groove—the assumption that everything we learn must be taught in courses.

While I would refuse requests for courses in education, I suggest that graduate schools consider the need for practice teaching and a seminar on college problems. To the educationists I suggest that they write some good books and include material they think college teachers should know and use but omit nonessential details. We think we are capable of reading and understanding, and we also think we are capable of judging values.

What I have said will not satisfy the critics. Their requests, however, are not supported by convincing evidence that better teaching would result if their advice were followed, or even that they themselves are better teachers.

After forty years of experience, I presume I should be wise enough to tell how science should be taught in college and how a science teacher should be educated, but the longer I live the less sure I am of the answers to such questions and the surer I am that no one else has final answers. This does not imply, however, that one answer is as good as another.

If I were to venture to make a few suggestions, not because they are new but by way of emphasis, they would run something like this. To young people thinking of entering the profession, I would say: Be sure you want to make teaching your life work and that you have the personal qualifications for success. If you are looking for an easy job, or can't be satisfied with a modest income, look elsewhere. If you decide to teach, educate yourself as best you can but there is no one pattern that must be followed. I suggest you emphasize both breadth and depth. Don't neglect the humanities, social sciences, and fine arts, for they too will add breadth worthy of acquisition, and add to your enjoyment of life. See and learn as much of the world and of peoples as possible. Learn to do research, for research activity will do much to keep you a live, virile teacher. Continue to be a student of the subject you teach and of teaching problems. You will never reach perfection, for it is neither in your genes nor in the environment. Know how good teachers teach, listen to suggestions and criticisms, then make your own decisions in the light of what you are attempting to do. Be yourself. Don't let your enthusiasm for your subject cause you to attempt to develop all students into specialists. Most students have other interests, and want science merely to add to their breadth of education or to satisfy their curiosity. Such students are worthy of your best efforts as a teacher. Never lose sight of the fact that a good teacher guides, and that a student learns by his own efforts. Encourage the reading of good books. Teach some things by example. By all means, remember your students will be citizens of the United States of America and that they will play significant parts in the development of our country. Differentiate sharply between propaganda, indoctrination, and education.

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# The Role of the Time Factor in Protein Synthesis[1]

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IT IS A FUNDAMENTAL CONCEPT that proteins are composed of amino acids and that the number and spatial arrangement of these building stones characterize the individual protein. This leads logically to the hypothesis that protein synthesis can proceed only if all the building stones involved are available.

This truism more or less exhausts our knowledge of the mechanism of protein synthesis. Cannon (*4*) recently emphasized the fact that in spite of our growing knowledge of the catabolic phase of protein metabolism, there are wide gaps in our understanding of its anabolic phase, and Northrop (*20*) called attention to our lack of knowledge concerning not only the energetics of protein synthesis but also the nature of the basic building stones of proteins: viz., whether proteins are synthesized directly from amino acids or from larger intermediary building stones, so-called plasteins.

The plastein theory of protein synthesis in its original naive form (Sawjalow, 1899), or in its recent revision, is based on the reversibility of protein hydrolysis *in vitro* and therefore can hardly account for the specificity of the synthesized proteins.

Even the epochal experiments of Schoenheimer and his colleagues shed little light on the problems of protein anabolism. The ease with which labeled amino acids have been incorporated into protein, however, forecasts a drastic revision of our current ideas on the rigid structure of living protein.

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[1] Based on a lecture given at the Gordon Research Conferences, AAAS, August 14, 1949.

The problem of protein synthesis was approached by the present author several years ago by feeding "incomplete" amino acid mixtures. W. C. Rose had shown earlier that protein synthesis occurs only if all the "essential" amino acids are present in the diet. This stimulated the investigation here of the metabolic fate of incomplete amino acid mixtures which, because of the lack of one or more essential amino acids, cannot be utilized for protein synthesis.

Three possibilities were considered: (1) that the amino acids can be stored in the body as such, representing thereby a stock pile from which protein synthesis can proceed as soon as the lacking building stones are supplied; (2) that they are stored in the form of osmotically less active peptides; and (3) that they are irreversibly further metabolized, and are therefore lost for protein synthesis.

