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WHOLE-BODY PROTEIN TURNOVER IN HUMANS—PAST, PRESENT, AND FUTURE

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ABSTRACT

Protein turnover is a fundamental biological process in all living organisms. The study of protein turnover in human subjects, and in the body as a whole, is of relatively recent origin. In this review, I summarize briefly how this field of work has expanded over the past 25 years, with emphasis on conceptual problems and controversies, particularly those related to methods of measurement. We cannot be certain that our estimates are accurate because no method of verification exists, but progress will be made through successive approximations. Some of the applications are described in different physiological and pathological states such as growth and trauma, and possible directions for fruitful future research are indicated.

INTRODUCTION

Protein turnover is characteristic of all living tissues, plants (143), and animals. The 1949 paper on this subject by Sprinson & Rittenberg (129) led to the slow and hesitant introduction of quantitative measurements of protein turnover in humans (152). In the 1960s, we found ourselves faced with a challenge: Children with severe malnutrition attributed to protein deficiency did not respond to treatment with protein, and many died. We therefore proposed that since the machinery for protein synthesis consists largely of protein, any damage to it could be irreversible. Methods had to be developed to test this hypothesis. The approach that we used (110) was in fact a variant of the original method of Sprinson & Rittenberg. This work caught the attention of Young & Scrimshaw at the Massachusetts Institute of Technology (MIT) (170) because of their concern with human protein requirements. The subsequent and dramatic rise in interest in protein turnover in humans thus had its origin in practical human problems, and the increasing availability of stable isotopes made it possible to tackle these problems.

The studies of the last decade may be roughly divided into four groups, with somewhat different aims: (*a*) to extend our understanding of amino acid kinetics and to validate the methods of measurement; (*b*) to examine the changes that occur in the components of protein turnover—synthesis and breakdown—in different physiological and pathological states; (*c*) to define quantitatively the fluxes through the metabolic pathways of amino acid metabolism; and (*d*) to gain information at the level of the whole body on the regulation of protein and amino acid metabolism by hormones and other effectors. Since it is not possible to cover all of these topics in a single chapter, and I confine myself mainly to the first two, relying on a number of excellent recent reviews (13, 113, 152, 164, 165) for background information.

A great deal of relevant data have been obtained from animal experiments, in

which measurements can be made on tissues at sacrifice (e.g. 5, 84). In humans, a popular tissue for the study of protein turnover is muscle, which can be sampled by biopsy (e.g. 10, 58, 59, 87, 116). Specimens of other tissues, both normal and pathological, have also been taken in the course of surgery (47).

The development of more sensitive instruments has allowed measurements to be made on leukocytes (107) and on samples obtained by endoscopy (12). The forearm and leg have provided useful systems for measurement in vivo based on arteriovenous differences in concentration and labeling (11, 20, 138). However, space limitations preclude mention of these studies, except in so far as they contribute to our understanding of events in the whole body.

NOTATION

Notation has always been problematic in the field of protein turnover. In 1969, Atkins (4, pp. 20–28) summarized the various systems in use up to that time, and since then many more have been proposed. An international meeting is needed to standardize the notation used in kinetic studies of metabolism. In this review I use the following notation:

1. Different letters are used for different quantities,: e.g. P (pool size) signifies the amount of tracee and p denotes the amount of tracer. Shipley & Clark (125) use Q and q, but this would require us to abandon $\dot{Q}$, which has become rather firmly established as the symbol for flux, and to use $\dot{F}$ instead.
2. As in other branches of physiology, time-dependent variables are denoted by a superscripted dot, e.g. $\dot{A}$ for R_a (rate of appearance). This allows the addition of a subscript, e.g. $\dot{A}_e$ for endogenous rate of appearance. The symbols R_a and R_d were introduced when investigators began to study the kinetics of amino acids rather than those of protein, which meant that synthesis (S) and breakdown (B) were no longer appropriate terms.
3. In equations, no initials, such as APE for atom percent excess, should be used. One cannot imagine Einstein formulating his famous law using SOL for the speed of light. Here, s is used for specific radioactivity or enrichment. A possible alternative to this abbreviation is ε.

MODELS

Models play an essential part in both the design and the interpretation of tracer studies on protein turnover. Many meanings have been attached to the term model (see e.g. 45); here, it signifies a simplified representation of a physiological system which has a structure (compartments) with routes of exchange of material between them. Importantly, such a model should represent a sim-

plification of a complex system; it is pointless to construct a model too complex to analyze. Green & Green have outlined two general approaches to the construction of a model (57). The first is to start with a system based on physiological concepts, e.g. of intra- and extravascular albumin, see whether the results fit, and alter the model accordingly if they do not. The second is to construct the model, with few preconceived ideas, on the basis of the results, which are usually analyzed by computer. In practice, these two approaches are often combined.

Compartmental Analysis

I use the terms compartment and pool interchangeably, although to do so is not strictly correct (4, pp. 8–9). However, the important point is that different compartments, or pools, can be distinguished. In compartmental analysis, a rule is introduced stating that the exchanges between compartments occur by first-order kinetics and that the rate constant is independent of concentration; this, however, may not always be the case (3). This rule leads to a series of differential equations whose number is determined by the number of pools in the system and whose parameters depend on the system's structure, i.e. the relationships between the pools. For simplicity, it is usually assumed that all pools are homogeneous and in a nonisotopic steady state. It is difficult, at least for a nonmathematician, to quantify the errors that may be introduced if these assumptions are incorrect, e.g. when fasting and feeding are compared. Wolfe has discussed this subject (158), particularly in relation to carbohydrate metabolism. The principles of compartmental analysis remain those formulated by Shipley & Clark (125) and by Steele (131), but in light of the increasing use of stable isotopes, the equations have been modified to account for the fact that the tracer is not without mass (24).

In recent years some very complex models, with up to nine pools, have been designed and analyzed (e.g. 70, 136, 141). Analysis is made possible by using several different stable isotopes and sampling sites (blood, expired CO_2, urine). The structure of the system is determined to be that which provides the best fit to the observed decay curves of enrichment against time after the administration of tracer as a single bolus. The curves are divided into a series of exponentials whose constants and coefficients provide the raw data for computation of pool sizes and exchange rates—a task that would be impossible without the computer. Bier has discussed such complex models and their interpretation (13). The practical drawbacks of these models are the very large number of blood samples needed and the extreme variability of the derived parameters, which is often so great as to make it impossible to draw any conclusions. For example, in a nine-pool comparison of lactating and nonlactating women (136), of all the parameters determined only one differed significantly between the two groups: the rate of exchange of amino acid between

a slowly turning over protein pool, provisionally identified as muscle, and its free pool. Although this finding is of interest, to apply such an approach more widely would be very difficult.

Stochastic Analysis

The Oxford English Dictionary defines stochastic as "pertaining to conjecture," which implies a probabilistic approach. However, compartmental analysis also involves probability in that it depends on the rule stating that the probability that a labeled molecule will be transferred from pool A to pool B depends on its concentration in A, and that this transfer occurs at a constant fractional rate. Conversely, the stochastic approach involves no such rule, and it would be better to call it "ruleless." In my opinion, we have no reason to suppose that the uptake of amino acids from the free pool into protein occurs by way of first-order kinetics. It is much more likely to be a zero-order saturable process, based on the extremely low Michaelis constants for the amino-acyl-t RNA transferases and the fact that protein synthesis is very insensitive to changes in plasma free amino acid concentrations. In contrast, Young (169) has shown that, at least for leucine, a close relation exists between plasma concentration and oxidation rate. One might therefore regard protein synthesis as having priority in terms of amino acid supplies, with anything left over being oxidized. This hypothesis has important implications for the precursor problem (see below) and for theories of regulation.

As Di Stefano (32) pointed out, a physiological model is just as necessary for stochastic as for compartmental analysis. A multicompartmental model can indeed be constructed if sampling is possible at more than one site or of more than one metabolite. In the simplest two-pool model with a metabolic pool and a protein pool, what is measured is the disposal rate $\dot{D}$*[1]. If the tracer is administered in a single dose, $\dot{D}$ is not obtained from the slope, as in compartmental analysis, but rather from the area under the enrichment-time curve divided by the dose (152). If the final measurements are made on urine, as in ^{15}N studies (see below), this area is simply the cumulative excretion up to a time t that is long enough for disposal of the tracer to be complete but not so long as to cause recycling to become a problem. This variant of the stochastic method has many advantages in terms of simplicity. For example, it could be used on an expedition across the Antarctic involving intense physical activity (133).

