressor to bind and inactivate the operator gene. While such a model would make an attractive hypothesis for the regulation of protein synthesis in all types of cells, there is as yet no conclusive proof that it is applicable to mammalian systems.

Protein end-product inhibition of its own synthesis would be a logical suggestion for both intracellular and secreted proteins. In the case of the
secreted, circulating plasma proteins, however, it is difficult to see how the concentration of protein molecules in the intravascular or extravascular space could affect the concentration within the cell, except perhaps by regulating release from the vesicles of the rough endoplasmic reticulum.

For transcriptional control to provide a sensitive means of response to environmental stimuli, the specific mRNA for that protein must have a relatively rapid rate of turnover. It would be of limited value to shut off the synthesis of new mRNA if preformed mRNA is stable enough to maintain translation for a significant period following the shut-off signal. It has been suggested that proteins, viz, enzymes, which are rate-limiting in a biochemical series of reactions, are most likely to undergo rapid rates of degradation. It would seem likely that the mRNA coding for such proteins would also have a short turnover time. Conversely, those proteins not essential for rapid modulation of biochemical reactions are likely to be slowly degraded, as are their specific messengers. The heterogeneity of degradation rates of hepatic mRNA has been elegantly demonstrated by Wilson and Hoagland. Following a period of starvation the polyribosome population of rat liver is severely reduced. If at the time of maximum reduction the animals were refed, a new population of ribosomes was synthesized. Actinomycin D was then administered in order to block synthesis of further mRNA and the polyribosome population allowed to turn over on the basis of mRNA degradation. The capacity of these ribosomes to synthesize albumin was studied at various time intervals during this process. The newly formed ribosomes decayed in a biphasic fashion; two-thirds had a half-life between three and three-and-a-half hours; one third were much more stable with a half-life of at least 80 hours. Analysis of the protein synthesized by the more stable ribosomes showed it to be predominantly albumin. In other words, albumin synthesis by the liver is more refractory to conditions which reduce mRNA synthesis than is the synthesis of most other liver proteins. While these observations preclude a transcriptional mechanism for rapid alterations of hepatic albumin synthesis, it is still probable that more long-term effects may have such a basis.

Evidence to support a transcriptional means of control has for the most part relied upon the observed effect of actinomycin D on the activity of a known inducer. Since actinomycin D inhibits DNA-dependent RNA synthesis, inhibition of induction implies that the increased rate of synthesis is dependent upon increased levels of mRNA. Possible examples of transcriptional control are the steroid induction of tryptophan pyrrolase and glutamic pyruvic transaminase, the induction of hepatic microsomal enzymes and organic anion-binding proteins by phenobarbitone and other drugs, the increase in acute-phase reactants in the plasma following injury and in the synthesis of caeruloplasmin which follows the administration of copper to rats. Similarly, a repressor-operator mechanism has been suggested for the regulation of the structural gene coding for the mRNA of δ aminolaevulinic acid (ALA) synthetase. In this schema, haem serves as a corepressor of its own synthesis, while certain inducing chemicals, notably 5β-H steroids, serve as derepressors by competing with haem for a binding site on the repressor, which is thereby inactivated. It has been suggested that derepression precipitated by certain drugs may be responsible for exacerbations of symptoms in patients with porphyria and for the induction of cytochrome P450 and other enzymes involved in hepatic drug hydroxylation.
Likewise, a good deal of conclusive evidence has been accumulated in support of the translational control of protein synthesis at the level of the polyribosome. Good correlation exists between the rate of protein synthesis and the proportion of the ribosomal population which exists in the form of polyribosomes, that is, messenger RNA molecules containing their full complement of ribosomes (Fig. 6). An analogy may be drawn between a goods train travelling from A to B (the length of the mRNA strand). Clearly it will transport more goods if it is drawing its maximum number of loaded wagons (polyribosomes carrying growing polypeptide chains) than if it is pulling fewer loaded wagons (functional oligosomes) and covering the journey in the same time (translation time). This applies both to polyribosomes bound to the rough endoplasmic reticulum and synthesizing proteins for export, eg, the plasma proteins and polyribosomes lying free in the cytoplasm which are synthesizing proteins for home consumption, eg, intracellular enzymes and storage, binding, transport, or membrane proteins. It is possible to analyse the ribosomal population of the cell by means of differential sucrose gradient centrifugation in order to gain some quantitative idea of the degree of aggregation of ribosomes. Baliga, Pronczuk, and Munro have shown in a cell-free system that the response to amino acid deprivation is a rapid disaggregation of polyribosomes into oligosomes. This process is reversible on restoration of the essential amino acid content of the incubation medium, an effect which cannot be blocked by inhibition of RNA synthesis. This is a clear example of amino acid supply acting on protein synthesis within the limits of preformed mRNA, ie, it denotes a translational mode of control. While similar acute effects have been noted in the intact liver, translational mechanisms cannot provide a complete answer to the defects in hepatic protein synthesis following upon chronic nutritional protein deprivation. In rats subjected to a low protein diet for two to three weeks, albumin synthesis, liver weight, and liver cell size fall markedly while the output of albumin per unit liver weight or body weight is reduced to a lesser de-
gree\textsuperscript{76,77,78}. However, polyribosome analyses of such livers have shown that aggregation may be preserved\textsuperscript{79}. This would suggest that the output of protein per cell is reduced and that this may be the result of an overall reduction in the polyribosome population consequent upon a primary transcriptional defect in RNA synthesis\textsuperscript{80}.