It was established first, by amino acid analysis of the tissues, that those amino acids which are not immediately utilized for protein synthesis are not stored as such in the body (*8*). Consequently an attempt was made to determine whether synthetic processes, such as plastein formation, could produce from the incomplete amino acid mixtures peptides of smaller or larger sizes which in turn could be synthesized into proteins as soon as the missing amino acids were added to the diet. However, it was found that feeding the missing amino acid several hours after consumption of an incomplete amino acid mixture did not promote protein synthesis as measured by the effect on growth (*8*). These experiments show that the incomplete amino acid mixtures are not utilized for the formation of intermediary building stones from which further synthesis could proceed at a later time, but are metabolized, probably irreversibly. Thus, there was found no support for the theory that protein synthesis is a piece-by-piece affair, but rather the experimental results favor a mechanism like the template method of formation in the sense of Langmuir-Schaefer or Pauling.

The fact already postulated by Rose, Mitchell, and Elman, that protein synthesis occurs only when all the essential amino acids are simultaneously available, has been thus experimentally established.

Cannon arrived at similar conclusions independently, using the repletion of protein-depleted adult rats as an indicator of protein synthesis. A further welcome confirmation was recently reported by Henderson and Harris (*14*), who found that "a delay of 3 hours or longer in the feeding of lysine as a supplement to a lysine low diet interfered with metabolism."

All of these findings are relevant to the *essential* amino acids. However, the nature of protein synthesis makes it mandatory to assume that the nonessential as well as the so-called essential amino acids must be available simultaneously and in ample quantities to permit protein synthesis. Although the animal does not require the nonessential amino acids in its diet, it is clear that they must be produced in the intermediary metabolism and provided as free amino acids in sufficient quantities to secure the optimal utilization of the essential amino acids (*2*). It is evident that the "law of minimum" is valid also for the nonessential amino acids, so that protein synthesis is limited not only by the supply of the dietary essential amino acids, but also by the speed with which the so-called nonessential amino acids are made available.

The experiments of Cox and Mueller (*6*) and of Rose (*22*) have actually shown that feeding all of the amino acids simultaneously is more effective than feeding of the essentials alone, and these tests therefore provide experimental proof for the rule just stated.

Speedy availability of the nonessential amino acids depends on the proper functioning of the conversion mechanisms and it seems probable that some of the pathological disturbances of protein formation—for example, in liver disease—may be the consequence of a disturbance in the formation of these nonessentials. It is evident that the formation of nonessentials depends also upon the supply of easily available precursors for these amino acids, such as sugar or fat. A somewhat older and as yet unexplained finding may be interpreted in this way. Larsen and Chaikoff (*16*), working with adult dogs, and Cuthbertson *et al.* (*7*), studying adult humans and rats, showed that the N-sparing effect of a single carbohydrate feeding was related to the time elapsed between the consumption of the daily meal and the ingestion of the extra carbohydrate. We found later (*9*) that the growth of infantile rats is likewise affected by the temporal separation of feeding of protein from the feeding of the remainder of the diet. It seems logical to assume that carbohydrates, which provide the necessary precursors for the formation of the nonessential amino acids, can be utilized for protein formation optimally only if fed simultaneously with the essential amino acids. To test this point, experiments are in progress in which we investigate whether a single dose of labeled sugar given simultaneously with the essential amino acids is deposited in the body proteins to a larger degree than when sugar is given four or five hours after the protein meal.

Considering the role of the time factor in protein synthesis, we have to conclude that optimal utilization requires the simultaneous availability of adequate amounts of all of the essential and nonessential amino acids participating in the make-up of the given protein.

Before drawing general conclusions from the results obtained so far, we should discuss the question of whether there are some basic differences between the role of the time factor in the use of amino acids for growth and its role in their use for maintenance metabolism. The existence of such foundamental differences has been assumed by several authors, but others—for instance, Wissler and his collaborators (*27*)—suggest that the requirement in respect to amino acids for maintenance does not differ essentially from the requirement for growth. Experiments with delayed supplementation were therefore undertaken to decide between the two possibilities. Cannon, in his investigations on repletion of protein-depleted adult rats, demonstrated in short term experiments that repletion occurs only if all the essential amino acids are supplied simultaneously. We recently repeated Cannon's experiments with a somewhat different technique, and found that, in long term experiments as well, repletion in adult rats occurs only when tryptophan is fed simultaneously with the other essential amino acids. Cannon's results, as well as our own experiments with repletion, shed little light on maintenance itself. The repletion process, i.e., the repair of lost tissue, is essentially equivalent to protein formation in growth. Repletion in adult rats, therefore, like growth promotion in infantile rats, requires the simultaneous presence of the essential amino acids.