Nowadays the tracer is generally given by continuous infusion. The enrichment in plasma reaches a plateau (more precisely a pseudoplateau) within a few hours. This time can be greatly reduced by giving a priming dose (78,

[1]The disposal rate is the outward flux of the tracee into its products, which in the steady state is equal to the inward flux, or appearance rate A.

158), but we then lose the information that could potentially be obtained from analysis of the rate of rise to plateau (29). The plateau represents an isotopic steady state in which the rate of entry of tracer, $\dot{d}$, is equal to its rate of uptake into protein synthesis and oxidation. The disposal rate of tracee, $\dot{D}$, is then given by $\dot{D} = \dot{d}/s$, where s is the specific activity or enrichment of the tracee at plateau. When we first used the constant infusion method, it had already been well established, e.g. for measurements of the production rate of hormones. We adopted this approach for measuring protein turnover because it allowed greater ease and accuracy in defining a straight line from a relatively small number of points over a few hours than would be possible by extracting the information from a multiexponential curve. Nissim et al (101), in a study with [^{15}N] glycine, showed that compartmental and stochastic analysis gave essentially the same results, as would be expected when the same data are used. The trade-off is between the potentially greater information content of the former and the simplicity and greater reliability of the latter.

METHODS OF MEASURING WHOLE-BODY PROTEIN TURNOVER

Precursor Methods

In the early studies using constant infusion, the value assigned to s in the calculation of $\dot{D}$ was the radioactivity or enrichment of the tracer amino acid in plasma, s_p. s_p was assumed to give a reasonable estimate of enrichment at the precursor site, but we now know that this assumption is incorrect. Nevertheless, this simple approach provided valuable information. For example, Bier (13) showed that the disposal rate or flux of different indispensable amino acids (IAAs) determined from their activities in plasma varied linearly with their concentration in muscle protein, which means that the IAAs all yield a similar value for whole-body protein turnover (WBPT). The major implications of this finding are that the fundamental approach is correct and that any amino acid not synthesized in the body de novo can be used to measure WBPT. The fact that the absolute values for WBPT are incorrect because s_p is an inaccurate measure of the precursor activity is unimportant in this context; the approximation has served its purpose.

Numerous animal experiments have shown that the enrichment of the labeled amino acid in the intracellular pool, s_i, or in the tissue pool as a proxy, is less than the enrichment in plasma, s_p, because of dilution of the incoming tracer by unlabeled amino acids derived from tissue breakdown. The difference can be very large; in the rat, s_i/s_p is –0.5 in liver and –0.8 in muscle (5, 84). The problem, then, is how to determine the intracellular precursor enrichment in vivo.

This subject has stimulated much research in recent years and has been well reviewed by Bier (13), Reeds (113), and others. The first approach to the problem was based on the theory that all direct products of the same precursor will have the same activity. Fern & Garlick (38) showed that after an infusion of labeled glycine the ratio of s (serine) to s (glycine) in mixed liver proteins was very close to the ratio in the tissue free pool, suggesting that this pool is indeed the site of the precursor. Moreover, they found that s (serine) in plasma was identical to that in the tissue pool, which thus could be sampled by measurements in plasma.

At about the same time, Matthews (90) suggested that the enrichment of the oxo-acid derived from leucine by transamination—alpha-keto isocaproic acid (KIC)—might provide a good estimate of precursor activity. In the current terminology, KIC is the reciprocal of leucine, and infusion of leucine with measurements on its reciprocal in plasma is now the most widely used method for measuring WBPT.

Because transamination of leucine to KIC occurs mainly in muscle, and because muscle is accessible by biopsy, attention has traditionally focused on KIC as a precursor for muscle protein synthesis. However, the experiments of Yu et al (171) in dogs showed that substantial amounts of KIC are also produced in the splanchnic region and liver. For albumin synthesis, KIC yielded a rate very close to that obtained from the plateau labeling of [^{15}N] guanidino-arginine after infusion of [^{15}N] glycine (104) and similar to that given by classical measurements with iodine-labeled albumin. Bennet et al (12) found that after [^{14}C] leucine infusion for 6 h, the specific radioactivity (SR) of plasma KIC was equal to that of newly secreted pancreatic enzyme. Similar results were obtained for fibrinogen (9). These findings suggested that KIC might provide a general solution to the precursor problem.

Further studies in which two isotopes, [^{3}H] leucine and [^{14}C] KIC, were infused showed that the ratio of enrichments of their reciprocals, [^{3}H] KIC and [^{14}C] leucine, was similar in the plasma free pool to that in plasma proteins in humans (123) and in a range of tissue proteins in rats (69) and dogs (83). These ratio studies are proof of consistency but not of the actual enrichment of the precursor. With leucine, the true precursor is leucyl-tRNA. In a study thus far unique in humans, Watt et al (154) found that in muscle, the enrichment of leucyl-tRNA was midway between that of plasma and tissue free leucine, suggesting some degree of compartmentation in the cell. A great deal of experimental evidence supports this observation. Thus, infusions of [14C] glycine resulted in a small but significant difference between the ratio of s (serine) to s (glycine) in albumin and ferritin, implying that these two proteins, both synthesized in the liver, must have been derived from precursor pools with a slightly different mix of intra- and extracellular amino acids (39). Moreover, we cannot be certain that the precursor activity is the same for

oxidation and for protein synthesis. Compartmentation may be particularly pronounced in the liver because of functional differences between cells in different parts of the lobule (61).

For amino acids other than the branched-chain type and the glycine-serine couple, no suitable reciprocal metabolite has been introduced. Alpha-amino-adipic acid has been proposed as a reciprocal for lysine, but it is present in such small amounts in plasma that it has to be measured in urine (1). A new variant of the reciprocal metabolite approach has been introduced for estimating precursor enrichment in the liver, with the idea that a protein that turns over very rapidly will come into isotopic equilibrium with its precursor within a short period. The very low-density lipoprotein (VLDL) apolipoprotein B–100, synthesized in the liver and secreted into the plasma, is a suitable candidate. Reeds et al (115) compared levels of labeling of plasma amino acids and of apo-B 100 during infusions of lysine, leucine, and phenylalanine. In the postabsorptive state, the enrichments were similar, but in the fed state they were substantially lower in the protein, suggesting intracellular dilution by unlabeled amino acids. In another study, Jahoor et al (76) found that the enrichment of [^{13}C] alanine derived from an infusion of [^{13}C] glucose was identical to that of apo-B 100, which represents in effect a double reciprocal approach.

The fact that in some recent studies the tracer amino acid has been given by nasogastric tube instead of intravenously presents another problem. When leucine is used as the tracer, some 30% of the label is sequestered in the splanchnic bed (65). This occurrence has been termed the first-pass effect. Estimates of whole-body flux from the enrichment of KIC in plasma give values some 15–30% higher than those obtained when the tracer is infused intravenously. According to my calculations, if the enrichment of the precursor were the same in all tissue pools, the two routes of administration would give the same value for whole-body flux. If in the fed state the enrichment is lower in the splanchnic pool than in the rest of the body, then intravenous dosage will underestimate the flux. The situation is further complicated by the fact that different amino acids behave differently. For example, when labeled phenylananine is given intragrastrically, a much larger proportion of the dose is sequestered than with leucine (14). It is difficult to be certain of the consequences of the first-pass effect, but with intravenous administration of tracer, fed-state fluxes may be consistently underestimated.

A different approach to the problem of precursor activity, originally proposed in the 1950s and more recently developed by McNurlan & Garlick (50), involves giving a "flooding" dose of labeled amino acid to rapidly bring all pools into isotopic equilibrium. The validity of the method is at present a subject of intense controversy (49, 116). Theoretically, the argument hinges on the kinetics of protein synthesis with respect to the precursor pool. If

synthesis is a first-order process, then the extra amino acid entering the precursor pool will automatically increase the rate of protein synthesis (21). If, on the other hand, it is of zero order, then expansion of the pool size is irrelevant. It has also been suggested, and disputed, that the flood may act indirectly by stimulating insulin secretion. Because of the short time involved (~1 h) the flooding dose method is particularly suited for measuring rates of protein synthesis in individual tissues, e.g. in muscle biopsies or samples taken during surgery (47). Comparisons in humans of rates of muscle protein synthesis by the constant infusion and flooding dose methods have led to conflicting results and interpretations (49, 116). The flooding dose method has not been applied to measurement of WBPT in humans, and probably cannot be (Garlick, personal communication); however, in rats, Obled et al (102) found that with lysine and threonine as tracers flooding dose and constant infusion gave similar estimates of whole-body fractional synthesis rates.

