Evidence has been presented from several laboratories that plastoquinone A is required for the Hill reaction activity of chloroplasts. Bishop (1) and Kroghmann and Olivero (2) have shown that extraction of plastoquinone A with hydrocarbon solvents stops the photoreduction of ferrocyanide or 2,6-dichloroindophenol, and that activity can be restored by adding back the PQ A. Arnon and Horton (3) have shown that the over-all indophenol, and that activity can be restored by adding back PQ A requirement. This localizes the function of PQ A between the first and second light reactions. Studies of oxidation-reduction changes of plastoquinone A under various conditions of illumination also indicate that it may function as an electron carrier between the first and second light reactions. Arnon and Crane (4), Redfearn (5), Witt et al. (6), Amesz (7), Vernon and Avron (8), and Trebst, Eck, and Wagner (9) have proposed that PQ A may function as the primary electron acceptor of the second light reaction.

Wood, Bhagavan, and Crane (10) have shown that additional factors besides PQ A in heptane extracts are necessary for full restoration of Hill reaction activity after mild heptane extraction. A large part of this additional requirement can be satisfied by addition of the reduced form of PQ A (11). We have previously shown that after more drastic extraction of chloroplasts with acetone, Hill reaction activity requires plastoquinone C in addition to PQ A (12). Unfortunately, the acetone extraction procedure removes a large part of the chlorophyll, so that, even when full activity is restored on the basis of chlorophyll remaining in the chloroplasts, the activity restored represents less than 10% of the total on a dry weight basis. As an alternative approach we have used long term extraction of lyophilized chloroplasts with heptane. This procedure removes less than 1% of the chlorophyll, but can remove up to 90% of the PQ A and PQ C. A large part of the DCI photoreduction activity can be restored after mild heptane extraction of lyophilized chloroplasts with heptane. This procedure removes less than 1% of the chlorophyll, but can remove up to 90% of the PQ A and PQ C. A large part of the DCI photoreduction activity can be restored to these extracted chloroplasts by addition of suitable combinations of plastoquinones A and C. These experiments will be described in this paper.

Recent studies by Das et al. (13) have shown that PQ C has the same quinoid ring as PQ A and has a 45-carbon prenyl side chain like PQ A, but has a hydroxyl group, probably in the second isoprenoid unit from the quinone ring. PQ B does not have a hydroxyl group, but does have an additional double bond in this second isoprenoid group. Thus any specificities shown by PQ C or PQ B will be related to these variations in the side chains.

**METHODS AND MATERIALS**

Preparation of Chloroplasts—Spinach chloroplasts were prepared by differential centrifugation of spinach leaf homogenate in 0.5 M sucrose, buffered to pH 7.3 with 0.05 M potassium phosphate. The isolated pellets of chloroplasts were washed three times to remove the sucrose by resuspension in 10 times their volume of distilled water and centrifugation at 3300 rpm for 10 min.

The washed chloroplasts were then lyophilized and the dried chloroplasts were extracted by shaking with purified heptane for various lengths of time on a reciprocal shaker at 22°C. The heptane extracts from 30 to 90% of the quinones from the chloroplasts, depending upon the extraction time and the number of times the chloroplasts are washed with heptane after separation.
of chloroplasts from the solvent suspension. This extraction procedure gives a yellow extract which contains less than 1% of the chlorophyll in the chloroplasts.

The chloroplasts were filtered from the heptane extract by pouring the solution through a funnel containing glass wool covered by a Kleenex tissue. Any heptane remaining on the extracted chloroplasts was removed by evaporation, and the chloroplasts were homogenized in 0.015 M NaCl, pH 6.8, just before use. The lyophilized chloroplasts retain their activity for 4 or 5 days if stored dry in a freezer.

The extent of extraction by heptane can be checked by a direct extraction on a small amount of the extracted chloroplasts having about 6 mg of chlorophyll. The direct extraction consists of homogenizing the chloroplasts in about 10 ml of ethanol, centrifuging the residue, and decanting the extract. The ethanol extraction is repeated three or four times, until the residue is colorless. The combined extract is evaporated to dryness, taken up in petroleum ether, and chromatographed on a thin layer plate coated with Silica Gel G-HR. The chromatogram is developed by ascending flow with chloroform, which separates a high zone of PQ A and a PQ C + D zone that is above the chlorophyll but about 2 inches below PQ A (14).

