Enzyme Induction in Higher Plants

Environmental or developmental changes cause many enzyme activities of higher plants to rise or fall.

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Introduction

As recently as 1964, it was correct to state that "no evidence has yet been obtained that the appearance of enzyme in response to substrate [or any other agent] in seed plants is actually because of induced enzyme synthesis" (*1*). Since that time, evidence has been obtained that enzyme induction—an increase in rate of synthesis of an enzyme in response to a change in a specific environmental parameter—occurs in higher plants.

Far more abundant, however, is the evidence concerning apparent enzyme induction—an increase in enzyme activity in response to a change in a specific environmental factor. These systems should be studied in greater detail because they are interesting examples of biological regulation, often with a strategic role in a developmental process.

The purpose of this article is: (i) to outline criteria by which increases in enzyme activity can be unequivocally identified as being due either to *de novo* synthesis or to enzyme activation; (ii) to recount the evidence that has definitely established the occurrence of enzyme induction in higher plants; (iii) to recount the instances of dramatic increases in enzyme activity in higher plants; and (iv) to consider the logic of controlling plant metabolism and development by control of enzyme degradation, in contrast to enzyme synthesis.

Criteria for Demonstration of Induced Enzyme Synthesis

All cases of induced enzyme synthesis have one common property. Amino acids are polymerized in a defined sequence to form one particular kind of protein more rapidly relative to other proteins under inducing conditions compared to noninducing conditions. This must be shown to prove induced enzyme synthesis.

There are two ways to show that the protein bearing an induced enzyme activity is synthesized during the apparent induction. The activity may be induced in the presence of radioactive amino acid or radioactive preexisting protein; the induced enzyme is then purified and the specific radioactivity is determined throughout the polypeptide to see if the polypeptide has the specific radioactivity of new or preexisting protein (*2*). The other method is to induce the enzyme in the presence of preexisting protein or free amino acid which has been density labeled with a stable heavy isotope (*3*). Old protein can then be separated from new protein on the basis of density difference. It can then be determined to which of the two classes the protein with the induced enzyme activity belongs.

An important part of a proof of induced enzyme synthesis is to show that an increase in rate of synthesis has occurred, rather than a decrease in the rate of destruction (*4*). This is a matter of concern which has received little experimental treatment up to now.

Any inhibitor of general protein synthesis should inhibit induced enzyme synthesis. Incorporation of an amino acid analog which renders newly synthesized protein nonfunctional should inhibit the formation of induced enzyme activity without necessarily inhibiting protein synthesis. Neither of these corollaries is itself proof of *de novo* synthesis of an induced enzyme, since activation of a latent enzyme may be indirectly dependent upon protein synthesis.

An absolute increase in the protein need not occur during induced enzyme synthesis, since an increase in the rate of degradation may also occur. In fact, an increase in the protein may reflect a decrease in rate of degradation rather than an increase in rate of synthesis. Detection of an absolute increase by purification or by reaction with specific antibody cannot alone prove the occurrence of induced enzyme synthesis.

Synthesis of messenger ribonucleic acid (mRNA) is not always necessary for protein synthesis, since in some systems mRNA is stable. Therefore the inhibition of RNA synthesis by actinomycin D or other agents may not always inhibit induced enzyme synthesis.

Possible Points of Control of Induced Enzyme Synthesis

After it is established that an induced enzyme activity is indeed due to *de novo* synthesis of enzyme, the site and manner of control should be found. Only those steps in protein synthesis which can be both rate limiting and specific for the synthesis of a given protein need be considered. Those steps are as follows: (i) the synthesis of mRNA, including initiation, termination, release, and activation; (ii) the functions of mRNA, including the formation of a complex with the ribosome, movement relative to the ribosomes, and the ability to resist degradation; (iii) the tRNA function, including the ability of unusual tRNA's to translate unusual nucleotide sequences; and (iv) polypeptide synthesis, including initiation, termination, release, and removal. There is published experimental evidence favoring virtually every one of the possibilities listed, in a wide variety of systems.

At present, there is perhaps one higher plant system in which the origin of induced enzymes has been well enough established to warrant an assault on the problem of where and how control of synthesis is exercised. This system is found in the aleurone cell of cereal seeds, which synthesizes several hydrolases in response to treatment with gibberellic acid.

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Evidence for Induced Enzyme Synthesis

As little as 10^{-10} mole of gibberellic acid per liter promotes release of reducing sugars by endosperm of barley half seeds (*5*) because of increased formation and secretion of α-amylase by aleurone cells surrounding the endosperm (Fig. 1) (*6, 7*). The α-amylase increase is inhibited by the following: (i) anaerobiosis and dinitrophenol (*8*), which suggests a requirement for phosphorylative energy; (ii) *p*-fluorophenylalanine and cycloheximide (*7*) and therefore requires protein synthesis; (iii) and actinomycin D and 6-methyl purine (*9, 10*), which suggests a requirement for RNA synthesis. Labeled amino acids are incorporated throughout the polypeptide chain (*9*). Thus at least part of the increased α-amylase activity is due to *de novo* synthesis of α-amylase molecule.

The density-labeling method has been used to show that the bulk of the new α-amylase is synthesized *de novo* (*11*). By allowing the in vivo proteolysis of reserve proteins of aleurone cells to occur in O^{18}-labeled water, O^{18}-labeled amino acids were formed (Eq. 1).

$$\text{Reserve protein} + H_2O^{18} \rightarrow (RCHNH_2CO^{16}O^{18}H \rightleftarrows RCHNH_2CO^{18}O^{16}H)$$

The incorporation of the O^{18}-labeled amino acids into proteins synthesized after the addition of gibberellic acid generated a difference in density between old and new proteins. The distribution in the equilibrium density gradient of α-amylase activity induced in H_2O^{18} indicated that all of the enzyme activity arose by *de novo* synthesis after addition of gibberellic acid.

Protease (*12*) and ribonuclease (*10*) activities produced by barley aleurone cells also increase in response to gibberellic acid. The increases are inhibited in parallel to the inhibition of α-amylase development, by the same inhibitors mentioned above. Protease has been shown to be synthesized *de novo* by the density-labeling method (*12*). It is not known what particular kind of RNA needs to be synthesized for the formation of α-amylase, protease, and ribonuclease.