To summarize, a great deal of progress has been made toward identifying a proxy for the precursor that will give reasonably accurate results in particular situations. However, measurements in the whole body remain subject to error because the precursor does not have the same enrichment in all tissues. No single metabolite in plasma can reflect these different enrichments. At best, it can provide an approximation to a weighted average, which should be adequate for most practical purposes. The leucine-KIC method, which has been by far the most thoroughly tested and widely used, may not be a gold standard, but we can use it as a silver standard. I must dissent from Bier's (13) view that progress can be made using more complex multicompartmented models. These models still provide only approximations unless they are combined with direct analyses at the sites of synthesis to give more accurate estimates of precursor enrichment.

End-Product Methods

Historically, the end-product (EP) method dates back to Sprinson & Rittenberg (129). It was first used with constant infusion of tracer in our studies of whole-body protein synthesis in malnourished children (110). The method, as its name implies, depends on measurement of the excretion of tracer in the urine. The assumptions involved have been defined by me and my colleagues (152) and by Bier (13). The most important of these is that for every amino acid, the proportion of ^{15}N to total nitrogen taken up into the end product is the same as the proportion of ^{15}N to total nitrogen taken up into protein synthesis. The assumption cannot be validated merely by measuring the labeling of the free amino-nitrogen pool, because its composition differs considerably from that of the amino acid mixture taken up into protein. Historically, [^{15}N] glycine is the most widely used tracer in the EP method. The nitrogen of glycine seems to have relatively few major metabolic pathways. It exchanges

freely with serine, but Jackson & Golden (72) were unable to detect any labeling of the transaminating amino acids alanine and glutamate after infusion of ^{15}N glycine. Using more up-to-date methods, Matthews et al (89) found that in plasma, glutamine + glutamate achieved 15% and alanine 7% of the labeling of glycine. The branched-chain amino acids were detectably labeled, presumably through transamination, whereas leucine and threonine were not labeled at all. Matthews et al calculated that if the basic assumption referred to above is correct, the enrichment of urea could be calculated from the weighted average of (enrichment of each amino acid in plasma × concentration of that amino acid in tissue protein). The average ratio of observed to predicted urea enrichment in 6 subjects was 1.15. This ingenious study provided some support for the basic assumption that when the tracer is glycine, protein and urea are synthesized from the same precursor pool.

However, when other amino acids and biologically labelled proteins were tested, the results were not as encouraging (42, 135). Alanine, aspartate, and glutamine gave high levels of labeling in urea, as might be expected from the biochemistry of urea formation, and hence yielded low levels of flux, whereas leucine and lysine had the opposite effect. If, in contrast to the precursor method, different amino acids give widely different results, the EP method clearly does not have a sound theoretical basis. It is purely a matter of chance that ^{15}N glycine, the amino acid originally chosen because it was cheap and easily available, behaves in a way that is reasonably consistent with the basic assumption.

In an attempt to find a better solution, investigators have performed experiments with biologically labeled proteins, i.e. yeast, wheat, soya, and egg. If glycine is taken as the standard, the best agreement in terms of flux is obtained with wheat (42) and egg (110). The studies with egg were done many years ago, with the labeled egg obtained from a hen fed biologically labeled leaf-protein concentrate. Since this process yielded only enough material for two measurements, this approach needs to be explored further.

A second problem is that with an unprimed constant infusion of ^{15}N glycine, urinary urea takes 30 h in children and 60 h or more in adults to reach an isotopic plateau. This inconveniently long time can be reduced with a priming dose (78), but as an alternative we suggested using ammonia, which has a much smaller and faster turning-over pool, as the end product (40, 153). Theoretically, two direct products of the same precursor pool should be equally labeled, but in practice they were not and gave different estimates of flux. This finding led to the idea of a two-pool metabolic model, shown in Figure 1, which differs considerably from the kinetic models used in the precursor method. In this model, the two measures of flux, $\dot{Q}_U$ (based on the labeling of urea) and $\dot{Q}_A$ (based on the labeling of ammonia), are independent, not additive, estimates of whole-body nitrogen flux. $\dot{Q}_U$ is biased by the flow of nitrogen

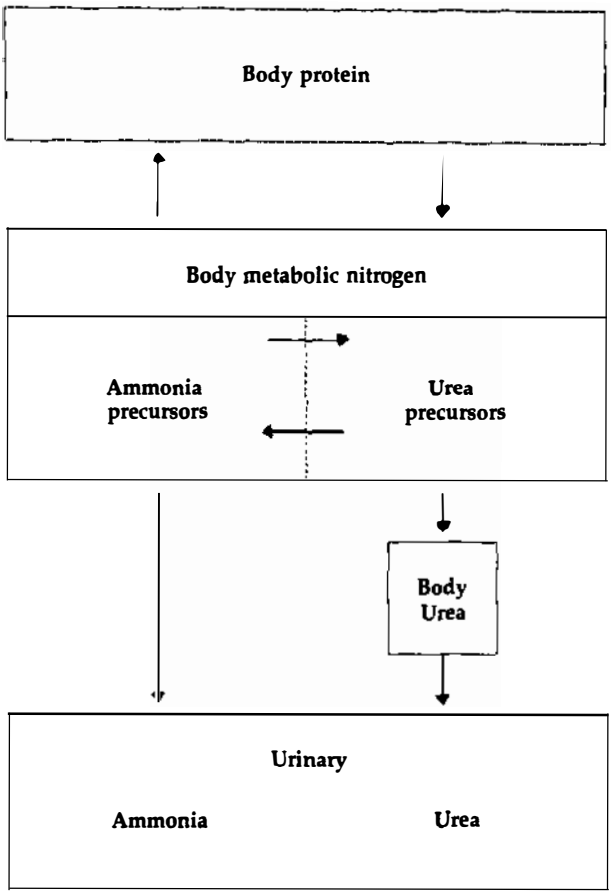

Figure 1 Two-pool model of metabolic nitrogen in the whole body. (From Reference 42. Reproduced by permission from *Clinical Science*.)

through the site of urea formation, i.e. the liver, whereas $\dot{Q}_A$ is biased by the flow of nitrogen through the sites of ammonia formation, i.e. mainly but not exclusively muscle, through the action of glutamine synthetase. A logical step is then to take as the best estimate of $\dot{Q}$ the average of $\dot{Q}_U$ and $\dot{Q}_A$. The arithmetic average assumes equal partition of the tracer between the two pools, whereas the geometric average is calculated from the mean level of labeling in the two pools (42). The two averages seldom differ by much, and both are very insensitive to wide variations in the partition of tracer or enrichment in the two pools (43). In general, the arithmetic average, denoted EPA (end product average), is used.

We must also recognize that the EP method relies on a complex metabolic

chain in which many reactions intervene between the tracer and the final end product. Results therefore sometimes reflect alterations in metabolic pathways rather than changes in flux. For example, in studies with [^{15}N] glycine on premature infants, urea was virtually unlabeled in some cases, and the estimate of $\dot{Q}_U$ was entirely unrealistic. This finding led to the suggestion that when growth is very rapid, de novo synthesis of glycine may be limiting, so that glycine is not available to donate nitrogen to urea. Therefore, there may always be a question about the interpretation of EP results.

There are serious differences of opinion as to the extent to which the EPA method may be considered valid and useful. Purists tend to ignore or reject it because of the arbitrary choice of glycine as tracer, the unproven assumptions about precursor and product, and the apparent illogicality of averaging the estimates of flux. However, I view the method as a challenge because it potentially provides more information about nitrogen fluxes in the body than can be obtained by the precursor method, unless multicompartmental models are used with multiple tracers. In particular, as discussed below, the pattern of variation of the two estimates of flux is logical, at least to some extent. For users not so concerned with theory, the EP method has great advantages and is particularly suitable for clinical and field work. Except for one blood sample at 12 h to determine the amount of [^{15}N] urea retained in the body pool, this method is noninvasive, and the measurements of ^{15}N enrichment require much less complex apparatus than is needed to determine isotope abundance in plasma amino acids or their derivatives. What may be called the standard version of the EPA method has been described by Fern et al (41, 42).