**Assay for DCI Reduction**—The 3.0-ml reaction mixture contained 20 µ moles of potassium phosphate buffer, pH 7.2; 0.09 µ mole of 2,6-dichloroindophenol, and chloroplasts containing about 0.01 to 0.03 mg of chlorophyll. For assays of longer than 1 min, as in Fig. 8, 0.18 µ mole of DCI was added. Chloroplasts were added to the assay mixture last. Before the chloroplasts were added, additions of quinones in isocyanate were made to the dry extracted chloroplasts, the solvent was removed by evaporation, and then the chloroplasts were homogenized in 0.015 M NaCl, pH 6.8, giving a chlorophyll concentration of about 1.0 mg per ml.

The 3-ml reaction mixture in a 16-mm test tube was exposed to 1 min of light by placing the test tube in a 250-ml Erlenmeyer flask filled with water, which provided a filter of 5 cm of water with a total distance of 10 cm from the 150-watt tungsten light source. After illumination, the sample was transferred immediately to a cuvette and the decrease in absorbance at 600 nm was measured against a dark control with a Beckman model DU spectrophotometer.

**Effect of Addition Conditions**—PQ C will restore activity if it is added to the extracted chloroplasts either by evaporation of a heptane solution on the dried chloroplasts or by addition of an ethanolic solution of the quinone to the aqueous suspension of extracted chloroplasts. In general the results with either procedure show similar activity, but we have found the dry evaporation to give more consistent results.

**Purification of Plastoquinones with Aluminum Oxide and Silica Gel G-HR**—Separation of quinones by column chromatography with aluminum oxide (acid-washed) as an adsorbent and a mixture of petroleum ether and diethyl ether for development provides a fast method of isolating quinones from large quantities of chlorophyll, sterols, and carotenoids in plant material.

An extract of chloroplasts containing as much as 2000 mg of chlorophyll can be chromatographed on one column of 200 g of aluminum oxide. With the use of large quantities of plant material, the quinones present in small amounts can be detected and isolated by concentrating the fractions from the column.

Mercer reagent grade aluminum oxide (200 g, acid-washed) was deactivated with 16 ml of distilled water, mixed with 184 ml of petroleum ether, and packed in a column, 2 x 61 cm. The column was eluted under nitrogen gas at a pressure of 2 p.s.i. After addition of the sample in a small volume of petroleum ether, the column was eluted with 1000 ml of petroleum ether, which removed most of the carotenes. Elution with six 500 ml fractions of a mixture containing increasing amounts of diethyl ether (0.3, 1, 5, 8, 12, and 25%) in petroleum ether will elute vitamin Ks, PQ A, PQ B, and PQ C + D. Further purification can be achieved by recrystallization on alumina, or the PQ C- and PQ D-containing fractions may be purified on thin layer Silica Gel G-HR.

The six fractions were evaporated to dryness, taken up in 5 ml of heptane, and spotted on thin layer plates of Silica Gel G-HR to check identity and purity. The six fractions usually still contain some carotene and can be further purified by streaking each on a thin layer Silica Gel G-HR plate, developed with a mixture of heptane and benzene (15:85, v/v) for vitamin Ks or PQ A and PQ B, and with chloroform for PQ C and PQ D. The spectrum of plastoquinone C prepared by this method is identical with the spectrum of PQ A (14). The E680 at 255 mu of our best preparation prepared by this method was 218.

