Core R10-10 was taken in the Newfoundland Basin. The upper half is a slightly silty lutite in various shades of gray and rose-gray. At a depth of 120 centimeters there is a gradational change from dark gray lutite to light gray calcareous lutite. The lower half contains several sharply defined color changes and two zones of glacial marine sediment—that is, lutite containing ice-rafted pebbles.

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Kinetic Aspects of Assembly and Degradation of Proteins

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Recent years have seen the development of highly refined techniques applicable to the study of protein synthesis. As a result, productive research on this formidable problem has grown considerably. Yet even today virtually nothing is known, except by inference, of the pathway between free amino acids, which are generally accepted as the ultimate precursors, and proteins. The catabolic aspects of protein metabolism have been almost completely neglected, and it is even questioned by some whether controlled intracellular degradation plays any role at all in normal protein metabolism (*1*).

Speculations with respect to the mechanism of protein biosynthesis have tended to focus on the problem of how a specific and definitive amino acid sequence is achieved. The result has been a deemphasis of the problem of the "pathway of biosynthesis" in the sense that this term has been applied in studies of other complex molecules. Many have concluded either that there are no intermediates in protein synthesis or that the intermediates must be so poorly defined or so transient that they defy characterization. It may be useful to consider the analogous situation that prevailed for a number of years in the field of fatty-acid synthesis. Even after isotopic evidence pointed overwhelmingly to a synthetic mechanism involving stepwise two-by-two condensation, intermediates were not detected by direct tissue analysis.

Studies from this laboratory have suggested the existence of intermediate compounds between free amino acids and completed protein molecules. Specifically, these studies have shown the nonidentity in terms of metabolic history of the different residues of a given amino acid species at different loci along the peptide chains of the proteins investigated.

Most of this work has been reported in detail elsewhere (*2–5*), and so we shall only outline here the general plan of the experiments and summarize the results for comparison with the findings of others. The two schemes shown in Fig. 1 serve as a basic outline for these considerations. In scheme A of Fig. 1, protein synthesis is pictured as an "all-at-once" assembly of individual amino acid residues on a sequence-determining template. Scheme B in Fig. 1 differs in that it includes intermediate compounds on the path between the free amino acid pool and the completed protein molecule. Many variants of these basic schemes can be devised by introducing reversible reactions, combining some of the features of each, multiplying the number of steps, and so on.

The design of our experiments can be made clear, however, without these refinements. We label amino acid A with carbon-14, incubate it with tissue *in vitro* or *in vivo*, and isolate from the tissue a sample of a pure protein. Synthesis according to scheme A should result in a protein having, for amino acid species A, the same specific radioactivity at all points along the peptide chain (*uniform labeling*). With scheme B, however, different residues of this amino acid species may have different specific radioactivities (*nonuniform labeling*). It should be emphasized that scheme B could also lead to uniform labeling if the pool sizes of the intermediates and the rates of the various reactions involved were favorable for this result. The finding of uniform labeling would therefore be ambiguous.

Experiments of this sort have been carried out in the case of three crystalline proteins—ovalbumin, insulin, and ribonuclease. In each case, the protein synthesized *in vitro* in the presence of a labeled amino acid has been rigorously purified. Samples of labeled amino acid derived from different loci in the peptide chain have been obtained by partially degrading the protein and separating the fragments. Ovalbumin has been studied most extensively of the three; the data obtained are summarized in part A of Table 1. Of particular interest is the comparison of the labeling data obained on the plakalbumin and hexapeptide fractions of ovalbumin which result from the treatment of this protein with the bacterial protease, subtilisin (*6*). Three of the residues in the hexapeptide—namely,

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Table 1. Summary of ovalbumin studies.

Amino acid residue isolated	Nonuniformity ratio* observed
A. Hexapeptide fraction (2, 3)	
Ala_1 (N-terminal)	2.7, 1.8, 1.5, 1.7†
Gly_2	0.9
Val_3	not studied
Asp_4	3.7, 2.8, 2.0, 1.4
Ala_5	1.0, 1.0
Ala_6 (C-terminal)	1.1, 1.1
B. Alanine-containing peptide fractions in pepsin digest (3)	
Ala from:	
Fraction 28 (0.31–0.39)‡	1.6§
Fraction 23 (0.65–0.72)	1.4
Fraction 13 (0.81–0.89)	1.0
Fraction 3 (0.72–0.80)	0.5
Fraction 1 (0.89–1.00)	0.4
C. Peptides in acid hydrolyzate of oxidized ovalbumin (5)	
Gly from $CySO_3H$-Gly fraction	1.4
Ser from Ser-$CySO_3H$ fraction	0.4

* Specific activity of indicated residue divided by the average specific activity of that amino acid species in the whole ovalbumin molecule.
† These values represent results in separate 4- or 5-hour *in vitro* incubations of oviduct.
‡ Fractions designated by R_f in 1:1 butanol and propionic acid, water saturated.
§ Representative examples from a total of 28 peptide fractions examined.

glycine and two of the three alanine residues—were found to have specific activities not significantly different from the average specific radioactivity for these amino acid species when they were isolated from complete acid hydrolyzates of ovalbumin. The aspartic acid and the third alanine residue, however, showed marked differences from the average, indicating nonuniform labeling of the molecule.