Evidence for synthesis *de novo* of isocitratase and malate synthetase (Fig. 2) in cotyledons of germinating peanuts has also been obtained by the density-labeling method (*13*). The data suggest that these two enzymes do not preexist in some inactive form in the cotyledons, but that they are completely synthesized after the onset of germination from a pool of amino acids which are probably not derived directly from hydrolysis of storage protein.

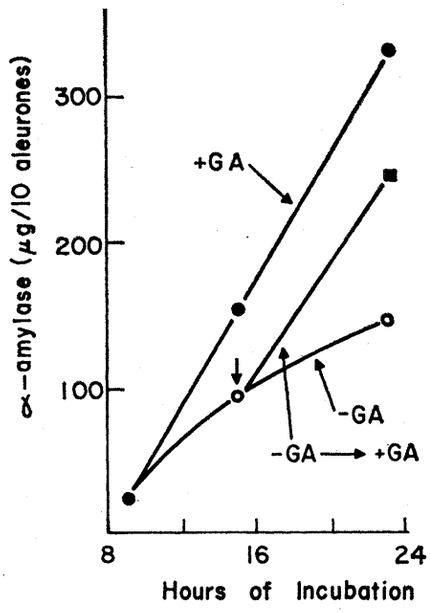

Fig. 1. Induction by gibberellic acid (GA) of α-amylase in isolated aleurone layers. The GA was removed after 9 hours of incubation, and then added back at 15 hours (arrow). The synthesis of α-amylase is dependent upon the continued presence of GA. [From Chrispeels and Varner (*10*)]

Hormonal Control of Enzyme Activities

There are several other examples of hormone-induced increases in enzyme activity which, like cereal hydrolases, also may be due to induced enzyme synthesis (Table 1). When applied to epicotyls of young pea, very small amounts of auxin (10 parts per million) induce a detectable increase in cellulase activity as measured in tissue extracts, and activity continues to increase with increase in auxin concentration up to 5000 parts per million. The addition of auxin to the extracts of pea epicotyls has no effect on activity, stability, or solubility of the cellulase. The increases are prevented by chloramphenicol, puromycin, 8-azaguanine, and actinomycin D (*14*), an indication of a requirement for RNA and protein synthesis.

Ethylene causes a tenfold increase in peroxidase activity in sweet potato root tissue (*15*). The increased peroxidase activity is in some way associated with disease resistance. Ethylene also induces an increase in polyphenol oxidase activity in sweet potato tubers (*15*). In white potato tubers, ethylene induces an increase in polyphenol oxidase but not in peroxidase (*15*).

Aerated disks of mature tubers of *Helianthus tuberosus* develop invertase activity (*16*). Disks of carrot root, potato tuber, and beet root behave similarly (*17*). At least part, and perhaps most, of the invertase is localized in the cell wall. In *H. tuberosus* tuber slices, the increase induced by aeration is inhibited by indoleacetic acid in the range of 10^{-7} to 10^{-3} mole per liter and is enhanced by small amounts of gibberellic acid (10 parts per million) (*18*).

Increases in invertase activity may result from an increased energy requirement. This is suggested by the correlation between invertase activity and rate of growth of the cell, perhaps best indicated by data on corn radicle (*19*). Invertase activity increases 40-fold from the meristematic region to the region of most rapid elongation (sevenfold based on cell volume), then decreases as the rate of elongation decreases.

An increase in invertase activity does not necessarily mean an increase in enzyme synthesis. For instance, potato tubers contain an invertase inhibitor (*20*). The inhibitor has been purified 1000-fold and shown to be a protein with a molecular weight of about 17,000 (*21*). It is most effective at the *p*H optimum (4.5) of invertase. The concentration of invertase inhibitor changes with environmental conditions. The inhibitor concentration is greatest in a potato tuber after storage at relatively high temperatures, and it decreases on storage at 4°C. Thus an increase in invertase activity is the result of both formation of active enzyme and decrease in the amount of inhibitor (*22*).

The activity of an unidentified enzyme involved in the hydrolysis of inulin also increases greatly in aerated disks of *H. tuberosus* and in aerated disks of chickory (*23*). This increase in hydrolytic activity is strongly enhanced by $10^{-5}M$ 2,4-dichlorophenoxyacetic acid (2,4-D). The analog, 3,5-dichlorophenoxyacetic acid (3,5-D), which is inactive as a growth substance at 10^{-5} mole per liter, is also inactive in promoting the hydrolysis of inulin.

Root and stem segments of pea (*Pisum sativum*) have the capacity to form certain acyl aspartates—conjugates with a peptide link between the amino group of aspartic acid and the

Table 1. Some enzymes which increase dramatically in response to a particular environmental parameter. Abbreviations are: Actinomycin (Act.); azaguanine (azaG); chloramphenicol (CAM); cycloheximide (CX); 2,4-dichlorophenoxyacetic acid (2,4-D); dinitrophenol (DNP); *p*-fluorophenylalanine (*p*fphe); gibberellic acid (GA); indoleacetic acid (IAA); phytochrome (phytoc.); puromycin (PM); streptomycin (SRP); thiouracil (thioU); purine (pur.); pyrimidine (pyrim.); naphthaleneacetic acid (NAA); and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU).