A comparison of results obtained by the two methods is shown in Table 1. This table may be regarded as biased because it does not contain all of the results documented in the literature. To make the results comparable, studies have been chosen in which protein intakes were in the normal range (0.6–1.5 g/kg per day) and in which the subjects were healthy young or middle-aged adults of either gender. A major problem with this comparison is that in most studies with the precursor method, the tracer has been given intravenously, whereas in studies using the EP method, it has usually been given by mouth. This distinction is important because of the first-pass effect described above. The results are given in terms of synthesis rather than flux in order to eliminate the effects of oxidation when protein intakes are not the same. They are expressed as rates per 12 h because when measurements have been made over only a few hours in either the fed or the fasted state, the results cannot legitimately be extrapolated to 24 h. For both methods, the variability between subjects is rather high, i.e. ~10% with the precursor method (88) and somewhat greater with the EP method. When the methodology was rigorously standardized, however, two studies of the within-subject variability by the EP method found variability to be quite low (41, 52).

Table 1 Comparison of rates of protein synthesis (g protein/kg per 12 h) measured by the (1-^{13}C)leucine-KIC method and the (^{15}N) glycine end-product method[a]

First author	Year	Reference	Synthesis rate Fasted	Fed	N	Comments
Leucine-KIC; intravenous tracer						
Cortiella	1988	27	—	2.00	5	
Pacy	1988	105	1.39	1.95	8	i.v. feeding
Conway	1988	26	1.13	1.55	7	female
Morrison	1988	95	1.70	—	11	
Bennet	1989		1.99	2.25	7	i.v. feeding
Beaufrère	1989	8	1.44	1.38	8	
Melville	1989	92	1.37	1.26	5	8-h infusions
"	"	92	1.12	1.51	5	12-h infusions
Melville	1990	91	1.11	1.15	9	
Carli	1990	18	1.85	—	6	female, fed i.v.
Pelletier	1991	108	1.49	1.55	11	adequate leucine
Hoerr	1991	65	2.06	2.35	5	
Yarasheki	1992	163	—	2.07	18	
Marchini	1993	88	1.60	1.63	6	egg pattern
"	"	88	1.43	1.52	7	MIT pattern
Hoerr	1993	66	—	2.48	6	
Jahoor	1989	77	2.48	—	6	
Pacy	1994	106	2.05	2.15	5	
Mean (SD)			1.514 (0.303)	1.787 (0.402)		
Intragastric tracer						
Cortiella	1988	27	—	2.16	5	
Melville	1989	92	1.97	2.02	5	
Hoerr	1991	65	—	2.87	5	
Hoerr	1993		—	3.02	6	
Mean (SD)				2.515 (0.346)		
(^{15}N) glycine, end-product average; oral tracer						
Fern	1981	40	1.30	2.00	4	
Glass	1983	51	—	2.19	11	
Fern	1984	41	—	1.89	5	
Swart	1988	134	1.36	1.77	12	
Badaloo	1990	6	—	1.60	6	
Robinson	1990	118	1.72	2.60	6	
Soares	1991	127	—	2.15	6	
Yarashaki	1992	163	—	1.58	18	
Soares	1994	128		2.50	6	
Mean (SD)				2.03 (0.343)		

Table 1 *(Continued)*

First author	Year	Reference	Synthesis rate Fasted	Fed	N	Comments
Intravenous tracer						
Fern	1981	40	1.47	2.12	4	
Fern	1984	41	—	1.89	1	
Jeevanandam	1986	80	0.87	1.12	7	
Pacy	1994	106	—	2.16	5	
Mean (SD)				1.82 (0.418)		

[a]The data relate to healthy adults, mainly young and mainly men. Protein intakes in the fed state ranged from 0.6 to 1.5 g/kg per day and were usually in the region of 1. The results with leucine were based on the enrichment of KIC and calculated on the assumption of 628 μmole leucine per gram of protein. The results with the EPA method were obtained by different protocols, with either single or multiple dose and with measurements over varying lengths of time. There do not appear to be any consistent differences between results from various laboratories.

The results of both methods and in both fed and fasted states fall in the same range of ~1.5 to 2.5 g protein/kg per 12 h. The small difference in fed-state synthesis between the leucine method with intravenous tracer (unweighted mean 1.79) and the EPA method with oral tracer (mean 2.03) is presumably a reflection of the first-pass effect.

The fact that the two methods give similar results does not, in Bier's view (13), constitute a validation of the EP method. In his words, "Demonstrating the identity of two parameters from incomplete or incorrect models validates neither the parameters nor the models." He argues that the gold standard is a "structurally and mathematically correct model." I believe, on the contrary, that no method of measuring WBPT in human subjects can be regarded as "true" and that even if we had such a model, we would not be able to determine that it was "correct." The only direct check is to perform tissue analysis of the uptake of tracer (102). Bier's complex models involve unproven assumptions about structure and basic mechanisms, e.g. that all transfers occur by way of first-order kinetics. Science advances by successive approximations, and if two methods that rely on somewhat different assumptions give similar results, we are probably getting closer to the truth. Bier's own findings (13) are an example of a fruitful approximation.

A third approach originally suggested by Golden & Waterlow (54) and developed by Walser (161) avoids direct confrontation with the precursor problem and uses a combination of precursor and EP methods. If, with a carbon-labeled amino acid, F is the fraction of dose excreted in expired CO_2,

then (1-F) is the fraction taken up into protein. The rate of protein synthesis, S, can then be determined from the relation $\dot{S}/\dot{E} = (1\text{-}F)/F$, where E is the rate of nitrogen excretion in urea + ammonia. This method assumes that the oxidation of tracer amino acid is representative of the oxidation of total amino-N. This point is considered below in the section on amino acid oxidation.

Recycling

What has been called internal recycling (152) is the appearance in the cell of amino acids derived from protein breakdown in that cell. As discussed above, by diluting the incoming tracer, this type of recycling has an important effect on estimates of protein turnover rates. Conversely, external recycling is the appearance in plasma of labeled amino acids derived from proteins that have already been labeled in other tissues. In continuous infusion studies in which the precursor enrichment reaches an apparent plateau, external recycling is assumed to be negligible within the time scale of the measurement. Indeed, numerous studies by both the precursor and EPA methods have shown plateaus in which the slope does not differ significantly from zero.

Most of these infusions have been for a few hours only. However, Carraro et al (19) infused labeled leucine for 5 days and found that the enrichment of plasma leucine increased by 15% over that time. In the experiments of Melville et al (92), the fasted flux (the inverse of enrichment) was 15% lower during the last 12 h of a 24-h infusion than after infusion for only 4 h. After stopping the long infusions, labeled leucine and KIC persisted in the plasma at a much higher level than would be expected from the turnover rate of the free leucine pool. These effects were attributed to recycling. Similarly, Schwenk et al (124) found that at the end of a 24-h infusion, the rate of appearance of tracer in plasma was 30% greater than the exogenous rate of infusion.

Carraro et al (19) argued, with justification, that these high rates of recycling cannot be reconciled with the kinetics of a simple two-pool model. I therefore made an approximate calculation of reentry in a three-pool system with the following characteristics: (*a*) 10g nitrogen/kg, turnover rate 1% per day (slow pool); (*b*) 5g nitrogen/kg, turnover rate 10% per day (fast pool); and (*c*) 1g nitrogen/kg, turnover rate 40% per day (super-fast pool).

After a 12-h infusion, recycling would add almost 5%/h to the exogenous rate of infusion. These results may seem exaggerated, but recycling will be much less serious with a short infusion because tissue proteins will be less highly labeled. In the above model, at the end of a 4-h infusion, recycling would occur at the rate of ~1.5%/h which, allowing for experimental error, is compatible with a plateau with zero slope. Furthermore, a super-fast pool even as large as that postulated above barely shows up in a multiexponential analysis of the rate of rise to plateau.

Another possibility is suggested by the observation, in EP studies, of a

significant step at 6–12 h in the rise to plateau of the enrichment of urinary ammonia (126). This finding cannot be explained by any model based on first-order kinetics, and the possibility was proposed of a fairly large protein pool turning over by lifetime kinetics, tentatively located in the gut. The data of Carraro et al (19) give a slight indication of successive steps in the enrichment curves of leucine and KIC. Another important but specialized form of recycling is that of urea in the colon, termed salvage by Jackson and his coworkers. This is considered below.

