

not measured, but the gibberellin-treated plants all had stems which were obviously more slender than those of the controls (Fig. 1). At the end of the experiment all plants that had been retained continuously under long days were still strictly vegetative, while all plants that had been given six or more photoinductive cycles had well-developed macroscopic flower buds. The control plants that had been given two and four photoinductive cycles had developed only microscopic inflorescence primordia—stages 2 and 3 of Salisbury (12)—while all but one of the gibberellin-treated plants given two or four cycles had well-developed macroscopic flower buds, which were farther along than the controls which had received eight inductive cycles (Table 1; Fig. 1). Gibberellin also accelerated reproductive development in the six-, eight-, and ten-cycle groups, particularly with regard to pistillate inflorescences in the latter group.

These results substantiate Lang's conclusion that gibberellin does not substitute for any short-day requirement of *Xanthium* as far as initiation of reproductive development is concerned. Various investigators, including Mann (13), Naylor (15), and Salisbury (12), have shown that, while one photoinductive cycle is sufficient for the induction of *Xanthium*, additional cycles increase the rate of reproductive development. Gibberellin can substitute for such additional photoinductive cycles in promoting the reproductive development of induced *Xanthium* plants.

VICTOR A. GREULACH

JOHN G. HAESLOOP

Department of Botany, University  
of North Carolina, Chapel Hill

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## Note on Absorption Spectra of Hill Reaction Oxidants

The selection of oxidants for the Hill reaction has been somewhat empirical heretofore. Mainly, complex inorganic ions and quinones have been found to be good Hill reaction oxidants. Hill and Whittingham (1) have listed the oxidation potentials of common oxidants. These range from  $-0.44$  for ferricyanide to 0 for ferrioxalate.

Yet surely the oxidation potential alone does not determine the suitability of an electron acceptor for the Hill reaction. For one thing, some oxidants of really negative potentials, which therefore should be easily reducible, are not reduced by illuminated chloroplasts. Examples of this kind are permanganate and periodate, neither of which participates in the Hill reaction at all (2).

It became of interest, therefore, to investigate some of the other characteristics which might influence the activity of various oxidants in the Hill reaction. For this reason, we have investigated the absorption spectra of various Hill reaction oxidants (3). The pertinent data are collected in Table 1. It is clear that Hill reaction oxidants absorb light near the blue peak of chlorophyll *a*, whereas the light absorption of those compounds which are inactive as Hill reaction oxidants is negligible around 420 to 430  $m\mu$ . This appears to be true despite the fact that the latter group includes compounds which are good oxidizing agents, such as permanganate and periodate. One test of this prediction was the finding that cobaltioxalate, which possesses the required absorption band at 420  $m\mu$ , acts as an electron acceptor in the Hill reaction (4).

Unfortunately, a real physical explanation of these facts cannot be given now. Neither the term levels of chlorophyll nor the term levels of complex inorganic ions are well enough characterized that an elucidation of their absorption spectra is possible at this time. The following explanation of the correlation which appears in Table 1 may, however, provide a reasonable approach to the problem.

It is well known that the Franck-Condon principle requires an overlap of energy levels of the reactants, because the actual time taken by electron transfer and by electronic excitation energy transfer is short compared with the time for ordinary atomic motion. If we consider that electronic excitation energy transfer from chlorophyll to the oxidant occurs in the Hill reaction, then the Franck-Condon principle requires chlorophyll and the Hill reaction oxidant to have overlapping energy levels. In the absence of precise knowledge of

energy levels of chlorophyll and ordinary Hill reaction oxidants, which has already been referred to, the additional assumption has to be made that overlapping absorption bands in the absorption spectra of two substances also indicate overlapping energy levels. The data of Table 1 indicate that good Hill reac-

Table 1. Absorption peaks of chlorophyll *a* and of various hill reaction oxidants.

Hill reaction		Absorption		
Oxi-dant	Ref.	mμ	Log ε	Ref.
		<i>Chlorophyll a</i>		
		250	~ 4.43	10
		325*	4.41	11
		375*	4.69	11
		427.5*	5.08	12
		660*	4.96	12
		<i>Ferricyanide</i>		
Yes	13	260	3.28	14
		303	3.36	14, 15
		420	3.04	15
		<i>p-Benzoquinone</i>		
Yes	16	240	4.7	17
		300	2.5	17
		425	1.4	17
		<i>Cobaltioxalate</i>		
Yes	4	245	4.33	18
		420	2.34	18
		596	2.22	18
		<i>1,2-Naphthoquinone</i>		
Yes	19	250	4.35	20
		340	3.40	20
		400-500	3.4-2.0	20
		420	~ 3.0†	20
		<i>1,4-Naphthoquinone</i>		
Yes	19	250	4.4	21
		335	3.5	21
		400	1.7	21
		425	1.6	21
		<i>Phenol-indophenol</i>		
Yes	2	420	3.0†	22
		<i>Ferrioxalate</i>		
Yes	23	420	1.4†	24
		<i>Chromate</i>		
Yes	2, 25	280	3.58	26
		380	3.66	26
		420	2.7†	4
		<i>Vanadate</i>		
No	2	310-370	cutoff	27
		<i>Thionine</i>		
No	2	240	4.08	28
		285	4.64	28
		585	4.60	29
		<i>Permanganate</i>		
No	2	310	3.23	30
		525	3.35	30
		545	3.33	30
		<i>Nitrate</i>		
No	2	200	4.1	31
		300	2.53	27
		<i>Cobalticyanide</i>		
No	4	200	> 4	32
		259	2.15	32
		311	2.2	32
		<i>Tetrathionate</i>		
No	2	216	4.0	33
		<i>Hypochlorite</i>		
No.	2	290	2.54	34
		<i>Periodate</i>		
No	2	222.5	4.0	35
		<i>Methylene blue</i>		
Yes	36	250	4.30	28
No	2	290	4.65	28
		615	4.57	29
		662	4.83	29