The nonuniformity of labeling is not limited to the hexapeptide portion of ovalbumin. After pepsin digestion of alanine-labeled ovalbumin, as many as 28 alanine-containing peptide fractions were separated, and the specific activity of the alanine in each was determined. As shown in part B of Table 1, the variation in specific activity among these different alanine fractions was of a rather high order. Significant differences have been demonstrated between glycine residues and also between serine residues (Table 1, part C).

The data presented in Tables 2 and 3 show that nonuniformity of amino acid labeling can also be demonstrated in other proteins. Specific degradation of both insulin and ribonuclease labeled *in vitro* yielded amino acid species, derived from different parts of these proteins, which showed marked differences in specific radioactivity.

Besides demonstrating the occurrence of a mechanism of protein synthesis that can lead to nonuniform labeling, these experiments furnish convincing evidence that radioactive amino acids are incorporated into the warp and woof of the protein molecule. The isolation and degradation of well-characterized peptides from the proteins studied (for example, the hexapeptide and the cysteic acid peptides from ovalbumin, and the heptapeptide from insulin) leave little doubt that incorporation has occurred by true peptide-bond synthesis.

The finding of nonuniform labeling of proteins is basically a kinetic result and, as is generally true of kinetic results, does not by itself define a mechanism. It may, however, permit us to define within broad limits the conditions that any proposed mechanism must satisfy. We have examined several plausible schemes for protein biosynthesis, and, where possible, we have derived equations that would describe the expected labeling results.

Stepwise Synthesis; Template Not Obligatory

Scheme B of Fig. 1 presents an ultrasimplified schematic representation of protein synthesis through free intermediate compounds. We limit our considerations to two of n intermediates, and we imply nothing with respect to the chemical nature of these intermediate conjugates except that they are kinetically distinguishable. Amino acid A, destined for positions A_1 and A_2 in the completed protein, passes through the nonequivalent intermediate forms X_1 and X_2 prior to incorporation. It is assumed (i) that both steps follow first order kinetics, (ii) that a constant steady-state concentration of intermediates is maintained, and (iii) that the specific activity of the free amino acid pool remains constant. On the basis of these assumptions, we can show that the radioactivity in the intermediate compounds follows a time course described by Eq. 1 and plotted in Fig. 2. Since steady state is assumed, the total radioactivity remains proportional to specific radioactivity.

$$\frac{dC_{X_1}}{dt} = k_1 C_A - k_2 C_{X_1}$$

$$C_{X_1} = \frac{k_1}{k_2} C_A (1 - e^{-k_2 t}) \qquad (1)$$

$$\frac{dC_{A_1}}{dt} = k_2 C_{X_1}$$

$$C_{A_1} = k_1 C_A \left[t + \frac{1}{k_2} (e^{-k_2 t} - 1) \right] \qquad (2)$$

where C_A is the radioactivity in amino acid A in free amino acid pool; C_{X_1} is the radioactivity in intermediate X_1; C_{A_1} is the radioactivity in residue A_1 in protein; k_1 is the rate constant for intermediate formation; and k_2 is the rate constant for incorporation of intermediate into protein.

The specific activity of the intermediate rises very rapidly at first and approaches the specific activity of the free amino acid precursor asymptotically. How rapidly this plateau is reached depends on the size of the intermediate pool and the rate of protein synthesis. The specific activity of the residue in the protein follows a time course described by Eq. 2 and plotted in Fig. 2. It is assumed that the amount of new protein synthesized is small relative to the amount of preformed, unlabeled protein so that specific activity will be directly proportional to total radioactivity in any particular residue. The specific activities of the protein residues rise at first with a positive acceleration and then, at the point at which the intermediates ap-

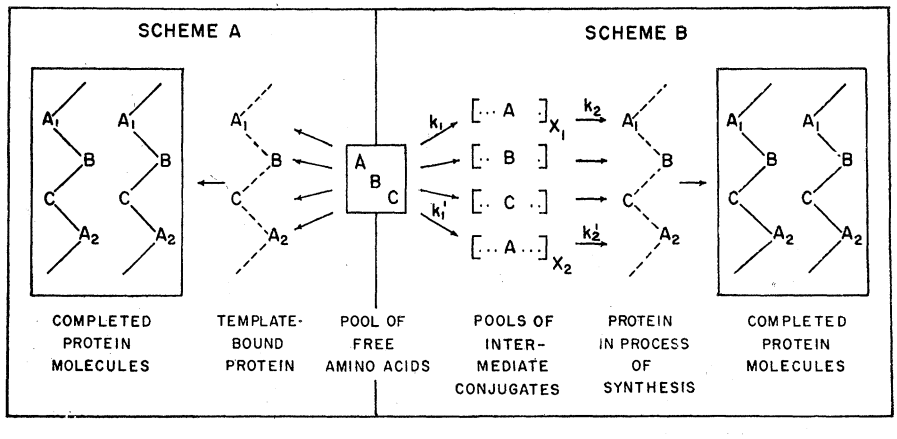

Fig. 1. Two basic schemes for assembly of amino acids to form protein: (scheme A) Simultaneous assembly onto a template; (scheme B) stepwise synthesis through intermediate forms, chemical nature unspecified.