Non–Steady States

That the metabolic or precursor pool is in a steady state is implicit in the equation flux $\dot{Q} = \dot{S} + \dot{O} = \dot{A} + \dot{I}$, where $\dot{S}$ is uptake into synthesis; $\dot{O}$ is oxidation; $\dot{A}$ is endogenous appearance; and $\dot{I}$ is intake from food. In some situations, however, there is clearly not a steady state, as in the transition between fasting and feeding, or in severely traumatized patients whose condition may change from hour to hour. An interesting example is a study by Jahoor et al (77) on patients with sepsis and burns. That such patients tend to be resistant to insulin is well known, and the authors wished to examine the response of leucine kinetics to a hyperinsulinemic euglycemic clamp. Without the clamp, these patients exhibited the increased rates of leucine appearance ($\dot{A}$), oxidation ($\dot{O}$) and uptake in protein ($\dot{S}$) that are usual in such severe states. With the clamp, which increased plasma insulin concentrations 100-fold, $\dot{A}$ and $\dot{O}$ fell, and from the expression that in the absence of food $\dot{S} = \dot{A} - \dot{O}$, it could be inferred that the synthesis rate also fell. That insulin should reduce the rate of protein synthesis seems intuitively unlikely, though perhaps not impossible. In this study, the clamp resulted in a drop of ~50% in the plasma leucine concentration. Therefore, the estimated rate of outflow to synthesis should be corrected for the change in pool size. Calculation from the data of Vinnars (144) for the free leucine concentration in muscle in surgical patients indicates that the change in pool size, spread over 5 h, might account for about half the apparent reduction in protein synthesis. Of course, we can only estimate changes in pool size from plasma concentrations; changes in intracellular concentrations may be much greater.

A recent study by El Khoury et al (37) provides another example of a non–steady state. Subjects were infused with labeled leucine for 24 h, during which time they consumed three discrete meals. These produced quite large, although temporary, changes in plasma leucine concentration, with rather smaller changes in KIC enrichment. Calculations based on an assumed steady state gave rates of uptake into protein that were rather constant over the 24 h, ranging only from 80 to 95 μmol/kg per h. A preliminary calculation from the equations of Shipley & Clark (125) that took into account the non–steady state gave much larger swings in synthesis rate, i.e. from 60 to 110 μmol/kg/h, in

response to meals. It may therefore be necessary to rethink some of the conclusions that have been drawn about the effects of feeding on whole-body protein synthesis.

AMINO ACID OXIDATION

One of the most important applications of amino acid kinetics in recent years has been the work of Young and his coworkers at MIT toward establishing the requirements for indispensable amino acids (166). The concept is analogous to the classical nitrogen balance: If an IAA is fed at various levels and if all other amino acids are present in adequate amounts, a point will be reached at which oxidation of the test amino acid exactly equals intake. The basic technique is as follows: After a week's stabilization on each level of intake, a primed infusion is given of the test amino acid labeled in the 1-carbon position. The infusion is maintained for a total of 8 h (3 h in the fasted and 5 h in the fed state), and oxidation is measured over the final hour in each state. These measurements over 1 h are then extrapolated to a 24-h day (12 h fasted and 12 h fed). The validity of this extrapolation, as mentioned above, was checked by 24-h infusions (35, 36). This approach has come to be known as the tracer balance method.

Most studies performed to date have been with leucine and have determined that the leucine requirement is almost three times the conventional estimate based on nitrogen balance. The evidence thus far suggests that the requirements for other IAAs, such as lysine, will be similarly increased (167). The important practical implications of these results have made it necessary to examine the method to determine the potential reasons for the discrepancy (149).

To calculate the amount of amino acid oxidized, one must know the precursor enrichment. With leucine as tracer and with the reciprocal pool as proxy for the precursor, an error of two- to threefold seems impossible. Another potential source of error is the proportion of the labeled CO_2 derived from amino acid oxidation that is retained in the body. This proportion, measured by a separate infusion of labeled bicarbonate, ranges from 15 to 30% and varies somewhat with fasting and feeding, duration of infusion (34), and exercise (159). It has been suggested that this method may not give a true estimate of CO_2 production when amino acids are oxidized within the mitochondria, but this effect does not seem to be significant (60). Again, therefore, an overestimate of CO_2 production cannot account for the discrepancy.

The general correspondence (Table 1) between the values for protein synthesis obtained by leucine-KIC and those given by the EP method provide support for the validity of the tracer balance method. If this latter method is indeed valid, one would expect to find a close match between oxidation of the tracer amino acid and that of total amino-N, as determined by the excretion

of urea + ammonia. If the leucine content of body protein is taken to be 8%, leucine kinetics can be converted to protein kinetics by multiplying by 100/8, to give a predicted value for total amino acid oxidation. In the experiments of Clugston & Garlick (23), if CO_2 outputs are corrected for KIC as precursor, there was on average excellent agreement between predicted and observed nitrogen excretion. If the amino acid pattern in food differs significantly from that of body protein, any excess amino acids will be oxidized. Pacy et al (106) found that when this discrepancy was corrected for, the ratio of predicted to observed nitrogen excretion averaged 0.8 and did not change significantly over a wide range of protein intakes. El Khoury et al (35, 36) found good agreement in 24-h infusions between leucine oxidation and nitrogen excretion after correcting for fecal and other losses. I am not sure that these corrections are justified, and I question whether the proper comparison should not be with urea production rather than urea excretion (see below), but these are minor points. The correspondence also depends on the value taken for the leucine content of body protein. The figure of 8% is derived from the leucine content of beef muscle (17) and of stillborn human fetuses (155). An alternative figure of 6.7% has been proposed (114) based on analyses of pig and sheep carcasses, but these analyses include connective tissue, which contains very little leucine and turns over very slowly.

With exercise, leucine oxidation increases with rising intensity of work (93, 159) but whether nitrogen excretion is similarly increased over the same time scale is unclear. These, however, are relatively unimportant problems. In general, comparison between leucine oxidation and nitrogen excretion reveals no major discrepancy, and one must ask not why the tracer balances give such high estimates of IAA requirements, but why the early nitrogen balances gave such low estimates (167). It should also be noted that the general agreement between expected and observed nitrogen excretion supports the validity of Walser's approach for measuring whole-body nitrogen flux and protein synthesis.

Urea Metabolism

In a normal diet, ~30% of urea produced, as measured by the dilution of [$^{15}N{\bullet}^{15}N$] urea in plasma or urine, passes into the colon, where the microbial flora break it down to ammonia. This ammonia reaches the liver, where part of it is recycled to urea. This urea can be distinguished from directly produced urea because only half of its nitrogen is enriched [$^{15}N{\bullet}^{14}N$]. The remainder of the ammonia enters the amino-nitrogen pool, where it is incorporated into nonessential amino acids or, by transamination, into the carbon skeletons of the indispensable amino acids. This process, called salvage, has been extensively studied by Jackson & coworkers (71, 73, 77), and its measurement is very reproducible (64). These authors have shown that the extent of salvage,

as a proportion of urea produced, is increased in subjects on low-protein diets, in young children and pregnant women, and in any other individuals who need to economize nitrogen. However, some aspects of the salvage process are difficult to understand. For example, when subjects were put on different levels of protein, intake was more closely related to urea excretion than to urea production. In the experiments of El Khoury et al (35), urea production was relatively constant, but urea excretion fell during the fasting periods, in accordance with the diurnal cycle of urea output recorded by Steffee et al (132). In fact, when Jackson's data are combined with those of El Khoury, salvage appears to be high in the postabsorptive state but very low in the fed state.

This evidence implies that the transfer of urea into the colon is a regulatory process, but it is difficult to visualize the mechanisms that could be involved. In their experiment with constant infusion of labeled urea, Wrong et al (160) found that the enrichment of ammonia in the colon was only approximately one tenth that of plasma urea, indicating a very large nitrogen flux through or in the colon many times greater than the entry of endogenous urea. Whether this intense activity, which must be ascribed to the colon microflora, can in some way regulate the entry of urea is at present a matter of speculation.