**Chemicals Used**—Chemicals were obtained from the following sources: heptane, Phillips Petroleum (redistilled before use); 2,6-dichloroindophenol and 

## Table I

<table>
<thead>
<tr>
<th>Extraction conditions</th>
<th>Quinone removed</th>
<th>DCI photoreduction activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PQ A</td>
<td>PQ C</td>
</tr>
<tr>
<td>1. 4½ hours at 22° and rinsed once with heptane</td>
<td>76</td>
<td>64</td>
</tr>
<tr>
<td>2. 4½ hours at 22° and rinsed three times</td>
<td>90</td>
<td>80</td>
</tr>
<tr>
<td>3. 4 hours at 22°</td>
<td>88</td>
<td>73</td>
</tr>
<tr>
<td>4. ½ hour at 22°</td>
<td>65</td>
<td>35</td>
</tr>
<tr>
<td>5. 1 hour at 22°</td>
<td>86</td>
<td>47</td>
</tr>
<tr>
<td>6. 1 hour at 22°, washed with water and re-extracted 1 hour at 22°</td>
<td>76</td>
<td>64</td>
</tr>
<tr>
<td>7. 1 hour with heptane followed by 1 hour with petroleum ether at 22°</td>
<td>94</td>
<td>93</td>
</tr>
<tr>
<td>8. 10 min at 22°</td>
<td>42</td>
<td>0</td>
</tr>
</tbody>
</table>

*a All extractions were performed with heptane, except that petroleum ether was used in Extraction 7.
The effects of heptane extraction vary with each batch of dried chloroplasts, with regard to both the relative amount of quinone extracted and the ability of quinones to restore activity. In general, shorter term extraction will remove only a small part of the PQ C and PQ A, and restoration effects are seen only with PQ C. Long or repeated extraction removes more of each of the quinones and induces a requirement for a combination of PQ C and PQ A to restore activity. The results of each extraction cannot be predicted exactly in advance and must be determined by the respective assays. Table I shows the results of a series of extraction experiments with different batches of lyophilized chloroplasts with regard to amount of quinone extracted and the activity lost. In general, loss of activity is proportional to loss of PQ C throughout the range of extraction. On the other hand, up to 40% of the PQ A is extracted by mild treatment without inducing a requirement for PQ A or a significant loss of activity.

**Effect of Quinone Addition to Unextracted Chloroplasts**—There have been reports of stimulation of activity by quinones added to unextracted chloroplasts (5, 15). We have checked the effects of the various quinones used in this study on DCI reduction activity in unextracted chloroplasts. Under the conditions which we have used for restoration by quinones, the addition of quinones to unextracted chloroplasts does not stimulate activity, and actually often tends to inhibit it. It should be noted that the levels of quinone which we find optimum for restoration are lower than levels used by others to show stimulation in unextracted systems.

**Restoration by Single Quinone Addition**—After short term extraction (30 min), plastoquinone C will often restore most of the activity. The results with this short extraction are variable, however, and for more consistent results we have routinely used a 44-hour extraction period. After this longer extraction some quinones, such as PQ C, will partially restore activity, but maximum restoration requires addition of a combination of quinones which will be described in the next section.

The effect of addition of single quinones to long term extracted chloroplasts is shown in Table II. Several quinones show stimulatory effects, including PQ A, PQ C, dimethyl- and trimethyl-benzoquinone, and coenzyme Q6. PQ D, PQ B, tetramethylbenzoquinone, a-tocopherylquinone, and coenzyme Q0 show very little effect. Samples of PQ C were also supplied by Dr. D. R. Threlfall and showed activity similar to PQ C prepared at Purdue.

**TABLE II**

*Effect of single quinone addition on activity of heptane-extracted chloroplasts*

<table>
<thead>
<tr>
<th>Preparations</th>
<th>Additions</th>
<th>Substance</th>
<th>Amount</th>
<th>DCI reduction rate</th>
<th>ug/min/mg chlorophyll</th>
</tr>
</thead>
<tbody>
<tr>
<td>Series 1</td>
<td>Lyophilized</td>
<td>None</td>
<td>0.00</td>
<td>2.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extracted</td>
<td>None</td>
<td>0.00</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extracted</td>
<td>PQ A</td>
<td>0.1</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extracted</td>
<td>PQ C</td>
<td>0.01</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extracted</td>
<td>PQ D</td>
<td>0.02</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>Series 2</td>
<td>Lyophilized</td>
<td>None</td>
<td>0.00</td>
<td>4.88</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extracted</td>
<td>None</td>
<td>0.00</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extracted</td>
<td>PQ A</td>
<td>0.01</td>
<td>1.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extracted</td>
<td>PQ A1,1</td>
<td>0.14</td>
<td>1.83</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extracted</td>
<td>PQ C</td>
<td>0.01</td>
<td>1.92</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extracted</td>
<td>PQ C</td>
<td>0.01</td>
<td>2.36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Extracted</td>
<td>PQ A</td>
<td>0.13</td>
<td>2.36</td>
<td></td>
</tr>
</tbody>
</table>