Table 2. Nonuniform labeling in insulin (*4*).

Amino acid residues isolated*	Specific radioactivity (count/min μmole)	
	Expt. 1	Expt. 2
A. Glycine residues		
A_1	27.2	21.0
B_{23}	11.3	9.0
$B_8 + B_{20}$	21.7	
B. Serine residues		
$A_9 + A_{12}$	8.8	1.9
B_9	12.4	5.6

* Designated by position in the A or B chain according to the notation of Sanger (*32*).

Table 3. Nonuniform labeling in ribonuclease (*4*).

Phenylalanine-containing peptides in pepsin digest	Phenylalanine specific radioactivity (count/min μmole)	
	Expt. 1	Expt. 2
Phe from:		
Fraction 1 (0.8)*	57.9	72.6
Fraction 2 (0.6)	33.0	38.0

* Designated by R_f in 4:2:1 *n*-butanol, glacial acetic acid, and H_2O, respectively.

proach their maximum specific activities, rise linearly. If there are one or more intermediate steps, we would therefore expect a "lag" phase followed by a linear rise in protein specific activity. Curves of this type have been observed by Peters (*7*). It may be worth noting that if the intermediate conjugates were in a bound form precipitated and counted along with the "protein fraction," one would then observe a specific activity curve rising linearly from time zero without a lag phase.

If we now consider two different A residues in the protein and assign numerical values for the sizes of the intermediate pools and for the magnitude of the rate constants, we can predict the extent and the course of the nonuniform labeling to be expected. In the example detailed in Figs. 2 and 3, we have assigned to intermediate X_1 a steady-state concentration of 1 percent of that of free amino acid A and to intermediate X_2 a steady-state concentration one-half that of intermediate X_1. Just after the addition of isotope, the specific activity of protein residue A_2 will be twice that of protein residue A_1, and the nonuniformity ratio will be 2. As incubation proceeds, the nonuniformity ratio falls, approaching unity asymptotically.

In the system defined by the aforementioned assumptions, the rate of decrease of the nonuniformity ratio can be shown to be a function of the total mass of protein synthesized. In other words, the abscissa labeled "Hours" in Fig. 3 could be replaced by an abscissa labeled "Total amount of protein synthesized." It follows that, in a system with given concentrations of intermediates, the more rapid the protein synthetic process, the earlier must samples be taken in order to detect nonuniform labeling.

If the reactions in the scheme B of Fig. 1 are made reversible, the kinetic equations become quite complex, but the general result should remain qualitatively unchanged. Furthermore, the utilization of breakdown products from one protein species in the synthesis of others can be visualized. This could lead to the introduction of fragments of very low (or very high) specific radioactivity, thus magnifying the degree of nonuniform labeling.

As we have pointed out previously (*2*), any conclusions drawn from studies of nonuniform labeling are independent of and shed no light on the chemical nature of the intermediates. It is perhaps especially important to reemphasize that the postulated intermediates need not be free. As in the case of the intermediates in fatty acid synthesis, they may only occur bound into a larger molecular complex. These kinetic considerations show that protein synthesis through one or more intermediate compounds, reversibly or irreversibly, could lead under appropriate conditions to nonuniform labeling and is therefore compatible with our experimental results.

Template Synthesis; Free Intermediates Not Obligatory

Two classes of substances with the complexity of pattern needed for a sequence-determining template are the nucleic acids and the proteins themselves. Both have been postulated to serve this function, and detailed consideration has

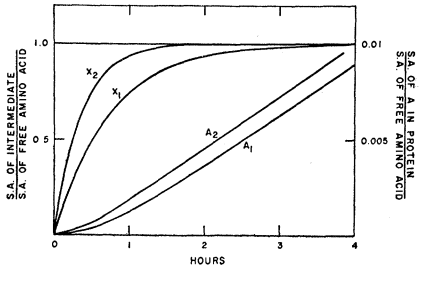

Fig. 2. Specific activity of the intermediate forms (X_1 and X_2) and of the residues in the protein (A_1 and A_2) as a function of time. The concentration of intermediate X_1 was taken as 1 percent of that of the free amino acid; the concentration of intermediate X_2 was taken as 0.5 percent of that of the free amino acid.

