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.

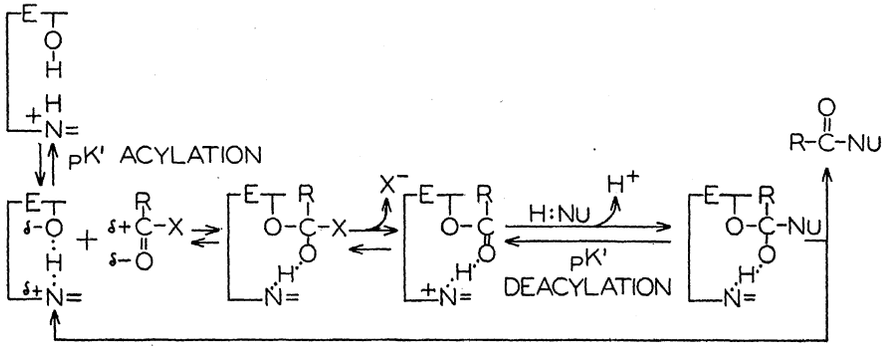

Fig. 1. Proposed mechanism for enzymatic hydrolysis.

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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-

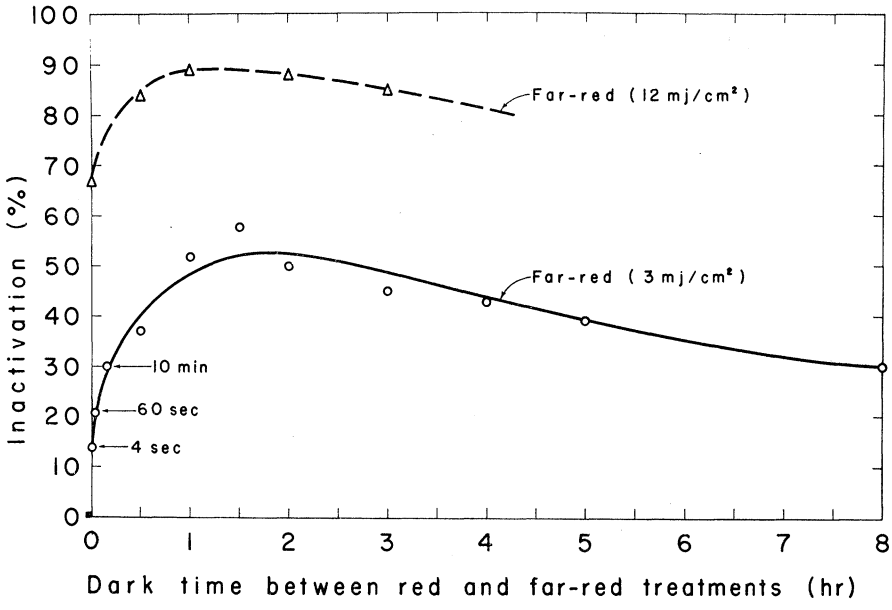

Fig. 1. Time course of far-red inactivation of red-induced morphogenesis in the hypocotyl hook of the bean seedling. Curves are shown for energy levels of 3 and 12 mj/cm², applied at 1000 μw/cm² for 3 and 12 seconds, respectively.

lecular collision in either direction and that both photoreactions follow first-order kinetics.

If such a simple reversal obtains, with no intervening collision step, it seems logical to assume that the system would be immediately reversible at the maximum rate, and that the time course would show a constant value of reversibility, to the point where the consequences of the red induction result in irreversible growth reactions.

In view of these considerations, it was decided to determine the time course for photoinactivation of red-induced photomorphogenesis in the bean seedling (*7*). A high-intensity carbon arc, in combination with interference filters, was used as the source. The irradiation periods were as short as could be obtained with this system, and were a matter of seconds. The bioassay was the rate of opening of the hypocotyl hook of bean, the technique of which has been described by Klein *et al.* (*8*). In this system, the induction response has a peak of activity in the red at 660 mμ, and photoinactivation peaks at 710 and 730 mμ (*3*). The angle of hook opening is proportional to the logarithm of the incident red energy over an irradiance range of at least 1 million for short periods of photoinduction, and the inactivating effect of the far-red is directly proportional to irradiance. A total energy of 10 mj/cm² of far-red energy distributed over a 30-minute period produces about maximal photoinactivation, the percentage of inactivation rising linearly with energy to approximately 85 percent and then leveling off rapidly thereafter.

The hypocotyl hooks were excised from 6-day old Black Valentine bean seedlings and arranged in a circle on moist filter paper in a 150-mm petri dish. The excised hooks were exposed to red energy at 25° with a carbon arc for 3 minutes at an irradiance of approximately 1500 μw/cm², which yielded a total energy of 250 mj/cm² at 660 mμ. The hooks were then kept in the dark until time for the far-red exposure, which was given at approximately 1000 μw/cm² at 730 mμ for 3 and 12 seconds for total energies of 3 and 12 mj/cm², respectively. The two filter systems were mounted in a sliding panel so arranged that the shortest dark time was 4 seconds.

The results are given in Fig. 1 as time-course curves. It will be noted that the capacity for photoinactivation by the far-red increases rapidly with time, reaching a maximum at between 1 and 2 hours and then decreasing gradually. The decrease is consistent with the hypothesis that irreversible growth reactions have occurred. For the shortest periods of time, the percentage inactivation is approximately proportional to the far-red energy. Data are available (*9*) which show that the far-red is incapable of producing significant inactivation at proportionately low energy levels in the presence of the red. Therefore, it is probable that the curve falls back to zero, either at zero time or slightly prior to the end of the red-induction period.

These results suggest that a thermochemical step intervenes between the absorption of red photons and maximum capacity for inactivation by the far-red. There are several possibilities about the nature of this step. It may involve the direct modification of the pigment itself to a far-red absorbing form so that inactivation cannot proceed until thermal conversion occurs. On the other hand, it may be concerned with synthesis of a reactant essential to the completion of the inactivation step, the concentration of which limits the far-red reversal process. The complete system is being studied with a complex of variables of incident energy, time, and temperature, with the view of determining the nature of the intervening step.

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13 March 1957

Some Characteristics of Cortical Recruiting Responses in Unanesthetized Cats

In 1941, Morison and Dempsey (*1*) demonstrated that trains of 8- to 12-per-second stimuli delivered to the intralaminar thalamic nuclei of the anesthetized cat evoked long-latency, surface-negative potentials which were widely distributed over the cerebral cortex. Because the first stimulus of the train evoked little or no cortical response, and because the responses to each successive stimulus showed a progressive increase in amplitude to a maximum, these potentials were termed "recruiting responses." Subsequent investigations have further defined the thalamocortical pathways and the character of the synaptic connections which mediate recruiting responses (*2–4*). It was shown that pentobarbital lowered the threshold for recruiting responses (*5*), and, since stimulation of the brain-stem reticular formation prevented their occurrence (*6*), it was assumed that recruiting responses could not be obtained in the waking animal.

The study described in this report employed the technique of chronic implantation of electrodes, which was developed by Sheatz (*7*), to explore this