---

**TABLE III**

*Comparative effect of high level of PQ A and combined PQ A plus PQ C addition on 2,6-dichloroindophenol photoreduction by extracted chloroplasts*

<table>
<thead>
<tr>
<th>Additions</th>
<th>Amount</th>
<th>DCI reduction rate</th>
<th>ug/min/mg chlorophyll</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>PQ A</td>
<td>0.015</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>PQ A</td>
<td>0.15</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>PQ A</td>
<td>0.45</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>PQ A</td>
<td>0.12</td>
<td>1.08</td>
<td></td>
</tr>
<tr>
<td>PQ C</td>
<td>0.014</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>PQ C</td>
<td>0.012</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unextracted chloroplasts</td>
<td></td>
<td>3.50</td>
<td></td>
</tr>
</tbody>
</table>

---

**TABLE IV**

*Combined effects of plastoquinones A and C on 2,6-dichloroindophenol reduction by extracted chloroplasts*

<table>
<thead>
<tr>
<th>Preparations</th>
<th>Additions</th>
<th>Amount</th>
<th>DCI reduction rate</th>
<th>ug/min/mg chlorophyll</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lyophilized</td>
<td>None</td>
<td></td>
<td>2.00</td>
<td></td>
</tr>
<tr>
<td>Extracted</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extracted</td>
<td>PQ A</td>
<td>0.01</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Extracted</td>
<td>PQ B</td>
<td>0.01</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>Extracted</td>
<td>PQ C</td>
<td>0.01</td>
<td>1.10</td>
<td></td>
</tr>
<tr>
<td>Extracted</td>
<td>PQ A</td>
<td>0.1</td>
<td>1.37</td>
<td></td>
</tr>
<tr>
<td>Extracted</td>
<td>PQ C</td>
<td>0.01</td>
<td>1.23</td>
<td></td>
</tr>
<tr>
<td>Extracted</td>
<td>PQ B</td>
<td>0.01</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Extracted</td>
<td>PQ C</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Perhaps the most notable effect in single quinone additions is the fact that it takes 10 times as much PQ A as PQ C to obtain the maximum stimulatory effect representative of each quinone.

Effect of Combinations of Quinones—Further study showed that the stimulations induced by PQ A and PQ C are additive. In any given extraction series, a small amount of PQ A will restore a part of the activity and increased PQ A will not greatly increase the stimulation. A small amount of PQ C in addition to the PQ A will give a further increase in activity (cf. Table III).

The various quinones were then assayed in systems to which PQ A or PQ C had been added to see which showed the PQ A type of stimulation and which showed a PQ C type of effect. The effects of these combinations are shown in Table IV. Among the other quinones which are found in chloroplasts, only PQ B shows a stimulation in the presence of PQ A. A combination of PQ B and PQ C, on the other hand, does not show stimulation. The other chloroplast quinones, such as α-tocopheryl-quinone, vitamin K₁, and PQ D, do not show a stimulatory effect when added along with PQ A.

Some quinones not found in chloroplasts also show an effect similar to that of PQ C. Thus, high levels of coenzyme Q₉ stimulate, and when Q₉ is added together with PQ A the stimulation is increased. In a similar fashion, trimethylbenzoquinone shows stimulation when added together with PQ A but not when added with PQ C.