Plant (*ref.*)	Enzyme	Fold increase	Control factor	Inhibitors
	Flower			
Stam. filaments corn (*106*)	Invertase	2	GA	Act. D, PM
	Fruit			
Apple (*107*)	Malic	2.5	Age	
	Mature seed			
Ipomea violacea L. (*108*)	Alkaloid synthesis		GA	
	Developing seed			
Kernel corn (*109*)	Amino acid incorp.	2000	Age	
Seed rye (*110*)	3′-Nucleotidase	2.5	Age	
Immature embryo				
Wheat (*63*)	Nitrate reductase	100	NO_3^-	
Orchid (*80*)	Nitrate reductase	30	NO_3^-, age	
	Expanding leaves			
Rye (*111*)	Carboxydismutase	3	Phytoc.	
	Transketolase	1.5		
	Ribose-5-phosphate isomerase	2		
Barley (*112*)	L-Arginine carboxylyase	2	K^+ def.	
Barley (*36*)	Ribulose-1,5-diphosphate carboxylase	2	Light	Act. D, PM, SRP, CAM, CX
	Phosphoribulokinase	2		
Bean (*33*)	Glyceraldehyde-3-phosphate dehydrogenase	2	Phytoc.	
Corn (*74*)	Nitrate reductase	5	NO_3^-, light	
Corn (*88*)	Nitrate reductase		NO_2^-	CAM
Corn (*113*)	Ribose-5-phosphate isomerase	1.5	Light	CAM
	Ribulose-5-phosphate kinase	1.6		
	Ribulose-1,5-diphosphate carboxylase	1.75		
Dwarf corn (*114*)	Peroxidase		GA	
Mustard (*115*)	Chlorophyll A synthetase	15	Light	Act. D
Mustard (*31*)	Phenylalanine deaminase	7	Phytoc.	Dark
Mustard (*30*)	Anthocyanin synthesis	100	Phytoc.	Act. D, thioU
Mustard (*116*)	Nitrate reductase		NO_3^-, MoO_4^{-2}	CX, polymyxin B
	Mature leaves			
Tomato (*117*)	Peroxidase	2	Phenylborate	
	Laccase	2		
	Tyrosinase	2		
	Catalase	2		
Cauliflower (*71, 72*)	Nitrate reductase	12	NO_3^-, MoO_4^{-2}	CX, many amino acid analogs
Ten species (*34*)	NADPH glyceraldehyde-3-phosphate dehydrogenase	1.7 to 1.8	Light	CAM
Pea (*35*)	Glyceraldehyde-3-phosphate dehydrogenase	4	Light	DCMU, salicylaldoxime
Cocklebur (*118*)	Nitrate reductase	45	NO_3^-, age	
	Fronds			
Spirodela (*32*)	Anthocyanin synthesis		Light	pur., pyrim. analogs
Lemna (*86, 87*)	Nitrate reductase	15	NO_3^-	CX
	Nitrite reductase	20		
	Shoot apex			
Pisum arvense (*119*)	Nitrate reductase	20	NO_3^-, age	
Pea (*120*)	Indoleacetic acid oxidase	2	Infrared light	Red light
	Hypocotyl			
Gherkin (*37*)	Phenylalanine deaminase	5	Blue light	CX
	Stem			
Sugar cane (*92, 94*)	Invertase	2	NAA	Glucose
Sugar cane (*94*)	Peroxidase	36		Act. D, *p*fphe
Pea (*26–28*)	Acyl aspartate synthesis	6	Auxins, some animal hormones	Act. D, PM
Pea (*14*)	Cellulase	6	IAA	CAM, PM Act. D, azaG
Bamboo (*121*)	Phenylalanine deaminase	20	Age	
	Tyrosinase	4		
Sweet potato (*122*)	*o*-Diphenol oxidase	7	Age	Act. D, PM, blasticidin S
Bean (*123*)	Aspartate transcarbamylase	3	Age	
	Stem tuber			
Jerusalem artichoke (*16, 28*)	Invertase	250	H_2O, GA	CAM, thioU, *p*fphe, IAA, RNAase
Potato (*124*)	Isocitratase	2	IAA	Act. D
Jerusalem artichoke (*23*)	Carbohydrase		2,4-D	

carboxyl group of certain acids, for example, benzoic, indoleacetic, or naphthaleneacetic acid (*24*). This activity is inducible, and can convert a high proportion of applied naphthaleneacetic acid to the conjugate (*25*). The activity is induced by several compounds with auxin activity: indoleacetic acid, naphthaleneacetic acid, 2,4-dichlorophenoxyacetic acid, 2,3,6-trichlorobenzoic acid (TCBA), and *S*-carboxymethyl-*N*, *N*-dimethylthiocarbamate. Of these inducers, only indoleacetic acid and naphthaleneacetic acid can serve as substrates. On the other hand, benzoic acid is a noninducing substrate (*26*).

The increase in the capacity of the pea stem segments to form benzoyl aspartate induced by pretreatment with indoleacetic acid is completely suppressed by 5 micrograms of actinomysin D per milliliter present during pretreatment (*27*). Although puromycin also greatly suppresses the induced in-

Table 1 (*continued*)

Plant (*ref.*)	Enzyme	Fold increase	Control factor	Inhibitors
		Root		
Corn (*19*)	Invertase	40	Age	
Field pea (*119*)	Nitrate reductase	20	Age, NO_3^-	
Beet (*125*)	Invertase	50	H_2O	
Sweet potato (*126*)	Phenylalanine deaminase	60	H_2O	Act. D, blasticidin S
Sweet potato (*127*)	Tyrosine deaminase	3	H_2O	
Sweet potato (*128*)	Peroxidase	25	H_2O	Act. D, CAM, DNP, *p*fphe, canavanine, blasticidin S, antimycin A, PM
Sweet potato (*15*)	Peroxidase	10	Ethylene	
	Polyphenol oxidase	2		
White potato (*15*)	Polyphenol oxidase	4	Ethylene	
Senecio viscosus (*129*)	Alcohol dehydrogenase	13	Flooding, anaerobiosis, ethanol	
		Cotyledons		
Pea (*59*)	ATP phosphatase	15	Age	CAM, DNP, *p*fphe
Mustard (*130*)	Isocitratase	7 to 116	Age	PM
Pumpkin (*49*)				
Sesame (*44*)				
Peanut (*47*)				
Cucumber (*49*)				
Peanut, sesame (*44*)	Malate synthetase	18 to 90	Age	
Peanut (*48*)	Aconitase	8	Age	
	Condensing enzyme	3		
Pea (*57*)	Amino acyl tRNA synthetase	8	Age	
Mungbean, cucumber, pea, sunflower (*49*)	Phosphatase	5 to 9	Age	
Soybean (*131*)	γ-Glutamyl transpeptidase	30	Age	
Vigna sesquipedalis (*132*)	Isocitrate dehydrogenase	3	Age	
Cotton (*133*)	Lipase	2.5	GA	Act. D, aflatoxin
Radish (*75*)	Nitrate reductase	37	NO_3^-	Act. D, PM, CAM, 8-azaG
Radish (*67*)	Nitrite reductase	6	NO_2^-, NO_3^-	Act. D, PM
Squash (*55*, *56*)	Protease	3	Benzyladenine	Act. D
Pea (*53*)	Hexokinase	7	Age	Act. D
	Glucose-6-phosphate dehydrogenase	4		
	6-Phosphogluconate dehydrogenase	4		
	α-Amylase	35		
		Endosperm		
Barley (*6–9*, *10*, *12*)	α-Amylase	35	GA	CX, Act. D, DNP, *p*fphe, abscisin, 6-methylpur.
	Protease	100		
	Ribonuclease	8		
Castor bean (*42*, *43*, *50–52*, *54*)	Hexokinase	16	Age	CAM, SRP, Act. C, PM
	Phosphoglucomutase	10		
	Pyrophosphatase	3		
	Adenosine triphosphatase	2		
	Fructose-1, 6-diphosphatase	7		
	Glucose-6-phosphate isomerase	3		
	Fructose-6-phosphatase	5		
	6-Phosphogluconate dehydrogenase	5		
	Glyceraldehyde-3-phosphate dehydrogenase	4		
	Malate dehydrogenase	24		
	Succinate-cytochrome c reductase	24		
	Cytochrome oxidase	12		
	Isocitratase	45		
	Malate synthetase	2		
	Fructokinase	2		
		Cell suspension cultures		
Tobacco crown gall (*134*)	α-Amylase	60		
Tobacco (*83*)	Nitrate reductase	1500	NO_3^-	Amino acids

