always found to be antigenically deficient when compared with Bence Jones proteins. No antiserums showed that these components form spurs over Bence Jones proteins. Additional immunoelectrophoresis experiments were performed with antiserums to Bence Jones protein that were absorbed with heterologous Bence Jones proteins. In one such experiment (Fig. 1*e*) the absorbed antiserum (upper trough) no longer shows the spur of Bence Jones protein over the second component; there is a line of identity between the two proteins. Antiserum to Bence Jones protein Ro proved of particular value after absorption with Bence Jones protein Cu. These proteins, intensively studied by Hilschmann and Craig (*7*), are homologous in the COOH-terminal half and show extensive differences in the NH_2-terminal half. The deficient component in the urine of Ro and of a number of other patients as well, continued to react with antibody to Ro absorbed with Cu; but the spur of the Bence Jones protein was no longer evident. It appeared that, in these instances, the deficient components were related to the variable portion of Bence Jones protein molecule in the NH_2-terminal half (*7*, *8*). However, two urines gave quite different results, and various similar absorption experiments always removed the line for the deficient component with only a diminution of the Bence Jones line.

The small components related to Bence Jones proteins were clearly detected in the urine of 9 out of 24 patients excreting κ-type, and 7 out of 22 patients excreting λ-type Bence Jones proteins. The detection of these smaller proteins in approximately one-third of the patients probably represents the minimum incidence in that, in some patients, not enough material was available and antiserums to each patient's Bence Jones protein were not available. The amounts of these proteins found in urine were usually quite low, in the range of a few percent of the quantity of Bence Jones protein; the largest amount was noted in patient Gr where it represented 10 to 15 percent of the Bence Jones protein.

These small components were detected in freshly voided urine and, in the case of Gr, were found at similar concentration in 20 different fresh urine samples taken over extended periods of time. In order to find out whether these proteins represented products of degradation in vitro of Bence Jones protein, isolated I^{131}-labeled Bence Jones protein (from patient Gr) free of small components was added to the urine which was then processed over a period of 72 hours. Starch-gel electrophoresis of the purified material revealed no radioactivity in antigenically deficient components. However, evidence was obtained for some increase in similar small components in other, older urine preparations, and such preparations were excluded from the study. These appeared to be enzymatic degradation products because incubation of concentrated urine preparations at 37°C, particularly at low *p*H, produced various new antigenic components in certain specimens.

The significance of the low-molecular-weight components remains obscure. They were readily separated from the dimer and monomer forms of Bence Jones protein and could be repeatedly demonstrated in fresh urine specimens from the same individual. However, it became evident that a number of precautions in the handling of the urine specimens was necessary to avoid loss of these components in the process of isolation of the Bence Jones proteins as well as to avoid production of low-molecular-weight material through degradation. It remains to be determined whether the deficient components might be synthesized independently or represent catabolic breakdown products of Bence Jones proteins.

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Flavin Mononucleotide Control of Glycolic Acid Oxidase and Photorespiration in Corn Leaves

Abstract. *Enzyme preparations from young corn shoots lacked the coenzyme, flavin mononucleotide, that is required for glycolic acid oxidation. When the coenzyme was added to the shoots, the rate of carbon dioxide production during photosynthesis increased. Shoots of wheat or oats did not lack the coenzyme.*

In air and in light of high intensity, detached leaves of tobacco, wheat, and oats can lower the concentration of carbon dioxide in a closed container to about 50 parts per million (ppm) by photosynthesis (*1*, *2*). This equilibrium is the carbon dioxide compensation concentration. It varies as a linear function of the concentration of oxygen around the leaf (*3*). On the other hand, young corn shoots have a carbon dioxide compensation concentration of less than 5 ppm in air (*2*, *4*). Apparently corn shoots produce little if any carbon dioxide during photosynthesis, so that the equilibrium value is very low, while the amount produced by leaves from other plants changes with the concentration of oxygen. The sensitivity of carbon dioxide compensation to high concentrations of oxygen and the known effect that a change in oxygen concentration has on glycine and glycolic acid metabolism in photosynthesis (*5*) suggest that glycolic acid oxidase (GAO) is important in carbon dioxide production during photosynthesis. The enzyme is present in high concentration in leaves (*6*). It has a low affinity for oxygen, which might be expected from the cofactor requirement of flavin mononucleotide (FMN) (*7*).

