division figures between treated and control plants were not significant at the 1-percent level. This effect extended over the uppermost 650 to 800 μ of the subapical region; below this distance, the number of mitotic figures drops rapidly to zero.

The increase of stem growth by gibberellin, the first gibberellin effect observed, and demonstrated in a great number of plants (*5*), is based on an increase in cell length, although in some cases a certain increase in cell number has been considered as probable (*6*). Our results show directly that gibberellin causes a great increase in the number of cell divisions in the subapical region of nonvernalized biennial *H. niger* rosettes, thus proving that gibberellin may function as a regulator not only of cell elongation, but also of cell division.

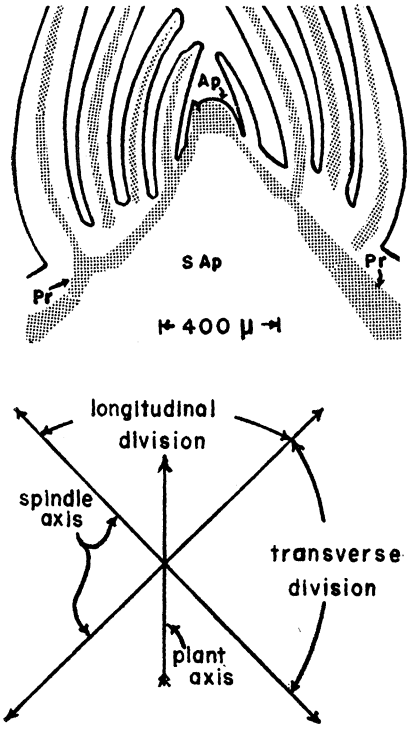

Fig. 1. (Top) Semidiagrammatic drawing of the shoot of nonvernalized, biennial *Hyoscyamus* plant. Ap, apical region; SAp, subapical region; Pr, provascular tissue. (Bottom) Sketch showing the basis for selection of longitudinal and transverse cell division figures.

It is conceivable that the effect of gibberellin on cell division is an indirect one, the division being induced by accelerated cell elongation. Dividing cells in the subapical region of *H. niger* are indeed 1.5 times as long as nondividing ones. However, this is equally true of treated and untreated plants, and the length of cells is not increased even after 9 days of daily gibberellin applications, although their shape is then quite different. It thus seems that increased cell elongation follows rather than precedes the effect of gibberellin on cell division.

The data presented in Table 1 further indicate a directional influence of gibberellin on cell division, longitudinal division figures being increased to a much greater extent than transverse ones. The factors responsible for this effect require further investigation.

It will also be necessary to check in further experiments whether, as suggested by the ones reported here, gibberellin fails to increase cell division in the apical region of the *H. niger* shoot. If this situation is generally true, it is suggestive of the hypothesis of Buvat (*7*), according to which the extreme apical region of the shoot meristem does not participate in vegetative growth, the actual meristematic activity being localized below that region. This area of meristematic activity, called the *méristème medullaire,* corresponds to the lower part of the apical and the upper part of the subapical region of *H. niger.* However, Buvat's theory goes further and describes the *méristème medullaire* and not the extreme apex as the origin of the initial cells of the entire stem (*7*, see particularly Fig. 12), whereas our results by no means exclude the apex as the primary source.

Table 1. Effect of gibberellin on cell division in biennial *H. niger*. All figures represent the number of division figures per 60-μ tissue slice. The errors quoted are the standard errors of the mean.

Hours after application of gibberellin	Apical region	Subapical region		Number of plants
		Longitudinal division figures	Transverse division figures	
		Controls		
0	12 ± 2	2 ± 1	3 ± 1	5
6	12 ± 1	3 ± 1	4 ± 1	4
12	15 ± 2	1 ± 0.5	4 ± 0.5	5
18	15 ± 3	1 ± 1	3 ± 1	5
24	9 ± 3	3 ± 0.5*	8 ± 2	10
		5 μg *of gibberellin*		
6	8 ± 1	3 ± 1	6 ± 1	5
12	13 ± 2	3 ± 2	6 ± 2	5
18	11 ± 1	12 ± 4	11 ± 2	5
24	12 ± 1	29 ± 3*	10 ± 2	9

* The difference is significant at the 1-percent level using the *t* test for unpaired samples in which the variances for the parent populations are unequal; described by Stearman (*8*, pp. 182 and 183).