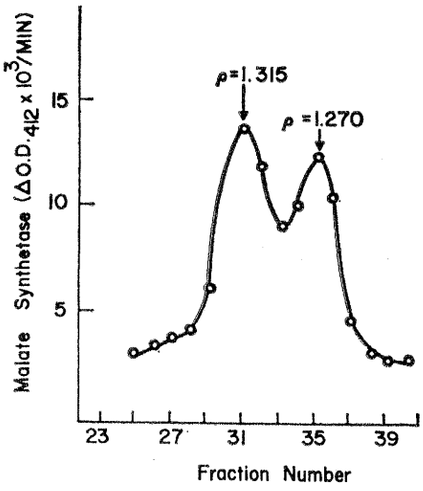

Fig. 2. Equilibrium distribution in a cesium chloride density gradient of malate synthetase present in a mixture of crude extracts from peanut cotyledons grown in 100 percent D_2O or 100 percent H_2O. The H_2O enzyme alone has a buoyant density of 1.270. The greater density of the 100 percent D_2O enzyme implies *de novo* synthesis from deuterated amino acids. [From Longo (*13*)]

creases, puromycin alone increases the level of acyl aspartate formation, in the face of 80 to 85 percent inhibition of general protein synthesis (*28*).

Animal hormones, L-thyroxine, hydrocortisone, and testosterone propionate also induce the formation of acyl aspartate in pea stem segments (*28*). Again, actinomycin D (5 micrograms per milliliter) completely suppresses the response.

Light Control of Enzyme Activities

Although little is known about the enzymes responsible for the synthesis of anthocyanins, at least one of the enzymes appears to be synthesized in response to environmental conditions which promote anthocyanin synthesis (*29*). The anthocyanin synthesis mediated by phytochrome in mustard seedlings is inhibited by puromycin and 2-thiouracil, and is prevented by prior treatment with actinomycin D (*30*). Activity of phenylalanine deaminase, an enzyme involved in anthocyanin formation, increases in response to light absorbed by phytochrome in mustard seedling (*31*). In *Spirodela*, 8-azaguanine and other analogs of pyrimidine or purine bases inhibit anthocyanin formation, and the inhibition is overcome by the corresponding natural bases, nucleosides, or nucleotides (*32*). These results suggest that an unstable nucleic acid is a catalyst or limiting factor in anthocyanin synthesis.

An example of an enzyme activity which appears to increase due to activation is the nicotinamide-adenine dinucleotide phosphate (NADP) glyceraldehyde-3-phosphate dehydrogenase of plastids of bean. The activity of this enzyme increases in etiolated tissue in response to red light (*33*). Chloramphenicol prevents this increase. Ziegler and Ziegler (*34*) have shown that, in the green leaves of a number of Angiospermae, white light at photosynthetic intensities caused a reversible increase in activity. Illumination did not have this effect on the nicotinamide-adenine dinucleotide (NAD)–linked enzyme. The NADP-linked enzyme activity increased about threefold in 20 minutes and decreased to the original amount in about 40 minutes. Chloramphenicol, but not actinomycin C, blocked the light effect.

A mutant of *P. sativum* lacking chlorophyll showed no increase in the activity of the NADP-linked enzyme after being subjected to illumination (*35*). The light-induced increase in enzyme activity is inhibited about 50 percent by $10^{-6}M$ 3-(3,4-dichlorophenyl)-1,1-dimethylurea, an inhibitor of noncyclic electron flow in photosynthesis. Higher concentrations inhibited totally. 3-(3,4-Dichlorophenyl)-1,1-dimethylurea had no effect on the enzyme activity of plants in the dark. Sodium azide, 10^{-3} mole per liter, inhibited photosynthesis and the light-induced increase of glyceraldehyde-3-phosphate dehydrogenase almost completely.

These results have been interpreted to mean that the change in activity of NADP-linked glyceraldehyde-3-phosphate dehydrogenase in light is dependent on chlorophyll and on intact photosynthesis, primarily photoreaction II, the noncyclic electron flow. The increased amount of enzyme activity may be caused by an increased supply of cofactors produced by photosynthesis.

Application of actinomycin D, puromycin, streptomycin, chloramphenicol, or cycloheximide to greening barley leaves, or to detached leaves prior to their greening, prevented the light-induced increase in ribulose-1,5-diphosphate carboxylase activity, while that of phosphoribulokinase was only partially suppressed (*36*). The increase in ribulose-1,5-diphosphate carboxylase activity is apparently more dependent on protein synthesis than the increase in phosphoribulokinase. Carbohydrate metabolites and substrates of the enzymes failed to induce either enzyme in the dark. No evidence was found for inhibitors in etiolated seedlings or activators in illuminated leaves of barley.