Another question that was recently reopened is whether the colonic bacteria can synthesize indispensable amino acids that can then be absorbed and utilized by the body. Preliminary experiments on the pig suggest that this process may occur to some extent in animals on low-protein, high-residue diets (140). Further developments will be awaited with interest.

EFFECT OF FOOD INTAKE ON PROTEIN METABOLISM

In terms of food intake, three different situations must be distinguished: the acute response to a meal; the effect of differences in what may be called the prevailing or habitual level of protein and energy intake; and the long-term effect of marginal food intakes, such as are seen in developing countries.

Response To a Meal

The early studies of Clugston & Garlick (23) with 24-h infusions of labeled leucine led to the concept of diurnal cycling, in which protein is deposited in the fed state and lost during fasting so as to maintain nitrogen equilibrium over 24 h. Pacy et al (106) later showed that the amplitude of the cycling increases with increasing protein intake. Clugston & Garlick originally concluded that the deposition of protein resulted mainly from increased synthesis in the fed state. Later results suggested, as discussed above, that with 24-h infusions, significant recycling of isotope could occur during the period of fasting, which followed that of feeding. As a result, the fasting flux and synthesis rate would be underestimated. Shorter infusions (8 or 12 h) showed that the main effect

of feeding was a reduction in protein breakdown, with very little change in synthesis (68, 92, 100). A general consensus that this is indeed the case has been reached (92).

On the other hand, El Khoury et al (35, 36) also gave 24-h infusions, with 12 h fasting preceding 12 h feeding of small frequent meals, and found no evidence of recycling; precursor enrichment remained virtually constant throughout. The discrepancy with the earlier studies can probably be attributed to the fact that, since feeding followed fasting, breakdown—and hence recycling—of isotope was suppressed. The original aim of these 24-h infusions was to validate the extrapolation to 24 h of the results obtained with fasting and feeding over shorter periods, and good agreement was indeed observed. In a third series of heroic 24-h infusions (37), three normal meals were given at intervals during the feeding period. Clear-cut variations, related to the meals, were found in plasma leucine concentration, leucine oxidation, and KIC enrichment. Thus with a normal meal pattern, a steady state cannot be assumed for the calculation of synthesis and breakdown rates. An example of an approximate calculation from El Khoury's data was given above.

The Prevailing Protein Intake

In experiments designed to examine the effect of protein intake on protein turnover, the usual procedure has been to keep subjects at each level of intake for 7–10 days, since previous work has shown that this is the approximate time needed to achieve nitrogen balance at the new level (112). Garlick et al (46) and McNurlan & Garlick (86) have discussed in detail the results of a number of such studies. They are rather variable. In the postabsorptive state we see a tendency toward higher rates of synthesis and lower rates of breakdown with increasing protein intake, suggesting that even during fasting there is a follow-on effect from the previous diet. In the fed state the changes are somewhat more pronounced but are still not significant in many cases. In some studies (25, 66, 97, 162) a clear-cut fall in fed-state synthesis was observed at protein intakes below 0.6 g/kg per day, which is approximately the level of the adult requirement for protein. Above this level, however, changes in synthesis do not parallel changes in intake.

Pacy and coworkers (106) are the only group to have examined the effect of the level of protein intake using both the leucine-KIC and the EPA methods. In this particular case, the two methods did not agree very well, probably because the changes with protein intake were not significant with either method. With that proviso, the EPA method showed increases in both synthesis and breakdown with increasing protein intake. Interestingly, at the lowest protein intake the ratio $\dot{Q}_U$ to $\dot{Q}_A$ was very high (106), suggesting a concentration of protein metabolism in the viscera when intakes are inadequate.

Young's group at MIT, in the course of their work on the requirements for indispensable amino acids, have generated many observations on protein kinetics when the test amino acid is fed at various levels and when total nitrogen intake is constant and adequate (summarized in 149). The general conclusion that can be drawn from these studies is that the rate of protein synthesis is not a sensitive or reliable index of whether requirements are being met. Young et al (168) also took steps to determine whether an adaptation period of one week is sufficient. In one experiment (168), when leucine intakes were inadequate as judged by the tracer balance method, a drop in synthesis was observed at one week, followed by a further significant drop at three weeks, but in a subsequent study this effect of a longer period on the diet was not confirmed (88).

A question of some interest is the site of postprandial protein deposition. In humans, attention has been focused on muscle because it is accessible. Measurements by constant infusion and with the forearm model have shown a variable but significant stimulation of the rate of muscle protein synthesis after feeding (10, 20, 59), whereas the flooding-dose method yielded no such increase (87). The results of constant infusion show an increase with feeding in the fractional synthesis rate of the order of 0.25% per day (i.e. from 0.5 to 0.75% per day) (59). This increased rate would allow deposition in muscle per kilogram of body weight of some 15 mg nitrogen/12 h. The data of Price et al (111) indicate that on a normal protein intake (0.77g/kg per day), the fed-state rate of deposition is much greater, averaging 40 mg nitrogen/kg per 12 h. It seems more likely that the main sites of postprandial protein deposition are the liver and other visceral tissues. Garlick et al (48) noted that in the rat, the protein content of liver might increase by 20% after a meal, and studies with labeled leucine in dogs showed a very large uptake in the splanchnic region after feeding (171).

During the fasting part of the cycle, the visceral organs must lose the protein they have gained. In fasting or undernutrition, the liver and gut are the first organs to lose protein, but after a short time peripheral tissues—muscle and skin—take over and bear the brunt of the loss (151). This result is what one would intuitively expect from a system with both rapidly and slowly turning over protein pools.

Energy Intake

Given the number of studies of the effect of altering protein intake, the effect of altering energy intake has by comparison been neglected. In normal subjects, moderate increases or decreases of energy of the order of 25% produced no detectable changes in leucine flux or in protein synthesis and breakdown (98, 162). The effect of much larger reductions in energy intake has been examined in obese patients. With a bolus dose of [^{15}N]-glycine and with ammonia as the end product, we were able to make 13 consecutive measurements of protein

turnover in a single patient over a 7-week period (52). Reducing the daily energy intake from 2000 to 800 kcal with adequate protein intakes had virtually no effect on the rate of protein synthesis, whereas the rate fell by 50% on a diet of 800 kcal but zero protein. Other studies have confirmed that the rate of protein synthesis changes very little or not at all in obese subjects on very low energy intakes (67, 109, 157). These studies are, however, subject to the criticism that the patients were not really energy deficient because they were drawing on their stores of fat.

One might tentatively conclude that protein turnover is well maintained on low energy intakes, but this cannot be the whole story. It has long been recognized that nitrogen balance is affected by energy intake, changing by 1.5–2 mg nitrogen per kilocalorie change in energy intake. The studies of Jackson et al (74) on children who had recovered from protein-energy malnutrition are relevant here. At constant and adequate levels of protein, energy intake was reduced from 108 to 87 kcal/kg per day. There was no change in synthesis, as measured by the [15N] glycine EPA method, but a 14% increase in breakdown was observed, and nitrogen balance fell from +81 to +46mg nitrogen/kg per day. Motil et al (98) likewise found an increase in leucine balance equivalent to +23 mg nitrogen/kg with an additional energy intake of 11 kcal/kg, exactly in agreement with the classical relationship. The N-sparing effect of extra energy may be related to the fact that higher energy intakes promote greater de novo synthesis of alanine (165).

Severe Acute Malnutrition

Evidence clearly shows that in young children with kwashiorkor or marasmus, protein synthesis is depressed (55). In these children the metabolically active tissues, such as brain and liver, are relatively well preserved, whereas the less active tissues, such as muscle, skin, and adipose tissue, are severely depleted (147). One would therefore expect protein turnover per unit body weight to be high. In reality, however, it is often low, indicating that essential vital functions are seriously compromised. In children who survive, both protein turnover and basal metabolic rate are rapidly restored to supranormal levels with adequate treatment (146); these functions are apparently so important that they take absolute priority in the recovery process. In two subjects who made an unaided crossing of the Antarctic continent, protein turnover was measured at intervals by EPA methods and was well maintained up to the end (95 days), when the subjects had lost more than a quarter of their body weight and were approaching the limits of human endurance (133; MF Stroud, AA Jackson & JC Waterlow, manuscript in preparation).