Taken together, these conclusions lead to the prediction that the glycolic acid oxidation pathway is not active in young corn shoots. There are conflicting reports on the GAO activity in corn shoots (*6*, *8*, *9*), and I report now on rates of oxygen consumption

Table 1. Glycolic acid oxidase from monocot shoots of wheat, oats, and corn measured as microliters of oxygen utilized per hour per gram fresh weight of tissue. FMN, flavin mononucleotide.

Addition	Glycolic acid oxidase (μl O_2 hr^{-1} g^{-1})		
	Wheat	Oats	Corn
None	17	19	15
Glycolate	80	93	13
Glycolate + FMN	80	93	37

Table 2. Glycolic acid oxidase from spinach and corn leaves measured as microliters of oxygen utilized per hour per gram fresh weight of tissue. FMN, flavin mononucleotide.

Addition	Glycolic acid oxidase (μl O_2 hr^{-1} g^{-1})			
	Spinach	Corn	Av.	Mixed (1 : 1)
None	7.5	14	10.5	42
Glycolate	220	16	118	150
Glycolate + FMN	220	48	134	150

by enzyme preparations in relation to the gas exchange by intact shoots.

Corn, oats, and wheat were grown in vermiculite in a greenhouse, and spinach was obtained at a local market. Crude preparations of glycolic acid oxidase were made by the method of Tolbert and Cohan (*8*). The assay mixture contained K_2HPO_4, 0.04*M*, and plant sap, 1.0 ml (equivalent to 3 g fresh weight), with a total volume of 5.0 ml. Sodium glycolate (0.006*M*) and FMN (0.001*M*) were added as indicated in the tables. Rates of oxygen absorption by the enzyme preparations, in a 4-ml vessel at the oxygen tension of air and at 33°C, were measured by a Clark polarographic oxygen electrode, with the circuit described by Kidder (*10*), and a Bausch and Lomb VOM 5 recorder. The sample was stirred magnetically with a Teflon-coated bar. To lower the rate of endogenous oxidation, a 10-minute aeration period preceded the assay. Rates of photosynthesis and carbon dioxide compensation by the shoots were measured at illumination of 11,000 lux in a closed system with an infrared carbon dioxide analyzer. Nitrogen gas was flushed through the system to lower the oxygen concentration to 1 to 2 percent.

Table 3. Effect of flavin mononucleotide (FMN) and oxygen on carbon dioxide compensation and on the rate of photosynthesis by corn and wheat leaves.

Inhibition of photosynthesis in air by FMN (%)	Treatment and CO_2 compensation (ppm)			
	Air and water	Air and FMN	Low O_2 and water	Low O_2 and FMN
		Corn		
65	<3	65	<3	3
55	<3	44	<3	<3
		Wheat		
60	37	90	4	7
45	38	68	3	3

The amount of glycolic acid oxidase in 14-day-old corn shoots, which did not produce carbon dioxide during photosynthesis, was determined. Wheat and oat shoots of the same age were used as control plants (Table 1). The rate of oxygen utilization by preparations from oat and wheat leaves was increased by the addition of glycolate, but FMN had to be added to the preparations from corn shoots before the glycolate increased oxygen utilization.