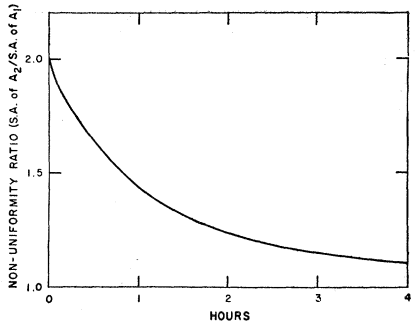

Fig. 3. Predicted time course of nonuniform labeling in the protein for the case in which intermediate pools differ in size by a factor of 2 (see Fig. 2).

been given to mechanisms by which they might operate (*8–11*). In the following paragraphs we wish to examine the template hypothesis from a kinetic point of view to determine what results might be expected in terms of the nonuniform labeling of protein. These kinetic considerations are independent of the chemical nature of the template.

1) *Simultaneous template synthesis.* If amino acids from the labeled free pool take up their appropriate positions on the template simultaneously, or almost simultaneously, uniform labeling or nearly uniform labeling will result. An instantaneous process of this type would be compatible with the apparent absence of intermediate compounds in protein biosynthesis. However, since it could lead only to uniform labeling, it is not compatible with the data that have been presented (*2–5*). In any case, the postulate of simultaneous condensation, interpreted literally, implies in terms of molecular collision an extremely improbable event.

2) *Stepwise template synthesis.* If the accumulation of free amino acids by a template surface requires a period appreciable in the time scale of the cell, then nonuniform labeling could result. This question of the time required for synthesis of a single protein molecule is a critical one. On the basis of the very short generation time of various microorganisms, one may guess that the period required *can* be extremely short. To make an estimate for mammalian tissues is much more difficult. We cannot be certain that the time required for synthesis of a protein molecule is fixed and unvarying. Increasing the rate of synthesis, in terms of net numbers of molecules synthesized per minute, could be accomplished by invoking a larger number of sites of synthesis, in which case the time per molecule might remain unaltered, or by speeding up the production of each individual protein molecule at each available synthetic site. The answer would be relevant in the evaluation of experimental studies on nonuniform labeling.

There appear to be two general ways in which synthesis on a template could lead to nonuniform labeling. The first of these depends on a changing specific activity in the free amino acid pool during the synthesis of a single protein molecule. As pointed out by Dalgliesh (*12*), if the specific activity of the pool falls significantly between the time the first and the last residue of a given amino acid species takes up its position on the template, there will be nonuniformity of labeling. The order in which the amino acids take up their positions would have to be completely or partially fixed, since random accumulation would tend to average out specific activity differences. The order of assembly, however, need not coincide with the amino acid sequence along the polypeptide chain.

Suppose that in our analysis of the protein we were to compare two amino acid residues, the first and the last to be attached to the template. In order to obtain a nonuniform labeling ratio of 2, we would require a drop in pool specific activity of 50 percent during the time of synthesis of each single protein molecule at a single template site. But if ten protein molecules are made per synthetic site during the experiment, a nonuniform labeling ratio of 2 could be obtained only if the pool specific activity fell by 2^{10} or more than 1000-fold. The assembly time per molecule is thus a crucial but unavailable datum needed to evaluate the plausibility of such a mechanism. It should be pointed out that, because of the finite time postulated for the build-up of the final protein, there would at all times be a significant population of template-bound intermediates in the cell.

There is a second way in which a template mechanism could lead to nonuniform labeling, even with unchanging specific radioactivity in the free amino acid pool. This mechanism is depicted schematically in Fig. 4. The template is visualized as a miniature assembly line designed for the gradual building up of protein molecules. At any given moment, the cell contains a broad spectrum of incomplete proteins. Some are virtually complete, lacking only the final residue, A_3; some are in the earliest stage of synthesis, bearing only the first residue, A_1. When assembly is completed, the finished protein dissociates from the template and enters the stable pool of completed proteins.

If, at any time, we add labeled amino acid A, the first molecule completed will have radioactivity only in A_3. After a time molecules having radioactivity in positions A_1 and A_2 will begin to appear in the pool of completed proteins, and finally all new molecules will be uniformly labeled. The A_1 and A_2 residues isolated from the completed proteins will always have a lower specific radioactivity than residue A_3, but uniform labeling will be approached asymptotically.

Combined Mechanisms

The process of protein synthesis could, of course, represent a combination of two or more mechanisms. For example, the formation of low-molecular-weight intermediates from amino acids might occur in free solution and then, by subsequent covalent exchange or transfer reactions, these could take up positions on the template. A mechanism of this type is suggested if compounds such as those described by Park (*13*), by Hoagland (*14*) or by Turba (*15*) lie in the pathway of protein synthesis. It is useful to contrast this type of mechanism, in which the template is not obligatorily involved in all phases of intermediate formation, with mechanisms in which the template is involved in both assembly and intermediate formation. In the latter, as typified by the schemes of Dounce and Lipmann (*10, 11*), amino acid activation and assembly proceeds by a series of successive reactions with sites on the templates themselves. The only intermediate forms between free amino acids and proteins are the template-amino conjugates themselves.