It may be assumed a priori, from the work of Schoenheimer and his group, that although formation of new protein in growth and repletion is an all-or-none matter, the existing, aging protein molecules may be mended by a piece-by-piece replacement of the individual amino acids. This would imply that the so-called "give and take" between dietary and tissue amino acids is not restricted to the simultaneous presence of all the building stones. To test this concept, adult normal rats were fed for 12 hours on a tryptophan-free diet and the missing amino acid was supplied 12 hours later. It was found that the adult rats receiving tryptophan as a delayed supplement lost weight as fast as or faster than the control rats receiving none of the amino acids in their diets. This observation shows that maintenance metabolism in adult rats requires the simultaneous presence of all the essential amino acids. Therefore, the concept of "dynamic equilibrium" has to be interpreted as a replacement of whole protein molecules destroyed in continuous metabolism. This process evidently requires also the simultaneous presence of all the amino acids.

The experiments of Sanadi and Greenberg (*24*) with labeled amino acids support this point of view. These authors found that animals reared on a diet deficient in one essential amino acid showed a reduced incorporation of $C^{14}$ from labeled tryptophan or glycine into their tissue proteins. It would be interesting to study tracer incorporation by applying the technique of delayed supplementation. Such experiments may show to what extent the exchange of the individual amino acids between tissue and dietary proteins is influenced by the presence or absence of certain essential amino acids. This problem requires further investigation because it has been shown recently (*3*) that the uptake of labeled lysine by guinea pig liver homogenates may occur *in vitro* in the absence of other amino acids. However, it is possible that the products of continuing protein hydrolysis *in vitro* are not removed and can, therefore, be reutilized for protein synthesis. The conditions *in vivo* are very different and this may explain why in adult rats, for all practical purposes, the time factor in the provision of amino acids is as important for maintenance metabolism as for promotion of growth.

The rule that all the amino acids have to be present simultaneously for protein synthesis does not imply that amino acids fed singly cannot be utilized by the organism at all. Besides participating in protein synthesis, amino acids are utilized by the organism for formation of vital factors such as vitamins, hormones, and coenzymes. Whether the utilization of the individual amino acids for these purposes may proceed also in the absence of the other amino acids has been the subject of investigation. In such experiments with tryptophan it was found that the method of delayed supplementation aids in distinguishing between the "protein function" of the amino acids and their "extraprotein functions," such as the formation of vitamins, coenzymes, hormones, biogen amines, or still other unknown compounds.

First the development of cataract in tryptophan-deficient rats was studied (*26*), and it was found that tryptophan had to be supplied along with the remainder of the protein moiety, rather than at a later time, in order to prevent the lesion. In the case of delayed supplementation, cataract formation occurred even though the animals consumed relatively large amounts of tryptophan. This clearly established the fact that cataract formation is a consequence of a disturbance in protein synthesis, and eliminated the possibility that tryptophan prevents the cataract formation by some specific action.

The same technique was applied to determine whether niacin synthesis from tryptophan occurs independently from protein synthesis.

In experiments on rats (*12*) in which a niacin deficiency had been produced by a diet imbalanced in amino acid—containing, for example, 9 percent casein and 6 percent gelatin—the tryptophan supplement proved to be equally effective in promoting growth whether fed simultaneously with or several hours after the imbalanced diet. Here we find that the corrective effect, contrary to that in growth promotion and cataract prevention, is manifested even when the amino acid is fed as a delayed supplement. Therefore, the transformation of tryptophan to niacin may be considered to be independent of protein synthesis.

It should be pointed out that the animals in the present experiments grew and excreted increased amounts of niacin even though tryptophan was given as a delayed supplement. This appears to contradict the experiments reported earlier in which animals on a diet *completely* deficient in tryptophan did not grow when the amino acid was given as a delayed supplement. In the present experiments, however, the deficient diet *per se* contains enough tryptophan in the form of casein to promote growth, and only the harmful effects of imbalancing the amino acid ratio by feeding gelatin had to be corrected by supplementation with tryptophan.