To determine whether FMN would be destroyed or precipitated during preparation of the enzyme from corn shoots, 2 μg of FMN, an amount sufficient to give about 50 percent of the maximum stimulation, was incubated with the crude enzyme preparation from 14-day-old corn shoots for either 1 or 30 minutes before the glycolic acid oxidase assay. The assay indicated that three-quarters of the activity remained after the 30-minute incubation. As a second test, 1 : 1 mixtures (by weight) of corn and spinach leaves were ground and assayed for glycolic acid oxidase (Table 2). Enzyme activity of the spinach leaves was not lost by grinding the two types of leaves together. The synergistic effects of mixing were removed by aerating the mixed samples for longer than the 10-minute standard period before the assay.

The cut ends of corn and wheat shoots were then placed in 0.001*M* solutions of either FMN or riboflavin. Control shoots were kept in water. With all six samples of wheat, 4-hour treatment in FMN resulted in no effect greater than 60-percent inhibition of photosynthesis. With all six samples of corn shoots, net carbon dioxide production in light occurred within 2 hours of feeding the FMN. Table 3 shows some effects of FMN on carbon dioxide compensation when inhibition of the apparent rate of photosynthesis was similar in wheat and corn. Inhibition was measured at 50 ppm of carbon dioxide above the carbon dioxide compensation concentration, with the concentration of oxygen at 21 percent. If carbon dioxide production were unaffected by FMN, a doubling of the compensation concentration might be expected with a 50-percent inhibition of the rate of absorption of carbon dioxide. This was approximately true for wheat samples. With corn samples, however, the percentage increase in the compensation concentration was many times greater than the effect on the rate of photosynthesis, which indicates a strong effect on the rate of production of carbon dioxide.

Table 3 also shows that, where carbon dioxide compensation concentrations could be measured at 21 percent oxygen, they were obviously lowered when oxygen concentration was lowered to 2 percent. Lowering oxygen concentration also reversed inhibition by FMN of the net rate of photosynthesis above compensation. Lowering the oxygen concentration inhibited carbon dioxide production in the dark by less than 20 percent; therefore, different carbon dioxide–producing processes were operating in light (*3*).

Riboflavin had no effect on wheat shoots, and, therefore, did not act as a competitive inhibitor of FMN. With corn shoots, riboflavin inhibited photosynthesis by 50 to 90 percent but increased the compensation concentration less than 10 ppm. Its effects stand in contrast to the effects of FMN on corn shoots. The effects of riboflavin could be reversed by lowering the oxygen concentration.

Production of carbon dioxide during photosynthesis by corn shoots was controlled by the availability of FMN. These shoots, which had a very low or nonexistent carbon dioxide compensation concentration, yielded enzyme preparations of glycolic acid oxidase that were inactive unless FMN was added. Shoots of both oats and wheat, which did produce carbon dioxide during photosynthesis, yielded active enzyme preparations without adding FMN. The oxidase system was activated in vivo by adding FMN to corn shoots, although there was an additional inhibition of photosynthesis by FMN. The effects of FMN were removed by lowering the concentration of oxygen.

These results support the hypothesis that glycolic acid oxidase is responsible for the production of carbon dioxide

during photosynthesis by green leaves. The amounts of all FMN-requiring enzymes that cause oxidation and decarboxylation are not known, but other studies (*11*) strongly indicate the importance of glycolic acid oxidase in the respiratory metabolism of leaves.

The importance of this carbon dioxide production to the carbon balance of the plant can be seen if we take carbon dioxide compensation of leaves as about 50 ppm (in many leaves it is higher) and the amount of carbon dioxide in the atmosphere as 300 ppm. Under these conditions carbon dioxide production is responsible for a 17-percent reduction in the net rate of photosynthesis when availability of carbon dioxide is limiting the rate. Corn shoots were the exception to this; carbon dioxide compensation was zero.