From these kinetic considerations two generalizations emerge. First, nonuniform labeling is incompatible with a synthetic process so rapid that there are no finite concentrations of intermediate compounds. As a corollary, then, such intermediates should be demonstrable, given the appropriate techniques, whether they are simple molecules in free solution or high-molecular-weight complexes with a "template." The same equations might describe the kinetics in either case. Taken together, the necessity for transmitting the extremely complex information required for synthesis of a specific amino acid sequence, and the observed results of nonuniform labeling, lend strong support to the hypothesis that protein synthesis occurs by what we may call a "stepwise template mechanism." Second, the degree of nonuniform labeling would be expected to decrease with increasing time from the introduction of the labeled amino acid. This apparently general characteristic is especially relevant in the interpretation of some of the negative studies on nonuniform labeling which are discussed in a subsequent section.

In concluding these immediate considerations, mention must be made of the possibility that free amino acids may "exchange" with their like in the fabric of completed proteins by a process different from that leading to net protein synthesis (*16*). If they are nonrandom, such exchange reactions could lead to nonuniform labeling. The exchange observed by Gale is dependent on the availability of an energy source and is in this sense distinct from simple transferase reactions such as transpeptidation and transglycosidation, reactions with free energy changes close to zero. If "exchange" implies only isotopic exchange, it seems probable on the basis of previous experience that, whatever the nature of the reactions involved, they may well reflect reversible reactions involved in net protein synthesis. While it has not yet been demonstrated to operate at the protein level, transpeptidation could represent an additional mechanism leading to nonuniform labeling, in this case by true exchange or transferase reactions.

Other Evidence for Synthesis through Intermediates

Precursors of protein more complex than free amino acids have yet to be unequivocally demonstrated. Hoagland has shown that a soluble liver enzyme system can, in the presence of adenosine triphosphate, catalyze the formation of activated amino acids presumably conjugated with adenylic acid (*14*). A similar activation mechanism has been demonstrated in several bacterial systems by DeMoss and Novelli (*17*). More work is needed to show that the amino acids activated by these systems lie on the pathway to protein, but it is quite possible that they represent the earliest intermediates in protein synthesis.

Turba has recently reported the isolation and partial characterization of a large number of peptides of high specific radioactivity from *Torula utilis* that was exposed briefly to labeled acetate (*15*). The time relationships of the distribution of radioactivity were compatible with the assumption that the peptides were precursors of protein. Rigid proof of this is still lacking.

Peters' studies on the synthesis of serum albumin by chicken liver slices strongly suggest the presence of intermediates (*7*). He observed that there was as much as a 20-minute lag between the introduction of a labeled amino acid and the appearance of radioactivity in serum albumin. When the slices were washed and transferred to a nonradioactive medium, radioactivity continued to appear in the serum albumin fraction even though there was no further increase in the radioactivity of the total trichloroacetic acid-precipitable protein fraction.

There are a number of less direct but possibly pertinent studies that should be mentioned. The synthesis of the tripeptide glutathione has been shown to be a two-step process catalyzed by two distinct

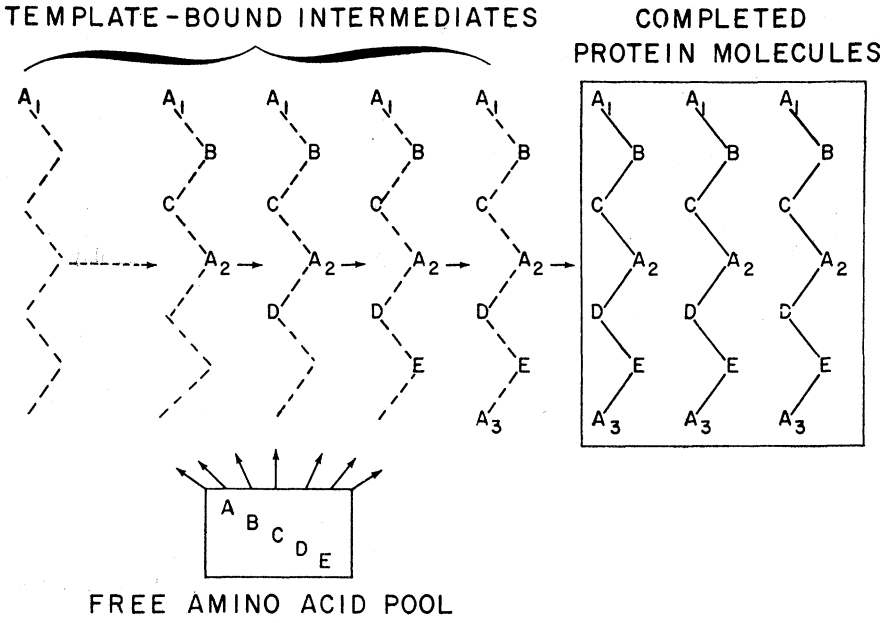

Fig. 4. A stepwise template mechanism for protein synthesis which could lead to nonuniform labeling.