Relative Effects of Plastoquinone A and C Concentrations—Relatively larger amounts of plastoquinone A than of plastoquinone C are required for maximum stimulation of activity. The effect of increasing concentration of plastoquinone A in the presence of a constant optimum level of PQ C is shown in Fig. 1. Maximum stimulation by PQ A under these conditions is achieved at about 0.1 to 0.2 μmole of PQ A per mg of chlorophyll. This is about the level of PQ A found in intact chloroplasts. On the other hand, the maximum stimulation by PQ C is at about 0.013 μmole per mg of chlorophyll in the presence of optimum levels of PQ A (cf. Fig. 2). This level of PQ C is in the range found in the chloroplast, or perhaps slightly lower. The inhibitory effect of higher levels of plastoquinone A is observed when the PQ A is added in association with the optimum levels of PQ C. In systems where PQ A is added alone, as shown in Table III, we do not see the inhibitory effect. It would therefore appear that the inhibition by PQ A observed in Fig. 1 is an effect of excess PQ A on the ability of added PQ C to reach an effective site.

Properties of Restored System—The DCI photoreductase activity of lyophilized chloroplasts has been compared with the activity found after addition of plastoquinones A and C to the extracted chloroplasts. The rate characteristics, pH optimum, and inhibitor sensitivity of the untreated and restored systems have been compared. In general, the restored activity resembles the original activity.

Rate Characteristics of Untreated and Restored Systems—When the rate of DCI reduction is followed over a period of time in untreated chloroplasts, it shows a regular increase up to 5 min, with a slightly faster rate in the 1st min. The restored system at maximum PQ A and PQ C levels shows similar characteristics, with a little more decay with time. At higher levels of PQ A or PQ C the initial rate may remain high, but the activity declines more rapidly than in the original after 1 min. The rate of DCI reduction over a period of several minutes is shown in Fig. 3.

Effect of pH—The untreated system shows a decrease in the rate of DCI reduction with increasing pH from the optimum at pH 7.0. Assays below pH 7.0 are impractical because of the difficulty induced by color changes of DCI below pH 7.0. The rate in Tris buffer is 50 to 100% higher than the rate in phosphate buffer.

The extracted system shows a more dramatic decline in activity with increasing pH. The rate of reduction is about the same in Tris or phosphate buffer except around pH 7.0, where the restored system is slightly more active in Tris buffer. The restored system has very little activity at pH 8.0 in either buffer.
FIG. 3. Effect of various combinations of plastoquinones A and C on DC1 reduction. Time indicates exposure to light. △-△, lyophilized chloroplasts; O—O, extracted chloroplasts plus 0.01 pmole of plastoquinone C and 0.13 pmole of plastoquinone A per mg of chlorophyll; ●-●, extracted chloroplasts plus 0.01 pmole of plastoquinone C and 0.83 pmole of plastoquinone A per mg of chlorophyll; ▲-▲, extracted chloroplasts plus 0.1 pmole of plastoquinone C and 0.83 pmole of plastoquinone A per mg of chlorophyll; ○-○, extracted chloroplasts, no addition. Ordinate is rate of reduction of DC1 in micromoles per min per mg of chlorophyll. The 3 ml reaction mixture contained 0.18 μmole of chlorophyll; O—O, extracted chloroplasts, no addition. Or-

The preparations suspended in sodium chloride were then aged in sodium chloride as described under "Methods and Materials." None of the lyophilized chloroplasts was used in these experiments. Heptane was then evaporated and the dry chloroplasts with the respective plastoquinones added were taken up in sodium chloride as described under "Methods and Materials." The preparations suspended in sodium chloride were then aged in an ice bucket in a refrigerator at 0°C.

<table>
<thead>
<tr>
<th>Additions</th>
<th>DC1 reduction activity</th>
<th>Amount (μmole/mg chlorophyll)</th>
<th>After 48 hours (% loss)</th>
<th>After 72 hours (% loss)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PQ A</td>
<td>0.10</td>
<td>1.12</td>
<td>0.22 (77)</td>
<td>0.22 (77)</td>
</tr>
<tr>
<td>PQ C</td>
<td>0.01</td>
<td>1.37</td>
<td>0.76 (48)</td>
<td>0.50 (57)</td>
</tr>
</tbody>
</table>

* In micromoles per mg of chlorophyll.