We must conclude from these experiments that tryptophan can be utilized along at least two pathways: first for protein synthesis and then secondly, probably independently, for niacin synthesis. In order to test the possibility of diverting the tryptophan utilization predominantly in one of these directions, niacin formation was investigated in animals where protein synthesis was depressed by the omission of cod liver oil from the diet. Such experiments have shown, however, that lack of cod liver oil interferes not only with growth but also with urinary nicotinic acid excretion; after the addition of cod liver oil, growth and nicotinic acid excretion increased nearly equally, independent of whether the tryptophan was mixed with the diet or fed as a delayed supplement. This shows that when growth ceases on a diet free of cod liver oil, the formation of nicotinic acid from tryptophan is not increased, but is in fact decreased. So it was not possible to demonstrate in this way any preference or competition in utilization of tryptophan either for protein or for niacin formation.

We should like to call attention to the fact that the curative effect of tryptophan in niacin deficiency can be prevented by the absence of cod liver oil as well as by the lack of the B vitamins, as reported before. This finding reemphasizes the complex interdependence of the different processes of intermediary metabolism.

Further experiments are in progress in which the deficiency symptoms and pathological changes in rats kept on a diet entirely free of methionine are compared with the symptoms developed by rats that receive methionine as a delayed supplement to the remainder of the diet. The results may indicate which of the symptoms are connected with altered protein synthesis and which are related to extraprotein functions of this amino acid. These experiments may also test the assumption of Salmon (*23*) that "one would expect more of the methionine methyl to be available for choline synthesis when methionine is fed as the sole supplement than when fed along with the other essential amino acids in the protein molecule."[2]

In this connection, attention should be called to the experiments of Paloheimo (*21*) who, incidentally, seems to be the first to have investigated the effects of delayed amino acid supplementation. Based on the findings of Osborne and Mendel that added cystine has a casein-sparing effect, he fed rats a diet low in casein at 48-hour intervals, and between meals supplied small amounts of cystine. He found a definite increase in the N-retention and assumed therefore that either the amino acids of casein or the cystine itself may be stored in the organism for a period of 12 hours or longer. However, more recent experiments fail to support any such amino acid storage. Paloheimo suggested, as a further possibility, that the cystine might also form some lower molecular compounds of biological importance, such as glutathione, which improve the animals' general condition. The author fails to explain why, in spite of improved nitrogen retention, the rats did not grow.

All of the experiments discussed thus far were performed with amino acid mixtures and therefore their results are primarily of theoretical interest: their practical value is restricted to those instances where mixtures of amino acids are fed or injected. To extend this type of study to more practical situations, the significance of the time factor in the feeding of supplementary proteins had to be investigated. The factors of slow digestion of the protein and slow absorption of the amino acids might equalize the effects of delayed supplementation. It was found (*10*) that feeding proteins with individually low biological value yielded mutual supplementation only when they were given simultaneously. If the same pairs of proteins were fed temporally separated they failed to supplement each other. That is, delayed provision of missing amino acids is ineffective not only when the amino acids are fed in the free state, but also when they are supplied in the form of proteins. These experiments confirm the results of Henry and Kon (*15*), who found that such complex foods as

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[2] To facilitate the study of such problems, a mechanical device for controlling the time of feeding of rats has been constructed. The apparatus was built by the Precision Instrument Shop of the University of Southern California Medical School, with funds generously provided by the National Vitamin Foundation.

milk, potato, bread, and cheese exhibit supplementary relationships only if fed together and not if fed separately on alternate days.

The practical consequence of all these findings may be very briefly summarized. First, in the feeding of proteins of low biological value, as in relief feeding or in emergency diets, the proteins should be fed together to secure the maximum benefit of any possible supplementary action. For example, according to Adolph (*1*), the Chinese consume different cereals mixed in definite proportions. In this fashion they have developed empirically a protein mixture of high biological value. An empirical wisdom similarly developed may be responsible for the timing, sequence, amount, and composition of the meals consumed throughout the world. It is an intriguing task for further research to verify or disprove the value of such practices developed throughout the ages. A very important step has been made recently in this direction by Leverton and Gram (*17*).