enzymes (*18*). The intermediate compound, gamma-glutamylcysteine, can exist in free solution, and the reactions are reversible. The mechanism of synthesis in this case would be classified as stepwise synthesis with free intermediates. This synthesis depends on adenosine triphosphate as the energy source but involves no other known cofactors. This is true also of pantothenic acid synthesis (*11, 19*). Synthesis of benzoyl-glycine, on the other hand, does involve an intermediate complex, benzoyl-coenzyme A (*20*). Finally, it should be emphasized that the widespread occurrence of peptides, even if at low levels, in animals and plants amply testifies to the ability of tissues to synthesize peptides of various lengths (*21*).

Evidence against Synthesis through Intermediates

Work and his collaborators have carried out studies similar to those reviewed here in an attempt to demonstrate nonuniform labeling in beta-lactoglobulin and casein synthesized by the goat. A few of the peptides isolated from the labeled protein contained amino acids differing from the average in specific activity. However, the preponderance of the samples isolated showed, within the limits of error of the methods, identical amino acid specific activities (*22*). The apparent discrepancy between these results and ours could, of course, be due to fundamental differences between the basic mechanisms for protein synthesis in the different tissues studied. On the other hand, in the light of the kinetic considerations described in a previous paragraph, the difference may simply be one of rates. The mammary gland produces protein at a phenomenally high rate, and the nonuniformity ratio might be expected rapidly to approach unity. Nonuniform labeling might therefore be demonstrable only in samples taken very shortly after introduction of isotope.

Muir, Neuberger, and Perrone injected labeled valine into rats, isolated hemoglobin, and then compared the specific activity of N-terminal valine with the average specific activity of valine isolated from the body of the molecule (*23*). They observed no significant differences. The question of relative rate of synthesis must be considered here as in the case of the similar *in vivo* experiments of Work and his collaborators. In addition, it is necessary to recognize that the sample of N-terminal valine isolated represented several residues and only their average specific radioactivity was determined. Differences between individual residues cannot be ruled out.

Finally, the studies of Velick, Simpson, and Heimberg must be mentioned (*24*). These workers injected labeled amino acids into the rabbit and isolated several crystalline enzymes from muscle. Then, after acid hydrolysis, they isolated a number of labeled amino acids from each enzyme and compared specific radioactivities. It was found that the specific radioactivities of the amino acids isolated from one enzyme all bore the same relationship, within the limits of error of the methods, to the specific radioactivities of the corresponding amino acids in the other enzymes. From this it was inferred that within each enzyme all the residues of each amino acid species were of identical specific radioactivity. While the conclusion may be correct, the evidence is by no means rigid or compelling. The proteins studied were of rather high molecular weight, and therefore the number of amino acid residues represented in each sample isolated was rather large. As in Neuberger's study, then, only an average specific radioactivity was measured. Now the constancy, or relative constancy, of the specific activity ratios observed is not unexpected even if synthesis occurred through intermediate compounds. *On the average,* the rate of incorporation of amino acids into a protein with a turnover rate twice that of another protein will be twice as great. Certainly, after the pools of intermediates become saturated with isotope, and therefore equal in specific activity, the rates of isotope incorporation must be precisely in the ratio of the turnover rates throughout. However, during the early stages of synthesis, on the basis of the kinetic arguments presented here, it is not at all unlikely that the rates of incorporation might differ considerably for individual amino acid residues. Even then, the mean rates of incorporation could easily be in a definite ratio related to the turnover rates.

Another line of evidence that has been interpreted by some to rule out intermediates in protein synthesis is to be found in the studies of induced enzyme synthesis such as those of Monod (*1*) and Spiegelman (*25*). These investigators have clearly shown that, under certain conditions, protein synthesis can proceed with no significant contribution from preexisting proteins or readily demonstrable peptides. Their evidence conclusively establishes that, again under the conditions used, more than 97 percent and possibly all of the precursor material for protein synthesis consists of free amino acids. These results, however, do not shed any light on the pathway by which these free amino acid precursors find their way into the protein molecule. The negative evidence that no intermediate compounds could be found by the rough survey methods used cannot be considered in any sense crucial evidence. The further implications of the studies with respect to the stability of bacterial protein are also of interest, particularly since it has been proposed by Monod that a similar "static state" of intracellular proteins may apply in the case of mammalian tissues (*1*).