\* In ethyl ether. † Continuous absorption, not a maximum.

tion oxidants do indeed have absorption bands which overlap the blue absorption band of chlorophyll. The evidence that methylene blue is an electron acceptor is conflicting in the literature.

It may well be asked where the energy for electron transfer at an energy level corresponding to the 420-m $\mu$  absorption band (68 kcal/mole) comes from, since the Hill reaction proceeds, as does photosynthesis, in red light (44 kcal/mole). It will be noted that at least 24 kcal/mole remain unaccounted for.

Another light absorption step may provide the additional energy needed. No such two-step process which uses only the short-lived singlet states can occur. Excitation of chlorophyll to a "chemically active species" may, however, be explained as occurring in a two-step process analogous to the one occurring in the flash photolysis of anthracene and related substances (5). This is based on the existence of a metastable state (triplet) of chlorophyll with a life-time of about  $10^{-8}$  sec (6). The energy of the metastable state is slightly below that of the first (singlet) excited state of chlorophyll; experimental evidence which indicates its participation in photosynthesis has been obtained by Calvin (7) and by Commoner (8).

Thus we picture radiant energy to be converted to chemical energy in the course of the Hill reaction in the following two-step process. The first step is excitation to either the first or second excited singlet state, but only the molecules remaining in the long-lived metastable state are of further interest. These may be promoted by absorption of another quantum of red light to provide the additional energy required for ultimate reduction of the oxidant (9).

One consequence of this two-step energy absorption process is that the quantum requirement for the formation of molecular oxygen cannot be less than eight. Two quanta are required to promote each of the four electrons involved for wavelengths corresponding to either the red or the blue absorption bands of chlorophyll (10).

RUDOLPH J. MARCUS  
JAMES L. HATCHETT  
KENNETH M. SANCIER

Chemistry Department,  
Stanford Research Institute,  
Menlo Park, California

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#### Serotonin, Norepinephrine, and Related Compounds in Bananas

The present study was initiated following a personal communication from J. A. Anderson (1) that ingestion of bananas produces an increased urinary excretion of the serotonin (5-hydroxytryptamine) metabolite, 5-hydroxyindoleacetic acid. This observation was reported recently by Anderson, Ziegler, and Doeden (2). The chemical studies

Table 1. Serotonin and catecholamines in banana. The values presented are the averages of a number of analyses on ripe, yellow bananas. In the case of serotonin, 14 assays were done on pulp (range, 8 to 50  $\mu$ g/g) and 5 on peel (range, 47 to 93  $\mu$ g/g). In the case of norepinephrine and dopamine, the values represent the results obtained on three bananas.

Pulp*		Entire peel†	
$\mu$ g/g	mg/banana	$\mu$ g/g	mg/banana
<i>Serotonin</i>			
28	3.7	65	3.9
<i>Norepinephrine</i>			
1.9	0.25	122	7.3
<i>Dopamine</i>			
7.9	1.0	700	42

\* Average wt. of pulp, 130 g.

† Average wt. of peel, 60 g.

reported here were undertaken to explain this phenomenon; they have led to the rather surprising finding that bananas contain large amounts of two physiologically important agents, serotonin and norepinephrine.

The studies started with an attempt to find serotonin in bananas. Extracts of banana pulp were prepared by homogenization in 0.1N HCl followed by alkalization and extraction as described by Weissbach *et al.* (3). Homogenates of banana peel were precipitated with  $Zn(OH)_2$  and the filtrates were used for assay (3). Spectrophotofluorometric analysis of these extracts revealed large amounts of material having the characteristic activation and fluorescence spectra of serotonin (4). The amounts found are shown in Table 1. The serotonin in these extracts was identified further by subjecting it to chromatography on paper. As is shown in Table 2, the  $R_f$  values in two solvent systems and the colors obtained with several spray reagents were identical with those obtained with an authentic sample of serotonin.

The presence of related 5-hydroxyindole compounds was also investigated. No 5-hydroxyindoleacetic acid was found by use of the quantitative assay procedure (5). Chromatography of acetone-HCl extracts (see below) revealed the presence of at least two other 5-hydroxyindole substances. No further identification of these substances was attempted in the present study.

The presence of serotonin, coupled with the fact that bananas produce melanin-like material on ripening, suggested the possibility of oxidation of 5-hydroxyindoles to "melanin-precursors," 5,6-dihydroxyindole compounds (6). The presence of "melanin-precursors" was further suggested by the ob-