Second, in compounding amino acid mixtures for therapeutic uses, it is not sufficient to simulate the composition of good quality proteins; instead, the relative amount of the individual amino acids should be chosen in such a way that, in spite of differences in absorption, excretion, and destruction, optimal utilization for protein synthesis should be secured (*13*). This requires much further work on the fate of the individual amino acids and amino acid mixtures. In this connection, an important problem of general interest is the question of the significance of the time differences in the gastrointestinal enzymatic liberation of the amino acids as a factor in the effective utilization of certain dietary proteins. Melnick and his collaborators (*19*) were the first to suggest such a mechanism as an explanation for the poor utilization of raw soybean protein; they showed that methionine is released more rapidly from the heated than from the raw meal. They suggest that the methionine is released from the raw meal so late in the intestinal transit that this amino acid, as well as the other incompletely supplemented amino acids, is not optimally utilized for protein synthesis. In the light of recent studies (*18*) it seems possible that some of Melnick's results may require a somewhat revised interpretation. The heuristic value of Melnick's conclusions, however, cannot be overemphasized, and in all further investigations on the digestibility of proteins it will be compulsory to consider also the time relations of enzymatic liberation and intestinal absorption of the constituent amino acids. We do not believe that conclusions based solely on digestion *in vitro* are of much value because the released amino acids are absorbed at different speeds from the intestinal tract.

Results of experiments with zein (*11*), which we tried to explain according to Melnick's theory, called our attention to still another factor which may influence the adequacy of a protein, viz., peptides formed by incomplete enzymatic digestion may in certain instances interfere with growth.

Attempts to determine the maximum time interval at which a missing amino acid may still be effectively supplied yield results which have to be further investigated. It was found that effective supplementation of the missing amino acid is possible only if the time interval is less than four or five hours, in spite of the fact that there are still considerable quantities of the deficient protein present in the intestinal tract of the animal at this time. This phenomenon may be explained either by the assumption that the original, imbalanced protein produces a continuous damage (Allison, Elvehjem), thus blocking the effective utilization of normal proteins when absorbed at a later time; or, alternatively, we may assume with Christensen (*5*) that the exessive concentration of certain amino acids may block, by a mechanism of competitive inhibition, the utilization of the missing building stones fed later. This problem is now being studied.

Another fundamental problem which arises from the studies of the time factor in amino acid supplementation is the ultimate fate of those amino acids that could not be utilized for protein synthesis. We have already pointed out that they are not stored in the body as amino acids or as peptides. Experiments of Schweigert and of Sauberlich and Baumann (*25*) showed that feeding incomplete proteins increases the urinary amino acid excretion, and that "there is a rough inverse correlation between the growth stimulating power and the percentages of ingested amino acids excreted in the urine." Since only 15 percent to 25 percent of the ingested amino acids of a deficient mixture can be recovered from the urine, it is still necessary to account for the remainder. A possible transformation and deposition in the form of fat or carbohydrate has to be ruled out because it would obviously also increase the body weight. The metabolized end products must be excreted in the form of organic compounds in addition to urea, or destroyed by catabolic processes. The possibility of an altered respiratory metabolism as a consequence of feeding incomplete proteins has not yet been investigated, but we suggest that the elimination of these unutilized amino acids may account, at least in part, for the so-called "specific dynamic action." An experimental investigation of this relationship may clear up many controversies in this field.

The investigations discussed in this review indicate that the method of delayed supplementation of amino acids can be successfully employed for the study of the mechanism of protein synthesis as well as for the study of the specific "nonprotein" functions of the individual amino acids, and for the identification and separation of the two functions. Experiments performed with this technique show that incomplete amino acid mixtures are not stored in the organism or used for the formation of intermediary building stones (plasteins), from which protein synthesis could proceed at a later time.

The results of several authors suggest that the law of minimum is valid not only for the essential, but also for the nonessential amino acids, so that protein formation is dependent not only upon the simultaneous presence of the essential amino acids, but also upon the speed with which the nonessential amino acids are made available. The study of the mechanisms by which formation of the nonessential amino acids is controlled seems therefore to be an important, though neglected, field for investigation.

These experiments with delayed supplementation fail to support the theories which assume that protein metabolism may be basically different in processes of growth and of maintenance.

As we see it, the whole problem of the time factor in supplementation seems to center around the puzzling question of how the organism determines whether a given amino acid mixture is to be retained for protein synthesis, or instead, because of an amino acid inadequacy, to be disposed of. Does this selection occur in the liver, or is the amino acid fixation and retention determined by some protein-forming templates? We hope that this problem may be approached experimentally in the near future, but in the meantime the "wisdom of the body" must be given credit for this selective retention.

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