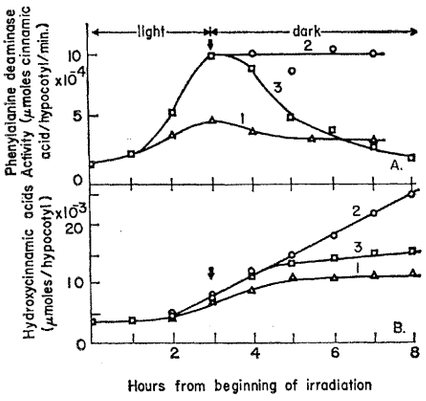

Fig. 3. Blue light induction of phenylalanine deaminase in hypocotyls of dark-grown gherkin seedlings. This enzyme is believed to be rate limiting in the pathway leading to hydroxycinnamic acids. Curves 1, cycloheximide applied at time zero; curves 2, cycloheximide applied after 3 hours; curves 3, no cycloheximide. Both the increase and decay of phenylalanine deaminase are inhibited by cycloheximide. [From Engelsma (*37*)]

Exposure of gherkin seedlings grown in the dark to blue light causes an increase in activity of phenylalanine deaminase after a 90-minute lag (Fig. 3) (*37*). About 180 minutes after the beginning of the irradiation, activity declines again. Cycloheximide inhibits both the increase and the decrease, suggesting that both processes depend upon protein synthesis. A similar phenomenon has been observed in potato tuber disks (*38*). Some animal enzymes also do not decay when protein synthesis is inhibited (*39*). The physiological significance of these intriguing phenomena is uncertain.

Temporal Control of Enzyme Activities

In synchronously developing microspores of lily, DNA synthesis begins about 21 days after completion of meiosis. Thymidine kinase activity is found at appreciable levels only during one day, immediately before DNA synthesis (*40*). Exogenously supplied thymidine enhances thymidine kinase formation, but only during a very small portion of the cell cycle close to the

interval when the enzyme normally appears. Temporal control of inducibility of thymidine kinase does not seem to involve thymidine uptake, since the accumulation of thymidine is constant throughout the cell cycle. The effectiveness of the inducer within the cell seems to be controlled (Fig. 4) (*41*). The inducibility of nitrate reductase in immature orchid embryos is also temporally controlled (see below). Thymidine also causes an increase in the activity of thymidine kinase in 2-day-old wheat seedlings, in which cells develop asynchronously. Both actinomycin D and chloramphenicol inhibit this increase (*40*).

Increases in enzyme activities are common during germination and seedling development. Isocitratase and malate synthetase appear chiefly in the fat storage tissue after germination of seeds high in fat. As the seedling develops, the activities increase and subsequently decrease (*42–44*). From the developmental point of view, the immediate cause of the rise and fall of these activities is of great interest. In sesame (*44*) both malate synthetase and isocitratase are localized in subcellular particles which sediment with the mitochondrial fraction. However, in castor bean endosperm, these enzymes of the glyoxylate cycle are not present in mitochondria purified by sucrose gradient sedimentation. They are specifically associated with a distinct particulate component of higher density which has been named glyoxysomes (*45*). Increases in enzyme activity in the cotyledons of watermelon seeds are prevented by cycloheximide (*46*). The mechanism which controls these increases is not known. In peanut, there is no indication of involvement of a factor from axis tissue (*47*). Removal of an inhibitor or end product (*48*) also does not appear to be involved.

Recently, it has been demonstrated by the density-labeling method that isocitratase and malate synthetase in the cotyledons of germinating peanuts are synthesized *de novo* (*13*). A report that the amino acid analog azetidine-2-carboxylic acid did not inhibit the development of isocitratase in pumpkin seedlings (*49*) is not necessarily in conflict with the result from peanut cotyledons. It is possible that insufficient azetidine-2-carboxylic acid was incorporated into the pumpkin isocitratase to interfere with its function. Alternatively, the isocitratase may be able to function even if azetidine-2-carboxylic acid replaces proline in the enzyme. No data were offered to indicate the proline content of the pumpkin isocitratase, nor the extent of replacement by azetidine-2-carboxylic acid, either in the isocitratase or the average pumpkin seedling protein.

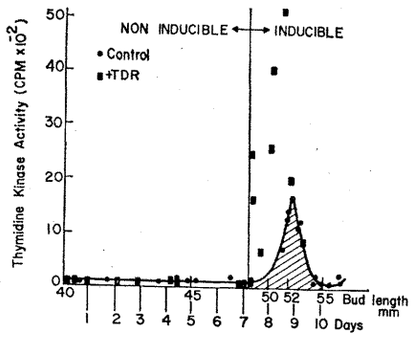

Fig. 4. Temporal and conditional substrate induction of thymidine kinase in synchronously developing microspores of lily. Thymidine can induce the formation of thymidine kinase (bars), but only around the period when the enzyme normally appears during microspore development (shaded area). [From Hotta and Stern (*41*)]

In the castor bean endosperm, the activity of several enzymes of the glycolytic and pentose phosphate pathways increases during the first few days of germination (*50*). Actinomycin D at 50 micrograms per milliliter completely inhibits the increases (*51*). The mitochondrial enzymes malate dehydrogenase, succinate-cytochrome c reductase, and cytochrome oxidase also increase in this tissue. Again, actinomycin D inhibits the activity increase (*52*). The increase in activity of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in the cotyledons of the pea are completely prevented by actinomycin D (*53*).

Marrè *et al.* (*54*) have shown that the activity of hexokinase and fructokinase in the cotyledons of the castor bean can be doubled by incubation in either glucose or fructose and that this increase is blocked by actinomycin D. These results suggest that substrates may regulate formation of some enzymes during development.

In squash, proteolytic activity increases in the cotyledons of intact embryos through the 3rd day of development and then decreases. The presence of the embryonic axis during the first 32 hours of germination is a prerequisite for the development of maximum proteolytic activity. Cytokinins in the culture solution could replace the embryonic axis (*55*). Puromycin and actinomycin D blocked the development of the proteolytic activity controlled by the axis (*56*).

The specific activity of the amino acid activating enzymes in the cotyledons of peas has been reported to increase eightfold during the first 3 days of germination (*57*). Protease activity remains constant during this time. Seeds allowed to germinate in *p*-fluorophenylalanine or chloramphenicol show only a slight increase in amino acid activating enzymes. The eightfold increase may be an artifact of the hydroxamate assay, however (*58*).