ROY M. SACHS
ANTON LANG

Department of Botany,
University of California, Los Angeles

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12 March 1957

Proposed Mechanism of Action of Hydrolytic Enzymes

Certain hydrolytic enzymes, notably chymotrypsin, trypsin, and acetylcholinesterase have been shown to react with reagents such as diisopropylfluorophosphate to yield inactive proteins which contain single diisopropylphosphate residues (*1, 2*). The inactive protein is relatively stable in water but may be converted into the active enzyme by treatment with a variety of nucleophilic reagents (*3*). Chemical or enzymatic hydrolysis of the inhibited protein has invariably shown the dialkylphosphate residue to be attached at the hydroxyl group of a single serine residue (*4*).

Recently, singly substituted, inactive, acyl derivatives have been successfully prepared by reaction of delta-chymotrypsin with compounds such as *p*-nitrophenyl acetate, acetic anhydride, and benzoyl chloride (*5*). These proteins appear to be very similar to the dialkylphosphate-substituted enzymes except that they are more readily reactivated by water. These facts, together with the close parallel of the *p*H dependence of acylation (*5, 6*), normal hydrolytic activity (*7*), and dialkylphosphorylation (*1*), have led to the conclusion that these reactions are closely related and involve directly at least a portion of the active center of the enzymes (*3*). Dixon and Neurath (*5*) have also shown that the *pK'* for acylation and deacylation of delta-chymotrypsin are different, being about 6.2 and 6.8, respectively. The latter value is very close to that reported

for the hydrolysis of acetyl-L-tryptophan and acetyl-L-tyrosine ethyl ester, 6.71, by the enzyme (*7*). All these values fall in a *p*H region associated with the ionization of imidazole nitrogen. This fact, together with the almost inescapable conclusion that the mechanism involves nucleophilic attack on the carbonyl function of the natural substrates (*2, 3, 8*) has led to the idea that the uncharged imidazole nitrogen is the primary nucleophilic agent.

The location of the dialkylphosphate residue on the serine hydroxyl has been explained as the result of a secondary transfer reaction (*9*). The fact that the heat of ionization of the group of *pK'* 6.71 referred to in the preceding paragraph is about 11 kcal mole^{-1} (*7*) rather than the 6 to 7 kcal mole^{-1} ordinarily encountered for imidazole would, however, suggest that a relatively uncomplicated nucleophilic attack by the imidazole nitrogen is not a satisfactory explanation. It would seem that a mechanism should be sought which includes the direct involvement of the serine hydroxyl.

Such a mechanism is suggested in the condensed scheme outlined in Fig. 1. The formation of the active center is postulated to involve hydrogen bond formation between the serine hydroxyl and an uncharged imidazole nucleus (*10, 11*). The two components could be located on separate peptide chains in the enzyme as long as the specific three-dimensional structure, formed during activation of the zymogen, brought them near enough for this interaction to occur. The polarization of the resulting structure would cause the serine oxygen to be the nucleophilic agent which attacks the carbonyl function of the substrate (*3, 8, 11*).

The complex is stabilized by the simultaneous "exchange" of the hydrogen bond from the serine oxygen to the carbonyl oxygen of the substrate. Another group capable of hydrogen bonding could be involved in this sequence, for we have so far been unable to determine satisfactorily whether or not steric considerations preclude the involvement of a single imidazole nucleus as shown.