The nature of the message to which the liver is capable of responding by changing its rate of protein synthesis is reasonably clear in the case of amino acid supply. It is probably based on a rate-limiting role for the amount of charged, i.e., amino acid-loaded, transfer RNA. In spite of the fact that the liver maintains much of its amino acid pool by means of endogenous degradation of hepatic intracellular protein\textsuperscript{81}, it is still dependent to a large extent upon exogenous dietary supply or amino acids released from extrahepatic protein degradation for the replacement of obligatory losses from the pool. While overall reduction in the supply of essential amino acids has the maximum effect on protein synthesis, a special role for tryptophan has been suggested\textsuperscript{85,89,84}. This is supported by the effect of amino acids, and in particular tryptophan, in preventing or reversing the reduction of hepatic protein synthesis produced by alcohol\textsuperscript{85,86}. It has been suggested that the unique rate-limiting role of tryptophan can be attributed to the very low levels of free tryptophan present in liver and plasma\textsuperscript{82,87}.

Since we have already seen that a ‘compensatory’ fall in plasma protein degradation may follow upon exogenous protein deprivation, it is possible that the fall in intracellular hepatic albumin catabolism\textsuperscript{86} and perhaps the catabolism of other proteins may produce an abnormal dependence of synthesis upon dietary amino acids. We have as yet no direct proof for this hypothesis. It does not conform with the sequence for circulating plasma albumin of, first, protein deprivation followed by reduced hepatic synthesis, and only then by reduced degradation\textsuperscript{29}, and therefore must await simultaneous measurements of hepatic protein synthesis and catabolism of both plasma and intracellular proteins\textsuperscript{88}.

In clinical practice hypoanabolic hypoproteinaemia resulting from inadequate amino acid supply is seen in the kwashiorkor syndrome and severe malabsorption states. A more subtle cause has been seen in the stagnant loop syndrome\textsuperscript{89}. Heavy bacterial contamination of the small intestine was associated with low levels of many of the essential plasma amino acids and a gross reduction in the hepatic synthesis (and degradation) of both albumin and fibrinogen. However, unlike defects in protein synthesis due to intrinsic liver disease, malabsorption or dietary deprivation, the associated rate of urea synthesis measured directly was considerably increased. Much of this urea was not recoverable in the urine. In this situation, the critical siting of a large mass of bacterial flora could result in the degradation of dietary amino acids, principally by deamination. Absorption of released ammonia may have resulted in the augmentation of urea synthesis by the induction of the potentially inducible urea cycle enzyme system\textsuperscript{81}. In addition, enterohepatic recycling of urea could provide increased substrate for intestinal bacterial urease activity resulting in diminished urinary urea and further absorption of ammonia nitrogen, some of which may be utilized for protein synthesis\textsuperscript{82,83}. The abnormalities in protein and urea synthesis and the levels of plasma amino acids were corrected by antibiotic therapy.