These observations are of great interest, because our general concept of pea cotyledons is that their only function is that of a storage organ. These experiments suggest that pea cotyledons are not only capable of protein synthesis, as already suggested by Young and Varner (*59*), but that they also may be capable of increasing at least a part of the protein synthesis apparatus.

Another enzyme of higher plants which appears to be partially under temporal control and partially under the control of a plant growth regulator is tyramine methylpherase (*60*), the enzyme which catalyzes the *N*-methylation of tyramine by *S*-adenosyl-L-methionine. This enzyme and its products, *N*-methyltyramine and hordenine, are absent from the dormant embryo. The enzyme activity in roots of germinating barley seedlings rises dramatically during the early days of germination. The initial rise in enzyme activity is not influenced by added kinetin, but the final enzyme activity attained is higher in the presence of kinetin. The high activity is maintained longer than in the absence of kinetin. The appearance of the enzyme is inhibited by arsenate, azide, and puromycin. The increase in enzymic activity is also inhibited by analogs of valine, lysine, and phenylalanine, while arginine, lysine, and glutamic acid enhance enzyme activity.

Substrate Control of Enzyme Activities

Nitrate reductase is the most thoroughly studied enzyme whose activity is substrate induced in higher plants. Hewitt *et al.* (*61*, *62*) showed that nitrate reductase could be extracted from cauliflower plants grown on nitrate or nitrite, but not from plants grown on ammonia. Others have demonstrated the phenomenon in a sufficient

number of plant species to indicate that induction of nitrate reductase activity by nitrate is probably a universal property of plants (Fig. 5) (*63–65*).

The induction of nitrate reductase in cauliflower is inhibited by cycloheximide, cycloserine, patulin, and polymixin B (*66*). In radish cotyledons, it is inhibited by actinomycin D, chloramphenicol, and puromycin, an indication that induction is dependent upon RNA synthesis and protein synthesis (*67*).

The nitrate reductase of *Neurospora* requires bound molybdenum for activity (*68*), and molybdenum is associated with soybean nitrate reductase through several purification steps (*69*). Furthermore, plants starved for molybdenum can grow better on ammonia than on nitrate (*70*). It is therefore not surprising that nitrate cannot induce nitrate reductase in plants deficient in molybdenum (*71*). It is surprising, however, that the kinetics of development of nitrate reductase activity are similar when molybdenum is infiltrated into leaf fragments deficient in molybdenum which are rich in nitrate, or when nitrate is infiltrated into leaf fragments deficient in nitrate which are rich in molybdenum (*71*, *72*). If nitrate reductase is an enzyme whose synthesis is induced, then apparently synthesis is controlled by both molybdenum and nitrate. If molybdenum merely activated a preexisting apoenzyme, a difference in kinetics would most likely have been found.

Burstrom (*73*) showed that nitrate reduction in wheat leaves is more rapid in light than in the dark. Nitrate reductase in cauliflower (*62*) and corn (*74*) increases in light and decreases in the dark. These effects appear to be more closely related to nitrate uptake than to enzyme synthesis (*75*). A similar loss of activity in the light occurs if nitrate (*71*), oxygen (*62*), or carbon dioxide (*76*) is removed. It is not known whether these various losses reflect decreases in the rate of formation or increases in the rate of degradation of functional enzyme.

Increases in nitrate reductase activity have also been observed in response to nitrite (*67*), and a greater response to nitrate has been observed in the presence of ammonium (*67*). Furthermore, the herbicide simazine has been shown to stimulate an increase in nitrate reductase in corn (*77*) and rye (*78*) under suboptimum conditions of nitrate nutrition. A smaller increase in nitrate reductase of corn has been reported to result from treatment with 2,4-D (*79*).

The ability of orchid seedlings to form nitrate reductase in response to nitrate appears to be under temporal control (*80*). Seedlings at first can develop on ammonium nitrate, but not on nitrate alone. During this early stage, nitrate reducase is not detectable. In the later stage, when nitrate reductase becomes detectable, the seedlings will continue to develop when transferred from ammonium nitrate to nitrate alone. In excised and cultured embryos of *Anagallis, Arabidopsis,* and *Sisymbrium* the ability to grow on nitrate was slight or absent (*63*), again suggesting that for some embryos, nitrate reductase is not inducible until a certain stage of development.

Formation of nitrate reductase is inhibited by ammonia in fungi and algae (*81*, *82*), but not in higher plants (*67*, *75*, *80*). Most amino acids inhibit the formation of active nitrate reductase in cultured tobacco cells, while a few (arginine, lysine, cysteine, and isoleucine) do not inhibit (*83*). Mixtures of amino acids which contain at least one noninhibitory amino acid are noninhibitory. Casein hydrolyzate inhibits formation of nitrate reductase in cultured tobacco cells in proportion to the ability of the amino acid mixture to meet the nitrogen requirements of the cells. These effects of amino acids on nitrate reductase formation are not readily demonstrable in excised plant tissues (*67*, *75*), although some effects of amino acids on nitrate reductase formation have been observed in systems other than cultured tobacco cells (*66*, *89*). A report that coumarin inhibits the formation of nitrate reductase has appeared (*85*), but the significance of this observation is obscure.

Nitrite reductase has been shown to be induced by nitrite in radish cotyledons (*67*). It is also induced by nitrate in this system, but the kinetics suggest that nitrite derived from nitrate is the probable inducer. Nitrate also induces nitrite reductase in *Lemna* (*86*, *87*). Again nitrate reductase developed slightly before nitrite reductase. Schrader *et al.* (*88*) have taken advantage of the greater sensitivity to chloramphenicol of protein synthesis in chloroplasts compared to protein synthesis in cytoplasm to demonstrate that nitrate reductase is synthesized by the cytoplasmic system, while nitrite reductase, which is localized in the chloroplast, is synthesized by the chloroplast system. In yeast, however, nitrate reductase and nitrite reductase have been reported to exist in a multienzyme complex (*87*).