The *p*H dependence of this step would be that associated with the acylation reaction. Thus, the *pK'* would be 6.2 and the heat of ionization might be expected to be low, near 1 or 2 kcal mole^{-1}. The intermediate acylated enzyme is written with the proton on the imidazole nitrogen. The deacylation reaction involves the loss of this positive charge simultaneously with the attack of the nucleophilic reagent, abbreviated Nu:H. This reaction would therefore be expected to show a *p*H dependence related to imidazole ionization, but the heat of ionization might be expected to be considerably different.

This, as has been noted, is the actual situation. The relative magnitude of the two *pK'* values is in accord with the demonstrated stabilization of the acylated enzyme at low *p*H (*12*). A possible alternative route of deacylation (*13*) would involve the nucleophilic attack of the imidazole nitrogen on the newly formed ester linkage of the postulated acyl intermediate, leading to formation of the acyl imidazole. The latter is unstable in water, hydrolyzing rapidly to give the product and regenerated active enzyme.

The reaction of alkylphosphates in such a scheme may be written in an entirely analogous manner, except that the resulting phosphorylated enzyme would be less susceptible to deacylation through nucleophilic attack (*3, 14*).

The validity of any mechanism rests on its agreement with available data, its success in predicting the results of subsequent new tests, and chemical and physical studies directed toward establishing the presence of specific molecular groupings invoked in the reaction scheme. The agreement of the proposed mechanism with existing data has been discussed. It is hoped that consideration of this alternative to what might be termed the "primary imidazole" hypothesis may lead to direct tests for distinguishing the two, though an approach does not seem to be immediately obvious. Although determination of the heat of ionization for the *p*H-dependent step preceding acylation may be of value, it does not seem likely that the formation of the hydrogen bond in the active enzyme would be detectable.

LEON W. CUNNINGHAM
Department of Biochemistry,
Vanderbilt University,
Nashville, Tennessee

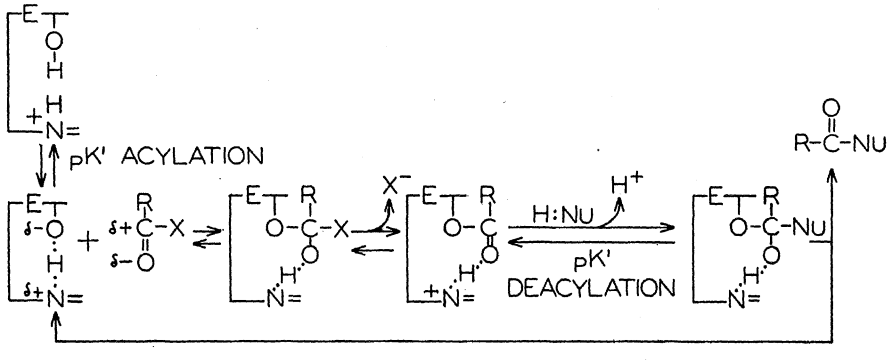

Fig. 1. Proposed mechanism for enzymatic hydrolysis.

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21 March 1957

Time Course of Far-Red Inactivation of Photomorphogenesis

Flint and McAlister (*1*) demonstrated in 1935 that red and far-red radiant energy produced opposing effects on lettuce-seed germination; the red stimulated germination and the far-red inhibited it. Borthwick *et al.* (*2*) later showed that, if the far-red were applied subsequent to the red, it prevented the consequences of the red reaction. They called this phenomenon photoreversal. The red induction and potentiality for its far-red photoinactivation also has been demonstrated in photomorphogenic development of seedlings (*3, 4*) and photoperiodism (*5*). Hendricks *et al.* (*6*) have advanced the hypothesis that the system involves a photoreversible pigment with two stable electronic energy states. When it is in the one energy state, the pigment can be transferred to the other if a quantum in the absorption band characteristic of the first state is absorbed. It can then be reversed to the original state, presumably by way of the same pathway, by the absorption of a quantum characteristic of the second state. Hendricks *et al.* postulate that the reaction rate is not dependent on a mo-