**Effect of Inhibitors**—The activity of both untreated and restored systems is inhibited by 10^{-5} M p-chlorophenol, 1,1-dimethyleurea. Relatively high levels of salicylaldehyde have also been shown to inhibit photoreductase activities (9). We find that both the untreated and restored systems are inhibited to the same extent by increasing concentrations of salicylaldehyde. Complete inhibition in both systems is attained at 10^{-3} M salicylaldehyde.

**Effects of Reduced PQ A**—Maximum restoration of TPN and ferricyanide photoreduction by reduced PQ A has been described by Wood and Crane (11). When reduced PQ A is added to the chloroplasts extracted for 4 hours it shows greater restoration of DC1 photoreduction than when PQ A is added. The reaction is not further stimulated by addition of PQ C. On the other hand, the amount of reduced PQ A required to obtain a high level of activity is 10 times greater than the amount of PQ C needed to achieve a similar increase over the level of activity produced by PQ A alone. These experiments are complicated by the fact that we do not know the amount of reduced PQ A that reaches the effective sites in the chloroplasts. Further studies of the role of reduced PQ A as compared to PQ C would be desirable. It should be noted that PQ C does not replace reduced PQ A in the ferricyanide or TPN systems described by Wood et al. (10).

**Effects of Other Quinones with Hydroxylated Side Chains**—Das et al. (13) have shown that PQ C contains a terpenoid side chain with a single hydroxyl group which is probably in the second isoprene unit from the quinone ring. This is an unusual position for the hydroxyl group as compared to terpenoid quinones formed by oxidation of the corresponding chromanol, in which the hydroxyl group is γ to the ring, as for example in the tocopherylquinones. We have already shown that α-tocopherylquinone is inactive in replacing PQ C. Actually a better comparison is found in γ-tocopherylquinone, since, like PQ C, it is trisubstituted. Another quinone of similar type has recently been described by Dunphy, Wittle, and Pennock (16) as the γ-hydroxy-
PQ A derived from the chromanol of PQ A which they found in rubber trees. Dr. Pennock has kindly provided a sample of this quinone. Although it has a hydroxyl group in the side chain it does not show activity equivalent to PQ C. Dr. Rita Barr in this laboratory has extracted a material from rubber tree leaves which appears to be plastochochromanol as described by Pennock. Oxidation of this material with gold chloride provides a quinone with an absorbance maximum in ethanol at 258 μg. This quinone, however, did not show the same chromatographic properties as the quinone obtained by Pennock by oxidation of synthetic plastochochromanol. The quinone prepared by Barr showed only slight activity at the level required for a PQ C effect, and did not cause significant extra activity when added with PQ A.

**Effect of Aging on Restored Preparations**—The best activity in extracted preparations is achieved if assays are performed immediately after extraction. If the extracted chloroplasts are resuspended in NaCl and stored at 0°C for several days, there is an abrupt loss of activity which tends to hold at the new low level. If PQ A or PQ C is added before the extracted chloroplasts are resuspended, the loss of activity is less abrupt. PQ A seems to prevent loss of activity better than PQ C, as is shown in Table V. The protective effect of PQ A against loss of activity on aging has been observed in several experiments. It should be pointed out that the relative restoration by PQ A and PQ C will appear to vary if assays are run at different times. After aging it usually appears that PQ A has restored more activity than if the assays are conducted immediately after extraction.

**DISCUSSION**

The experiments described were difficult to perform because of the need to handle the lyophilized and extracted preparations...
rapidly to avoid excessive water uptake and because of the need
for assays as soon as possible after extraction to avoid the decline
of activity with aging. In spite of the difficulties, certain con-
sistent patterns appear which seem to suggest a specific role for
PQ C in the chloroplast electron transport system which leads to
reduction of DCI.

The relation between the amount of PQ C removed and the loss
of activity gives the first indication that the PQ C site is actually
a rate-limiting site in chloroplasts. On the other hand, a part
of the PQ A can be removed by mild extraction without a cor-
responding decline in DCI reduction activity. After mild ex-
traction PQ C usually shows more stimulation than PQ A, which
again indicates that PQ C is limiting the reaction and that PQ A
is present in excess. Only after more drastic extraction does the
requirement for PQ A begin to become apparent, and at this
time the combined effects of PQ A and PQ C are seen.