End Product Control of Enzyme Activities

Very few examples of inhibition of enzyme formation by an end product have been reported so far in plants. Nitrate reductase has been discussed above. An interesting case is the acid phosphatase of *Euglena gracilis,* which is repressed by phosphate (*89*). When grown on a limiting amount of phosphate, the acid phosphatase activity is essentially zero until the onset of phosphate starvation, when activity rises rapidly. Addition of phosphate to stationary phase cells deprived of phosphate brings on a rapid loss of enzyme activity as growth resumes. A 20-fold increase in acid phosphatase has been reported to occur in phosphate-deficient tomato leaves (*90*). It would be worthwhile to see if addition of phosphate to such plants represses the enzyme.

The increase of phytase in the scutellum of germinating wheat embryo is completely abolished by 3×10^{-4} mole of puromycin per liter or 80 micrograms of actinomycin D per milliliter (*91*). Because inorganic phosphate also abolishes the increase in enzyme activity, it is concluded that phosphate represses the synthesis of phytase.

Maximum enzyme activity is reached at about the 13th hour of germination. The phytase increase is completely blocked only if actinomycin D is supplied within the first 6 hours, and it is completely ineffective after the 14th hour. Similarly, inorganic phosphate is ineffective if applied after the first 6 hours. The interpretation of these results is that inorganic phosphate represses phytase synthesis only during the period of phytase mRNA synthesis, that is, that inorganic phosphate acts at the level of transcription (*91*).

Invertase activity in a number of plant tissues seems to be regulated by the synthesis and destruction of the enzyme through control systems mediated by hormones or carbohydrates, or both (*92*, *93*), and in other tissues through reactions initiated by aeration (*16*). Evidence that the changes in invertase activity in tissue slices from rapidly expanding internodes of sugar cane depend upon protein synthesis is provided by the effects of glucose, chloramphenicol, puromycin, actinomycin D, and fluoride (*93*).

The two- to threefold increase in invertase activity during 8 to 12 hours of aeration of sugar cane slices is prevented by $1.5 \times 10^{-2}M$ chloramphenicol, $0.12M$ glucose, $1.1 \times 10^{-3}M$ puromy-

cin, or $8 \times 10^{-6} M$ actinomycin D. Tissue transferred from aerated water without glucose to water with glucose rapidly loses invertase activity, with a half-time of loss of about 2 hours. The rate of loss is the same in the presence of glucose or chloramphenicol, suggesting that glucose inhibits synthesis of invertase rather than accelerates destruction. Actinomycin D is not effective if the addition is delayed until the increase in invertase activity is well initiated. However, the glucose effect can occur when actinomycin D is ineffective (*93*). These results suggest that actinomycin D prevents the formation of a necessary RNA, perhaps mRNA, while glucose acts in some other way. Rapid formation of invertase occurs after a 2- to 4-hour lag, when tissues that had been treated with glucose and glycine to stop invertase formation are transferred to water. No invertase formation occurs if the transfer is to water containing actinomycin D. Incubation in glucose presumably destroys some required form of RNA. Auxin can be either promotive or inhibitory in respect to invertase activity, so the effects are not explicable in simple terms.

Glasziou, Waldron, and Most (*94*), by examining the effects of various hexoses and pentoses, have formulated a working hypothesis relating structure and activity of repressors of invertase formation in sugar cane. Glucose appears to be active either without modification or as the β-anomer in the D-pyranose form.

In isolated wheat embryos, glucose starvation apparently represses the formation of enzymes of glucose catabolism and enhances the formation of enzymes of gluconeogenesis (*95*). Experiments with actinomycin D suggest that in embryos deprived of glucose, the synthesis of hexokinase and phosphofructokinase is inhibited, while the synthesis of fructose-1,6-diphosphatase and glucose-6-phosphatase is stimulated. Glucose has the opposite effect (Fig. 6). It has been proposed as a working hypothesis that glucose-6-phosphate exerts concerted control of the biosynthesis of the two groups of opposing enzymes.

Control of Enzyme Degradation

The cells of a higher organism exist for relatively long intervals between cell divisions, and they may often have to undergo many physiological changes in response to changing environments during one cell cycle. If they were not able to specifically destroy enzymes, their ability to respond to the environment would consist only of sequentially adding enzyme activities. Such a system of response would give the cells a very limited range of capabilities when compared to the virtually unlimited range made possible by selective destruction of enzymes. Certainly the changes in cell behavior which occur during development would be greatly facilitated by a mechanism for specific destruction of enzymes.

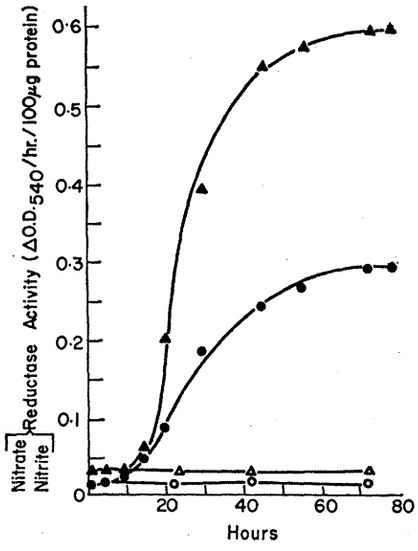

Fig. 5. Induction by nitrate of nitrate reductase (●) and nitrite reductase (▲) in duckweed. Cycloheximide inhibits the formation of both activities (open symbols). [From Stewart (*86*)]

Perhaps the most striking new generalization to come out of the survey of apparently induced enzymes of higher plants is that often an induced activity can be caused to decay, usually by removal of the inducing conditions. Some enzymes for which this is true are nitrate reductase, phenylalanine deaminase, fructose-1,6-diphosphatase, malate synthetase, isocitratase, γ-glutamyl transpeptidase, and thymidine kinase. In the latter four cases, the increase and decline are part of a developmental pattern which is not completely under the control of the experimenter.