Chronic Undernutrition

Studies of metabolic rate and protein turnover have been performed in India on very poor adult subjects who are stunted in height, have an average body

mass index (BMI) of only 16.7, and live on marginal intakes of protein and energy (127, 128). Per kilogram body weight or lean body mass, these subjects' rates of protein synthesis and turnover, as measured by the glycine-EPA method, were the same as those of controls, and their BMRs were actually higher. These results must be considered in the context of the subjects' body composition. In these undernourished men, muscle mass was only 16 kg, compared with 26 kg in the controls, whereas nonmuscle mass, at 23 kg, was the same in both groups. These changes in body composition were paralleled by changes in the estimates of flux calculated from urea and ammonia. $\dot{Q}_U/\dot{Q}_A$ was 1.18 in normal subjects vs 1.65 in undernourished subjects, suggesting once more that with marginal protein intakes, protein turnover in the viscera constitutes a larger proportion of WBPT.

GROWTH

Remarkably, the deposition of protein in the growing infant is characterized by a high rate of protein breakdown, with an even higher rate of synthesis. This high rate is usually attributed to the need for remodeling and flexibility in enzyme production. The process is wasteful, since protein turnover requires energy (see below). Some other organisms have adopted a different strategy. Varieties of edible mussel, for example, grow by completely suppressing protein breakdown (63), and in rainbow trout a lower rate of protein breakdown allows some fish to grow faster than others on the same intake (85).

In humans, many studies have been performed on premature infants. The following discussion is confined to infants with a postconceptional age of ~30 weeks and with a birth weight of ~1.5 kg, on whom measurements were made 7 or more days after birth. Studies within a day of birth on infants who have respiratory distress syndrome or require parenteral nutrition do not represent normal growth (94, 117). Most measurements have been made by the EP method with [^{15}N] glycine or, in some cases, with [^{15}N] yeast (28, listed in 130). The results are rather variable, and several authors have confirmed Jackson's original observation (75) that in prematures urea is sometimes very poorly labeled; this suggests that the availability of glycine is limiting. The range for protein synthesis may be taken to be 8–14 g/kg per day. The results of two studies with labeled leucine fall within this range (7, 30). Synthesis rate seems to be unaffected by the type of milk (i.e. human or formula), and in some cases an inverse relationship was found between synthesis and postconceptional age.

These premature infants were depositing protein at the rate of ~2g/kg per day, and their average synthesis rate could be taken as ~12 g/kg per day. If one accepts the estimate of Reeds (114) that the basal rate of protein synthesis in the absence of growth is of the order of 4g/kg per day, then it follows that

for 1 g of protein to be deposited, 4 g must be synthesized over and above the basal rate. This apparently futile synthesis makes an appreciable contribution to the energy cost of growth, and in some of the studies listed, the energy intake may have been too low to support maximal deposition of protein.

When the synthesis rate in premature infants is related to the so-called metabolic mass, i.e. the three-fourths power of the body weight, a close correspondence with adults is evident (in premature infants, $S/W^{0.75} = 13.3$; in adults, $S/W^{0.75} = 12.4$). At first sight, this similarity seems to be the expression of a natural law, bringing intraspecies comparisons in line with the well-known interspecies relationship—ranging from mouse to cow—between body weight, metabolic rate, and protein turnover (145). However, it is really a statistical curiosity, since a premature baby is not merely a tiny adult but differs greatly in body composition. For example, liver and brain are proportionally much larger and muscle mass much smaller in the premature infant. A finding of Beaufrère (7) provides evidence of the dominant role of the liver: With nasogastric infusions of labeled leucine in premature infants, 48% of the label was extracted in the splanchnic bed compared with ~25% in adults.

As might be expected from the rapidity with which the growth rate falls off after early infancy, in children 1-2 years of age the rate of protein synthesis is ~6 g/kg per day and is approaching the adult value (53, 55). Pregnancy and lactation are also forms of growth. Even in the third trimester of pregnancy, when protein deposition is greatest, it amounts to less than 1% of whole body protein per day. It is therefore hardly surprising that no very clear-cut changes in protein turnover have been found (31, 137, 156). During lactation, the average nursing mother produces ~8 g protein per day, which is ~4% of her whole-body protein synthesis. One remarkable study of lactation, mentioned above, involved multicompartmented analysis with [^{13}C]-lysine given intravenously and [^{15}N]-lysine given orally (136). Of all the parameters measured, in only one did lactating women differ significantly from nonlactating controls, but this difference is interesting enough to justify such an elaborate study: The lactating women had a reduced rate of exchange between free lysine and a slowly turning over protein pool, which might perhaps be regarded as an adaptation to the stress of lactation.

Energy Cost of Protein Synthesis

In mammalian species, protein turnover and metabolic rate vary as the three-fourths power of body weight (145). The ratio of the coefficients is ~1:5, so that ~5 kcal are expended per gram of protein turned over. Those concerned with animal production have estimated the energy cost of protein deposition during growth, but as discussed above, deposition is not the same as synthesis. Another approach has been to relate protein synthesis to metabolic rate within a group of individuals, or in the same subjects after a stimulus such as triio-

dothyronine. This approach has indeed often resulted in a correlation (150). However, this kind of correlation exercise is of little help because we have no logical reason to suppose that a change in energy expenditure is solely determined by a change in protein turnover. We therefore must rely on biochemical estimates, which have been discussed many times in the literature (e.g. 150). It is accepted that 4 moles ATP or GTP, produced at an energy cost of 19 kcal/mole (16), are needed for the formation of 1 mole peptide bond, which reduces to 0.87 kcal/g protein. This result will be an underestimate to the extent that many proteins are synthesized in a precursor form from which peptide chains are later detached. Additional costs are associated with amino acid transport, RNA turnover, and protein breakdown. The maximum cost of amino acid transport can be estimated if we assume, first, that 70% of amino acids used for protein synthesis enter the cell from the blood, and 30% are recycled within the cell and therefore need no transport. The second and worst-case assumption is that all amino acid transport is sodium dependent, with a stoichiometry of 1 mole Na per mole of amino acid and with 3 moles Na transported per mole of ATP (33). These assumptions lead to an energy cost of 0.05 kcal per gram of amino acid transported.

Schöch and his colleagues have developed methods for estimating the turnover rates of ribosomal, transport, and messenger RNA from the urinary excretion of specific methylated bases (121, 122; S Schöch & H Topp, personal communication). They calculate that 94% of the energy cost of RNA turnover is associated with mRNA because mRNA has a much shorter half-life than tRNA or rRNA. The turnover rate of mRNA in an adult was found to be ~1 mol/kg per day. If we assume an average of 8000 bases per molecule precursor mRNA and a cost of synthesis of 2 ATPs per base, the energy cost of mRNA turnover would be ~0.3 kcal/kg body weight per day. If this cost is associated with a protein turnover rate of 4g/kg per day, the energy cost amounts to 0.075 kcal per gram of protein turned over, or less than 10% of the total. Incidentally, it follows from these calculations that an average molecule of messenger will translate ~100 times before decaying.

Finally, we must consider the cost of proteolysis. According to Varshavsky et al (142), most of the selective breakdown of intracellular proteins under normal metabolic conditions is ATP dependent and nonlysosomal. The signal for degradation is the attachment to the protein of one molecule of ubiquitin. However, as this requires only three molecules of ATP per protein molecule, the cost is negligible compared with that of peptide bond synthesis. On the other hand, Mortimore & Pösö (96) claim that, at least in liver, lysosomes are primarily responsible for protein degradation. The maintenance of lysosomal activity will also require ATP for the transport of protons across the membrane (103), since the intralysosomal milieu is more acidic than the cytosol, but the cost involved is unknown.

All these numbers add up to ~1 kcal/g protein synthesized which, from a

physiological point of view, may be taken as a reasonable estimate for the time being. Thus, in the normal adult, protein turnover at 300 g/day accounts for ~20% of the basal metabolic rate; the proportion may be substantially higher as a result of growth or injury.

INJURY AND INFECTION

"The classical researches of Cuthbertson ... provide the background to all the current work on protein turnover in injury... He described the increased nitrogen loss after injury as a 'catabolic' loss and suggested that it was derived from muscle under the influence of corticosteroids. Subsequent work has in general supported these ideas" (145). These words were written 10 years ago, and the large amount of work that has been done since supports this conclusion.