It has been argued that the failure to find intermediate compounds means that the assembly of amino acids to form protein must occur simultaneously. If this argument is to be applied to the process of protein synthesis, it must likewise be applied to the process of protein degradation. Since no significant concentrations of protein fragments seem to occur in tis-

Table 4. Inhibition of protein degradation in rat tissue slices.*

Flask	Nonprotein N/protein N (mg/mg)	Protein N released (%)	Amino acid counts/ protein counts	Protein counts released (%)
Expt. 1. Phenylalanine-labeled liver slices				
Zero time control	0.093		0.008	
Incubated control	0.137	4.4	0.058	5.0
Incubated in N_2	0.116	2.3	0.027	2.1
$2 \times 10^{-3}M$ Dinitrophenol	0.108	1.5	0.034	2.6
Expt. 2. Alanine-labeled kidney slices				
Zero time control	0.244		0.012	
Incubated control	0.292	4.8	0.084	7.2
$2 \times 10^{-2}M$ *o*-Fluorophenylalanine	0.267	2.3	0.039	2.7

* 100 μc of C^{14}-amino acid intraperitoneally 3 days prior to experiment; 400 mg of slices incubated 4 hr at 37°C in *p*H 7.4 Krebs-Ringer phosphate containing 0.012*M* carrier amino acid.

sues under physiological conditions, it becomes necessary to postulate that protein breakdown also occurs by an "instantaneous" process. While nothing definite is known about protein degradation within the living cell, it can probably be safely inferred from studies on the kinetics of proteolysis that the process is stepwise, although possibly occurring at a rapid rate and in what may appear superficially to be an "explosive" degradation (*26*).

Degradation and the Dynamic State

Since the classical isotopic studies by Schoenheimer and his group, it has been accepted by most investigators that the protein in mammalian tissues is in a true dynamic state (*27*). By this we mean that most or all of the protein species are being constantly degraded and resynthesized at a rate characteristic for each. While the synthetic mechanism is receiving constantly growing attention, the nature of the equally relevant and possibly related mechanism of degradation has received little attention. The only established mechanism for protein degradation is hydrolysis of peptide bonds by digestive enzymes or by tissue cathepsins. However, the role of catheptic enzymes in "physiological" protein degradation remains to be established. During the past year we have studied this problem of protein degradation in living cells, and the preliminary results obtained suggest that this process may be more complex than direct catheptic hydrolysis (*28*). It is hoped that exploration of this degradative process may give information of value in understanding the process of synthesis as well.

Aside from a host of studies on autolysis, studies in which protein breakdown under decidedly nonphysiological conditions has been investigated, there are few reports in the literature germane to this problem. Simpson's provocative study represents perhaps the first attempt to apply modern techniques to this problem (*29*). He found that protein degradation appeared to have an unexpected and rather paradoxical dependence on energy. These results were obtained by isotopic methods, using the release of radioactive amino acids from prelabeled rat liver slices as the measure of protein degradation. The studies involved the use of high concentrations of carrier amino acid in order to "trap" the radioactivity released from protein. Consequently the observed inhibitory effects might have been due to decreased efficiency in the penetration of the carrier amino acid into the cell. Moreover, deprivation of an energy source might inhibit rather indirectly by bringing about disruption of cell function and structure at a basic level.

We have reinvestigated this problem, measuring both net protein degradation and release of labeled amino acids from protein. As shown in Table 4, net breakdown of liver slice protein is strongly inhibited by anaerobiosis and by dinitrophenol. The release of radioactive amino acids, simultaneously determined, is similarly inhibited. It is of interest that the values obtained by the two methods are in the same general range. The somewhat greater count release is what would be anticipated in view of the high concentrations of carrier amino acid present. Alternatively, it may be due to preferential degradation of protein components with high specific radioactivity.

Because interference with energy supply might well affect protein degradation indirectly and nonspecifically, inhibitors more likely to be closely related to protein metabolism per se were investigated. It was found that the phenylalanine analogs, *o*-fluorophenylalanine and *p*-fluorophenylalanine, which are effective inhibitors of protein synthesis (*30*), are almost equally effective inhibitors of protein degradation (Table 4). Again, this is true whether protein breakdown is measured by isotopic methods or in terms of net increase in nonprotein nitrogen.

The possibility that these inhibitors might simply decrease the rate of catheptic hydrolysis was examined by measuring "autolysis" of liver homogenates at *p*H 5, the optimum for many cathepsins. The rate of protein breakdown was not significantly influenced by concentrations of dinitrophenol and *o*-fluorophenylalanine that effected a 50-percent inhibition of breakdown in intact tissue slices at *p*H 7.4. If the cathepsins play a role in the physiological breakdown, it is presumably somewhat different from simple hydrolysis, or it involves synergism with other systems.

It is noteworthy that the several inhibitors of protein degradation described here are likewise inhibitors of protein synthesis. Reversibility at one or more stages in protein synthesis would be compatible with these results and with previous results on nonuniform labeling of proteins. The apparent energy requirement and the inhibition by amino acid analogs suggest that the breakdown process may involve a complex acceptor substance rather than water. For this there are analogies in the mechanisms for degradation of other macromolecules, such as glycogen and ribose nucleic acid (*31*).