The response of the DCI system to extraction as carried out
in these experiments is in contrast to the response of the ferricya-
nide photoreduction activity reported by others. Both Bishop
(1) and Redfearn (5) have shown that with highly active chloro-
plasts the decline of ferricyanide reduction activity is propo-
tional to the removal of PQ A and that the extracted system is
restored by PQ A alone. The implication in the differences
observed is that the ferricyanide system involves a different set
of carriers or that ferricyanide reacts with the electron transport
system at a different site from DCI.

Trebst et al. (9) have previously reported that the responses of
TPN photoreduction and ferricyanide photoreduction activities
to extraction differ. Thus ferricyanide reduction was lost
immediately with loss of less than 70% of PQ A, whereas TPN
reduction was lost only after most of the PQ A was removed.

Wood et al. (10) have also found that ferricyanide reduction and
TPN reduction are lost upon mild extraction of PQ A and that
activity can be partially restored by addition of PQ A. PQ
C did not restore any activity in short term extraction systems,
which would probably correspond to the short term extractions
described in this paper except that the extractions which Wood
et al. describe were carried out at 0°.

Redfearn (5), Shavit and Avron (15), and Krogmann and
Olivero (2) have reported stimulatory effects of quinones on
unextracted chloroplasts. The amount of quinone required to
cause the stimulation is much larger (0.27 as opposed to 0.10 mg
per mg of chlorophyll) than the amounts we have used; thus we
do not feel that the restorations we observe can be based on
quinone stimulation of the residual rate. We also do not find
any stimulation of the DCI reduction rate in fresh or lyophylized
chloroplasts with the amounts of quinone used for restoration.

Redfearn (5) has reported greater stimulation of ferriyanide
reduction by 2,3,5-trimethylbenzoquinone than by plasto-
quinone A. The effect which he observes may be similar to the
effect of 2,3,5-trimethylbenzoquinone which we observe. Since
it appears from our combination studies of 2,3,5-trimethyl-
benzoquinone and PQ A that the former is acting at the site of PQ
C or is in some other way replacing PQ C, Redfearn’s experiment
may indicate a site for PQ C function under certain conditions on
the pathway to ferriyanide.

One of the most striking effects in these experiments is the
small amount of PQ C required for maximum stimulation as
compared to the amount of PQ A required for maximum effect.
The proportion of 0.1:0.013, PQ A:PQ C, is reasonably close to
the ratio 0.1:0.03, PQ A:PQ C, found in intact chloroplasts, and
suggests that the amount of PQ C required in the chloroplast
electron transport system is significantly less than the amount of
PQ A. One must suspect, in view of the losses to be expected
with the addition procedure, that the effective amount of PQ C
is less than the 0.013 amole per mg of chlorophyll added.

The PQ C effect is quite specific in relation to the other
quinones actually found in chloroplasts. The major quinones in
chloroplasts are PQ A, PQ C, α-tocopherylquinone, and vitamin
K1. Of these, PQ C is the only one which shows added stimula-
tion of the indophenol system when added with PQ A. The
other quinones, which are usually found in minor amounts, are
PQ B, PQ D, and β- and γ-tocopherylquinones. Of these, PQ B
shows an effect similar to that of PQ C, but the relative amounts
of the two would favor PQ C as the functional element unless
there is an interconversion between these two closely related
quinones.

In previous studies we have presented evidence of a stimulatory
effect of PQ C for DCI photoreduction by acetone-extracted
chloroplasts (12). The present results are consistent with the
earlier studies. Furthermore, Mr. Kyle Fink in this laboratory
has found that PQ C shows maximum effect in the acetone-
extracted chloroplasts at a level, 0.015 μmole per mg of chloro-
phyll, similar to the maximum levels found in these experiments.

The acetone extraction experiments also showed a combined
effect of PQ C and PQ A for TPN reduction and a requirement
for PQ C for reduction of TPN when reduced DCI is used as an
electron donor in the presence of p-chlorophenyl-1,1-dimethyl-
urea to prevent transfer of electrons from water. Similar types
of experiments with the heptane-extracted chloroplasts will be
desirable in locating the site of PQ C function.

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