In other situations, it appears that synthesis is able to respond to depletion of the protein pool resulting from extrinsic loss of protein from the plasma.
This is the case in the nephrotic syndrome and in the protein-losing gastroenteropathies. In these examples, the nature of the feedback stimulus to protein synthesis remains unsolved. It is difficult to imagine how a reduction in pool size alone could be detectable by the hepatocyte. The plasma concentration of protein has been suggested as an alternative mediator. However, no consistent reduction in hepatic albumin synthesis was observed in patients in whom low plasma albumin concentrations were corrected by intravenous albumin infusions. Nevertheless, Rothschild and coworkers have demonstrated that the isolated perfused liver is capable of responding to a reduced circulating plasma albumin by increasing its rate of synthesis, and to an increased concentration by reducing its rate of synthesis. However, they also showed that isosmolar replacement of albumin by sucrose had the same effect. It was suggested that the effect was exerted on the liver cell by changes in the oncotic pressure of the plasma via secondary but more profound direct effects of the hepatic interstitial fluid oncotic pressure.

Amino acid supply and plasma concentration effects are translational regulatory mechanisms that may be common to many proteins of hepatic origin. Hormonal effects may be more complex. Corticosteroids may have a marked anabolic effect on hepatic albumin synthesis in patients with active liver disease, although no comparable effect was noted on fibrinogen synthesis. This may in part have been due to an action at the level of the polyribosome. It is hardly likely, however, that this anabolic role of corticosteroids prevails throughout all tissues, since their effect on pool sizes and plasma concentrations of albumin were far less than that expected from the increase in hepatic synthesis rate and their role in the whole organism is predominantly catabolic. Clearly, there must be highly specific control mechanisms which dictate which effect a hormone will produce in different tissues and even within the same cell.

Other regulatory mechanisms may also exhibit a high degree of specificity. The role of iron in the control of synthesis of its storage protein, ferritin, and its carrier protein transferrin, or of lipid in the regulation of synthesis of carrier apolipoprotein may be examples of 'function-related' regulation. In this situation one may expect that eventually roles will be defined for prosthetic groups, drugs, hormones, and anions in the regulation of the synthesis of their carrier apoproteins, which may have rate-limiting effects on the metabolism of these substances.

Since albumin makes the largest single contribution to plasma protein turnover and the data on its metabolism are most complete, it would be valuable to place this contribution quantitatively within the context of the protein economy of the whole organism (Fig. 7). The evidence for some of these data has been reviewed by Fauconneau and Michel. The average albumin synthesis of about 12 g per day (in a 70-kg man) represents about 25% of the total protein synthesized by the liver and should be seen against the background of total body protein synthesis of about 300 g per day. Roughly 1 g per day of albumin is lost intact from the intravascular albumin pool into the gastrointestinal tract. This represents a little less than 10% of endogenous albumin catabolism under steady-state conditions, and is minute in relation to total exogenous and endogenous protein passing down the gut. Even if this were to increase by an order of magnitude it would produce no drain on the total nitrogen economy since intestinal digestion and absorption would have little difficulty in dealing with it. It would, however, throw a
considerable strain on the hepatic synthesizing capacity which would have difficulty in doubling its total output of albumin. The effect would be an hypoalbuminaemic state without the consequences of overall nutritional protein deficiency.

Summary

In summary, the available evidence suggests that regulatory mechanisms in hepatic protein metabolism fall into two main categories. Specific control of the synthesis and degradation of individual proteins may be related to a vital and unique metabolic role, while non-specific mechanisms such as amino acid supply may range across the whole spectrum of liver-produced proteins. Other factors such as hormones may play a dual role. In the case of plasma proteins of hepatic origin, homeostatic feedback mechanisms must logically be postulated to account for the remarkable stability of concentrations and pool sizes. Although the synthetic machinery for a specific protein may have no direct control over the degradation of that protein it may compensate for the effects of degradation on pool size by adjusting its own rate of activity. Likewise, catabolic processes can respond to alterations in pool size resulting from defective synthesis by a reduction in their activity. The exact mechanisms of these responses remain to be elucidated.

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Fig. 7 The turnover of plasma albumin in a 70-kg adult seen in the context of the daily protein economy of the gastrointestinal tract and overall nitrogen balance. The total exchangeable albumin pool of about 300 g is distributed between the intravascular and extravascular compartments in a ratio of approximately 2:3. In this simplified schema the balance sheet is expressed in terms of g of protein (≈ 6.25 x g of N). Losses do not include relatively minor routes, eg., 2 g per day from the skin.
The synthesis and degradation of liver-produced proteins

References


The synthesis and degradation of liver-produced proteins


1East, A., and Hoffenberg, R. Unpublished observations.

Tavill, A. S., Nadkarni, D., and Hoffenberg, R. Unpublished observations.

Tavill, A. S., and Black, E. Unpublished observations.