Taken together, the present results suggest that there is a continuing, organized process of intracellular protein degradation, a concept recently questioned by Monod and coworkers (*1*). While a considerable part of the "turnover" of mammalian proteins is undoubtedly a reflection of cell renewal and replacement of secreted proteins, the present studies indicate that there probably is in addition a true dynamic degradation and resynthesis of protein within the cell.

Note added in proof. K. Shimura, H. Fukai, J. Sato, and R. Saeki (*33*) have recently reported the finding of nonuniform labeling of glycine in silk fibroin isolated from silkworm larvae which were sacrificed 3 and 4 hours after the injection of C^{14}-labeled glycine. In two experiments, the specific radioactivity of the N-terminal glycine residue was, respectively, 9 and 10 times the average specific radioactivity of the glycine residues in the remainder of the molecule.

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J. M. D. Olmsted, Physiologist and Historian

James Montrose Duncan Olmsted, emeritus professor of physiology, University of California, died 26 May 1956 in his home in Berkeley at the age of 70 years. Sincerely mourned by a host of colleagues and pupils, Olmsted combined marked gentleness of demeanor and character with high scholarly standards. Under his aegis, his department, which had lacked luster after the departure of Jacques Loeb, regained distinction in both teaching and research. Olmsted had been a pupil of G. H. Parker, a Rhodes scholar at Oxford, and a former colleague of McLeod at Toronto. He mediated satisfactory relationships with the University of California School of Medicine as well as with the Berkeley undergraduate and graduate curricula. The success of Olmsted's department can be attributed not only to his urbanity but to his vision and perspective, and his appointments to his staff indicated a capacity to correctly estimate talents in quite diverse personalities. His field of personal preference in the medical teaching program concerned the physiology of the organs of special sense, a subject to which he contributed by researches on the eye and a subject reviewed by him in the *Annual Reviews of Physiology* and handled by him in the Bard-McLeod textbook.

His scientific contributions, as senior author and coauthor, include considerably more than 100 papers. Beginning with a chemical contribution from Oxford, he quickly entered the realm of general physiology in experiments on lower organisms at Bermuda, Wood's Hole, and elsewhere, and, in this field, he published a beautiful paper ["The nerve as a formative influence in the development of taste buds," *J. Comp. Neurology* **31**, 465 (1920)] on the effects of cutting the branches of the seventh cranial nerve to the barbels of the catfish. Here he established the degeneration of the taste buds subsequent to nerve section, their regeneration only after regeneration of the nerves, and nerve penetration into the germinative layer of the epidermis, leading to the formation of dermal papillae and then taste buds.

On joining the staff at Toronto, he enthusiastically entered the field of mammalian and human physiology, at the time that the Toronto discovery of insulin, its preparation, and the study of its effects created a classical epoch in insulin research. Olmsted's examination of the effect of insulin on the nervous system was an important part of the pioneer Toronto program. Indeed, two of the most important subsequent discoveries in this field—the hypersensitivity to insulin of hypophysectomized animals and the "diabetogenic" effect of the anterior pituitary—were clearly recognized in the paper by Olmsted and Logan of October 1923.

Olmsted is to be credited with outstanding achievement in his studies on the history of physiology and especially in four enjoyable and authoritative biographies of Magendie, Claude Bernard, and Brown-Sequard. In one of these he was joined by his wife, Evangeline Harris Olmsted. The personalities described in these works [*Claude Bernard, Physiologist* (Harper, New York and London, 1938); *Francois Magendie, Pioneer in Experimental Physiology and Scientific Medicine in XIX Century France* (Schuman, New York, 1944); *Charles-Edouard Brown-Sequard, a Nineteenth Century Neurologist and Endocrinologist* (Johns Hopkins Press, Baltimore, 1946); with E. Harris Olmsted, *Claude Bernard and the Experimental Method in Medicine* (Schuman, New York, 1952)] were responsible for the rise of experimental physiology in France, and Olmsted's portrayal combined literary charm with scientific accuracy. It is a surprising fact that no French scholar had discerned or taken advantage of the opportunity to chronicle the lives of these scientists and significant that the Parisian Academy of Science recognized Olmsted's successful achievement by awarding him its Prix Binoux and the French National Academy of Medicine by awarding him its Prix de Martignoni.

How he came to write these biographies is told in a very charming way in a short article that he wrote for his undergraduate college, appearing in the Middlebury College News Letter. It may, perhaps, be mentioned that Olmsted contributed superbly succinct summaries of Claude Bernard's discovery of glycogen and its role in carbohydrate metabolism to *Diabetes* in 1953 and to the *Journal of the American Dietetic Association* in 1954.

Olmsted's cultural interests, shared with his wife, included participation with a small group of other faculty members in amateur theatrical performances. Professor Olmsted painted, and with talent, and was pleased to be a member of the San Francisco Association of Artists.

His life is surely a reminder that, although controversy and indeed hostility may arise in human affairs, they would not appear to be ineluctable and that modesty and kindliness ideally accompany all endeavor.

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