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Chain Initiation and Control of Protein Synthesis

Abstract. *Analysis of the enzymatic mechanism of chain extension during protein synthesis and studies with* N-*formylmethionyl-sRNA suggest that chain initiation requires formylation of the amino group of the amino acid destined to start chain growth. The existence of a set of starting triplets coding for a special set of* N-*formylaminoacyl-sRNA's is postulated. These triplets might be ambiguous in the sense that they specify different amino acids, depending on whether they are at the beginning of or within a message. A number of starting triplets and their* NH_2-*terminal amino acids are predicted from previously suggested ambiguities. The biochemical, regulatory, and genetic implications of a formylation step controlling chain initiation are discussed.*

Analyses of the effect of puromycin on the size distribution of polysomes in vivo led me and my associates to postulate that the initiation of a new polypeptide chain upon attachment of a ribosome to the beginning of messenger RNA requires a special mechanism that is separable from the read-out process or chain extension (*1, 2*). This idea has received further support from an examination of the interaction of transfer RNA with ribosomes during chain extension. Available evidence (*2*) is compatible with the scheme of chain extension (Fig. 1). An important aspect of this scheme is the postulate that during the steady-state process of chain extension the ribosomes oscillate between two states, A and B, characterized by the presence of the growing polypeptide chain linked to sRNA (*3*) in either of two sites, α or β (*4*). Thus, with the formation of each peptide bond the nascent chain is transferred to the incoming aminoacyl-sRNA, while the preceding sRNA, now discharged, remains firmly bound to the β-site (Fig. 1, *a* and *d*). In the subsequent step, the GTP-dependent enzyme translocase advances the messenger by one triplet and thereby returns the peptidyl-sRNA to the β-site on the 50S subunit, displacing the discharged sRNA into the loosely binding exit site γ (Fig. 1*b*). The α-, or decoding, site is now vacant until filled with the aminoacyl-sRNA specified by the newly exposed codon (Fig. 1*c*). Our failure to detect aminoacyl-sRNA bound to active ribosomes indicates that this state is very short-lived, evidently because the ribosome-bound peptide synthetase triggers peptide-bond formation at the very instant the correct aminoacyl-sRNA is captured.

If we now try to apply this scheme to the situation at chain initiation, a rather perplexing difficulty arises: what induces the translocase to act whenever the initial aminoacyl-sRNA has been selected by the first triplet in the decoding site? For, as evident from the right hand side of Fig. 1, this constellation differs radically from the situation encountered during chain extension. It would seem to violate all rules of enzyme specificity if an aminoacyl-sRNA with a predominantly charged NH_3^+ group could serve as a substrate for an enzyme that normally recognizes the uncharged amide group of peptidyl-sRNA in this position. A similar situation presents itself with respect to peptide synthetase in the subsequent step. This enzyme is likewise normally confronted with a neutral peptidyl-sRNA rather than with a protonated acyl-sRNA in the condensing site.

The simplest solution to this paradox would be to postulate that chain initiation requires a special derivative of the first amino acid, possibly coded for by a special starting triplet. Furthermore, the postulated derivative should have the charged NH_3^+ group masked to make it look more like peptidyl-sRNA which is what translocase normally recognizes. At the same time it should be rather labile. A derivative that answers this description would be obtained by *N*-formylation. The discovery of *N*-formylmethionyl-sRNA (*5*) lent substance to this hypothesis and suggested that it might function in chain initiation.

After we had started experiments designed to test this hypothesis, further data on *N*-formylmethionyl-sRNA appeared, which strongly indicated its role in chain initiation, although this possibility was not mentioned by Clark and Marcker (*6*). Among their findings the following are of particular relevance to the problem of chain initiation. (i) At least two methionyl-sRNA species exist in *Escherichia coli*, and at least one of them cannot be formylated; (ii) formylation occurs only after methionine has been linked to sRNA; (iii) when a mixture of formylated and nonformylated methionyl-sRNA was offered to a ribosomal incorporation system containing polyUG, methionine, and phenylalanine were incorporated in a ratio of about 1 to 8, and 80 to 90 percent of the TCA-insoluble methionine was recovered as the NH_2-terminal *N*-formyl derivative; (iv) addition of N^{10}-formyltetrahydrofolic acid increased the