The main question arising from Cuthbertson's work is whether the catabolic nitrogen loss results from an increase in protein breakdown or from a suppression of synthesis. Both recent and past studies support the pattern proposed by Clague (22): The body's response to injury, whether it takes the form of skeletal trauma, burns, sepsis, or multiple organ failure, is an increase in both synthesis and breakdown, with breakdown predominating (e.g. 2, 18, 77, 81). With moderate trauma, as in elective surgery, these changes may be small and transient (51), but with more severe injury the gap between synthesis and breakdown widens, resulting in increasing oxidative loss. The conclusion is the same regardless of whether the leucine-KIC or the EP method is used, as demonstrated in the early studies by Kinney's and Young's groups (15). The same pattern has been found in more chronic disease states, e.g. cirrhosis of the liver (134), sickle cell disease (6), and early lung cancer (79, 91). In the case of sickle cell disease, the increased rate of protein turnover presumably reflects the continuing demand for hemoglobin synthesis. In the case of cirrhosis of the liver and early lung cancer, whether the production of acute-phase proteins is increased remains unknown. We calculated from the data of Fleck (44) that when sepsis reaches its peak, the production of these proteins might be as much as six times the rate of albumin synthesis and could account for an appreciable increase in whole-body protein synthesis (147). To this result, we must add the extra synthesis required for the multiplication of leukocytes.

The body's ability to mount these responses is influenced by its nutritional state, as was recognized long ago by Munro & Cuthbertson (99). A striking example is provided by the work of Tomkins et al (139) on young children in Nigeria. Well-nourished children with an acute infection had a protein synthesis rate of ~10 g/kg per day, with an even higher breakdown rate. In contrast, in malnourished infected children this rate was ~6 g/kg per day, and in malnourished uninfected children it was only ~3.5 g/kg per day. Whether this low rate should be considered a compromised response or an adaptation is difficult to determine.

Cuthbertson's original suggestion that the nitrogen lost in injury was derived from muscle is supported by the finding of increased urinary losses of 3-methylhistidine after surgery (18, 82). In the Nigerian children who were well-nourished, infection was accompanied by massive increases in 3-methylhistidine and creatinine output, which were not seen in the malnourished.

The very sick patient provides a good model for examining the effects of hormones on the components of protein turnover because many authors have found that the hormonal concentrations in plasma are altered in such patients (2, 56). This subject has been reviewed by Young (165). In patients with multiple organ failure, Arnold et al (2) found raised concentrations of the catabolic hormones: cortisol, glucagon, and noradrenalin. Of these, cortisol was the best predictor of nitrogen loss. On the anabolic side, growth hormone has been found to reduce the losses of nitrogen and of 3-methylhistidine and to increase the uptake of amino acids in the leg (56). In a study with [^{15}N] glycine, growth hormone produced a significant increase in protein synthesis, with a smaller, nonsignificant increase in protein breakdown (82).

Particular interest has focused on insulin because of its well-known anabolic and anticatabolic actions. Insulin resistance is well recognized in traumatized patients, and various suggestions have been made as to how this resistance might relate to the catabolic loss. Jahoor et al (77) examined the maximum biological effectiveness of insulin on the rate of protein breakdown. In patients with sepsis or burns, insulin infusions with a euglycemic clamp produced levels of plasma insulin ~100 times greater than normal. Measurements with labeled leucine showed that use of the clamp resulted in a significant decrease in leucine oxidation and in leucine appearance from protein breakdown. However, severely injured patients are likely to be in a non–steady state, and as mentioned above (77, pp. 23, 24), account must be taken of this in analysis of the kinetic data. In thyrotoxicosis, as in trauma, the metabolic rate is increased. It was therefore not unexpected that in experiments on rats, thyroid hormone caused an increase in both synthesis and breakdown of muscle protein, indicating a more rapid rate of overall turnover (81a). In human thyrotoxicosis, however, the opposite seems to be the case: In the study of Morrison et al (95), protein synthesis was depressed both in muscle and in the whole body. As Jepson et al (81a) point out, the interactions between amino acids, insulin, and thyroid hormones are complex, and more work needs to be done on these pathological states before precise causal relationships can be established. The situation resembles that in metabolic control analysis, where a change in any one effector induces changes in all the others.

THE FUTURE

Looking ahead, we must ask ourselves not only how measurements of WBPT can be improved, but also whether these measurements are likely to contribute

to theoretical or practical knowledge. Ever since Schoenheimer's discovery of the dynamic state of body constituents, we have been faced with the question of how to maintain a steady state. The work on diurnal cycling (106) leaves us with the picture of a body protein mass, or at least a part thereof, that is continually expanding and contracting over several hours. Amino acids and hormones are effectors that can stimulate these processes, but we do not know how they are coordinated, i.e. who conducts the orchestra (148). The evidence reviewed above suggests that protein degradation is more sensitive than protein synthesis. In Young's (165) summary of the effects of insulin and amino acids on protein turnover, it is remarkable in how many studies the main effect was on protein breakdown.

A discussion of regulatory mechanisms cannot, of course, be confined to amino acids and proteins. As discussed by Young & Marchini (166), the rate of protein synthesis at any moment is determined by the amount of available mRNA, which in turn seems to depend primarily on its rate of breakdown (119, 120). One way in which the stability of macromolecules is influenced by their environment is illustrated by the elegant work of Häussinger and his colleagues (62). Their experiments show that the hepatocyte is extremely sensitive to the hydration state, which can be altered within minutes by hormones, amino acids, and other effectors. Swelling has an anabolic and shrinkage a catabolic effect. Perhaps we have here the germ of a regulatory system: Proteolysis results in an increase in intracellular amino acid concentration; the cell swells; and synthesis is stimulated.

Although it is satisfying to try to develop a generalized system of control, it would be misleading to regard the whole body as a collection of parts all operating in the same way, with the same reactions to effectors and the same biochemical control systems. Liver and muscle are prototypes of tissues with different responses. In the rat, in the course of ontogeny, the fractional synthesis rate of liver protein changes little between weaning and old age, whereas that of muscle decreases by a factor of approximately four (152). In liver, amino acids and insulin reduce the rate of proteolysis; in muscle, the main response, at least in young rats, is an increase in synthesis, although whether this conclusion applies to adult humans remains controversial (59, 87). Cortisol has long been known to have a catabolic effect in muscle and an anabolic effect in liver. Such examples could probably be expanded. Therefore, a limited but important objective of future work on WBPT would be to find ways of assessing, separately and noninvasively, the responses of what might be called liver-type and muscle-type components. We are not even certain whether these two different types of response correspond to the conventional separation of rapidly and slowly turning over protein pools.

On the basis of current work, we can define three possible approaches to the problem, all of which need to be confirmed by animal experiments:

1. An extension of multicompartmental models, probably with multiple tracers. The studies conducted to date have relied for the most part on single injections of tracers, but there is no reason why the multicompartment approach should not be used with constant infusions to produce isotopic steady states. Direct analysis of precursor-product relationships in different tissues should then be very rewarding.
2. The first-pass effect, with both oral and intravenous infusion of tracers, holds promise for examining separately the activity of visceral and peripheral tissues. Here, the different behavior of different amino acids makes further exploration difficult. How, for example, can a 50% retention of phenylalanine with only a 25% retention of leucine be reconciled with the idea of increased synthesis in the splanchnic bed? Of particular interest are the kinetics of lysine and threonine, in light of their very large pool sizes.
3. In the EP approach, the variation between the two estimates of flux based on urea and ammonia seems to follow some pattern (summarized in 148), indicating a change in the balance of protein turnover between the visceral and peripheral compartments. Further examination of this method, and particularly of the metabolic pathways of glycine that lead to the labeling of the end products, is necessary. A measurement of WBPT based on the metabolism of a single particular amino acid can never be satisfactory. Yet because amino acids have different metabolic characteristics, it is difficult to circumvent this problem. The solution is perhaps to use a uniformly labeled protein with the same amino acid composition as tissue protein, as we did in our early experiments with egg (110).

Another reason to develop and improve the EP approach is the ease with which it can be used in the field. Only through widespread measurements on subjects living in a range of different conditions will it be possible to answer the questions raised by Millward et al (93A) and by Young (164): Is there any advantage, in terms of health, well-being, and activity, in having a high or a low rate of WBPT? Is there a threshold that must be reached? The response to such questions would be a crucial contribution of metabolic studies to the definition of protein needs in humans.

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