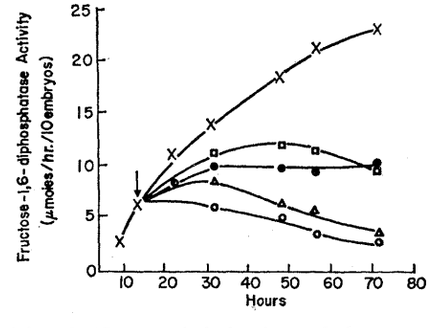

Fig. 6. Temporal induction of fructose-1,6-diphosphatase in wheat embryos (X) and inhibition of the enzyme increase by various agents added after 14 hours of germination (arrow); (●) 0.5 percent glycerol; (△) 50 mM glucose; (○) 0.2 mM puromycin; (□) 80 μg/ml actinomycin D. Glucose not only inhibits the enzyme increase, but it also promotes decay of the enzyme. [From Bianchetti and Sartirana (*95*)]

Very little is known about the factors which control the disappearance of enzymes in vivo. Rat liver tryptophan pyrrolase is stabilized in vitro and apparently also in vivo by its substrate, tryptophan (*4*). Yeast hexokinase is stabilized by glucose against trypsin attack (*96*). Presumably, the conformation of the enzyme-substrate complex is so different from that of the free enzyme that the enzyme is resistant to proteolysis (*97, 98*). The stabilizing effect of substrate or perhaps specific effectors could provide the basis for selective degradation of enzymes by proteases.

Cycloheximide stops the decline of phenylalanine deaminase which follows the light-induced elevation of this enzyme activity in gherkin hypocotyls (*37*) and in potato tuber disks (*38*). This suggests that the fall in activity is dependent on synthesis of protein *de novo*. Similar phenomena were observed earlier with mammalian liver enzymes and have been studied in greater detail. Thus, hydrocortisone induces both tryptophan pyrrolase and tyrosine α-ketoglutarate transaminase in rat liver, but the induced activities decay after a few hours. However, actinomycin D blocks the decay (*99*). Prednisolone, a corticosteroid hormone, accelerates both the rate of synthesis and the rate of decay of glutamate-alanine transaminase in rat liver (*100*). Because the rate of synthesis increases to a greater extent, the steady state activity of enzyme rises. The increase in rat liver arginase in response to a low protein diet has been attributed to a decrease in the decay rate of the enzyme (*97*). Tyrosine α-ketoglutarate transaminase of rat liver normally has a half-life of 1.5 hours, but when protein synthesis is inhibited by cycloheximide, the enzyme activity remains constant (*39*). This means that enzyme degradation as well as enzyme synthesis is dependent upon protein synthesis, perhaps upon the expression of a specific inactivation gene.

The established importance of the

control of enzyme decay rate in mammals, and the probable importance of such control in plants, contrasts sharply with the situation in vegetative bacteria. Enzyme molecule concentrations in bacteria are, in the main, controlled by the rate of synthesis (*101*), although intracellular protein of *Escherichia coli* is broken down at 0.6 percent per hour in exponentially growing cells and at 5 percent per hour in nongrowing cells (*102*). When the concentration of an enzyme molecule in a vegetative bacterial cell is to be lowered, usually synthesis is repressed, and the existing enzyme molecules are diluted to the new low concentration through subsequent cell divisions (*103*). Dilution through cell multiplication substitutes for degradation of enzymes. There are exceptions to this generalization, such as the glycerol dehydrogenase of *Aerobacter aerogenes* (*104*). It is noteworthy that in a situation where a bacterium undergoes an elaborate intracellular change without extensive cell division—that is, during the development of spores—enzyme degradation appears to play a large role (*105*).

It should be emphasized, however, that a loss of detectable enzyme activity may merely reflect a reversible inactivation or inhibition, rather than an irreversible degradation of enzyme molecules. The data available at present for higher plants are measurements of activity losses, not enzyme molecule losses.

Summary

There are many pitfalls in the path of the investigator who applies general inhibitors of protein and nucleic acid synthesis to the study of the synthesis of enzymes. These studies alone cannot prove that a new enzyme activity arose by synthesis *de novo*. Enzyme synthesis may be unequivocally demonstrated either by (i) radioisotope labeling coupled with enzyme purification, or (ii) density labeling with stable isotopes coupled with isopycnic equilibrium centrifugation.

Many cases of apparent enzyme induction are known in higher plants. Only the hydrolases produced by barley aleurone cells and isocitratase and malate synthetase of peanut cotyledons have been demonstrated to be synthesized *de novo*. Nevertheless, large fluctuations of enzyme activities in plants are encountered in response to such factors as substrates, hormones, light, dark, air, water, and development (time).

Many of the enzyme activities which can be induced to increase in plants also decay. The factors which control the decay of these enzymes are unknown. Specific control of enzyme degradation may be a significant process in the control of the metabolism of higher organisms, perhaps second in importance only to control of enzyme synthesis.

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Family Planning and Public Policy: Who Is Misleading Whom?

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Federal policies on family planning services and population research are currently under review as a result of the report of the President's Committee on Population and Family Planning (*1*). Judith Blake's article, "Population policy for Americans: Is the government being misled?" (*2*), which is presumably intended to influence this review, contains numerous errors of fact and interpretation which it is important to clarify. To support her position, she knocks down several straw men; ignores the bulk of serious demographic research on U.S. fertility patterns in the last 15 years, as well as research on differential availability of health care and the relative effectiveness of various contraceptive methods; and cites opinion-poll data in a manner that distorts the overall picture. The article's methodological limitations alone are sufficient to suggest that the question raised in its subtitle may more appropriately be turned around and asked of the article itself.

The article is based on six principal propositions.

1) That the reduction of U.S. population growth—indeed, the achievement of "population stability"—is "virtually unchallenged as an official national goal."

2) That, in pursuit of *this* goal, the "essential recommendation" by official and private groups has been a program of publicly financed family planning services for the poor.

3) That this program of family planning for the poor will not achieve the goal of population stability.

4) That advocates of this policy contend that the poor have been denied access to family planning services because of "the prudery and hypocrisy of the affluent."

5) That the poor desire larger families than higher-income couples do and are significantly less inclined to favor birth control.

6) That the estimate of 5 million poor women as the approximate number in need of subsidized family planning services is exaggerated.

With the exception of proposition 3, each of these statements is seriously misleading or in error. Let us examine the evidence on each point.

A Consensus on U.S. Population Stability?

If the United States had as a national goal the reduction of its population growth and the achievement of population stability—and if the program of publicly funded family planning services for those who cannot afford private medical care had been advanced as the principal or only means of achieving population stability—Judith Blake's contention that the government is being misled would have much validity. However, neither proposition is sustained by the evidence.

We have individually and jointly been associated with the evolution of

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