In an earlier paper (1) it was reported that etiolated barley seedlings when exposed to visible radiation suffer a large decrease in catalase activity and a simultaneous increase in chlorophyll. Eyster (4) found that dark-grown genetic albino corn seedlings, completely devoid of chlorophyll, also suffer a loss in catalase activity when illuminated. There are several earlier studies such as Knott (7) that report the effects of light on the catalase activity of growing plants. In all these studies light of the whole visible spectrum was used. Since it is apparent that the decrease in catalase involves a photochemical reaction, it is reasonable to assume that certain regions of the spectrum may be more effective than others. We initially planned to determine the action spectrum for this process, but difficulties involved in obtaining light of relatively narrow spectral bands of sufficient intensity forced use to confine our studies for the present to red and blue. This paper reports the results of these studies.

MATERIALS AND METHODS

Barley, variety Atlas, was used in most of the experiments described in this paper. The seeds were soaked overnight, placed on stainless steel screens fitted into 400-ml beakers (fig 1), and kept in a saturated atmosphere in a dark room. Within six days the seedlings had attained a height of 4 to 6 cm; two lots were now removed from the dark chamber and placed in special chambers irradiated with red and blue light respectively. A descriptive sketch of the light chamber is given in figure 2. The sources of radiant energy were fluorescent tubes GE F14T12. The characteristics as given by the manufacturer are as follows: the red tubes do not emit any light shorter than 6000 Å and have a maximum emission at 6600 Å. The blue tubes have a maximum at 4400 Å and extend partially into the green to the region of about 5000 Å. Measurements were made of the irradiance as well as the total energy of the fluorescent tubes. In the center of the chamber eighteen red tubes gave a total irradiance of 180 fc and a total energy of 0.0308 cal/cm²× min (2.15×10⁴ ergs/cm²× sec). Since 18 tubes are the maximum number that our chamber can accommodate, it was necessary to adjust the blue to the same total radiant energy by using a smaller number of tubes and/or colored glass filters. The temperature in the chambers was generally maintained at 24 ± 1°C unless otherwise stated.

After the plants were placed in the light chambers samples were periodically taken by cutting off the upper 2/3 of the first leaf blades. Weighed portions of these samples were used for determinations of dry weight, nitrogen, chlorophyll, and catalase. The methods used in these determinations are described elsewhere (1, 2). We define the catalase unit as the amount of catalase required to liberate from H₂O₂ one ml O₂/sec at 0°C, under our experimental conditions. Several other plants were used to compare with barley, also some other determinations were made in addition to those mentioned. These are described in their proper places.

RESULTS

Typical effects of red and blue light are presented in figures 3 and 4. A comparison shows that percentage dry material and total nitrogen, calculated on a fresh weight basis, are approximately the same under both types of irradiation. In fact, the changes in both of these were very slight during the 8-day period that they were studied. The chlorophyll accumulates at approximately the same rate in red and blue grown plants during the first 2 days. After that there is no further accumulation in the plants grown in red light; however, the plants grown in blue light continue to accumulate chlorophyll until the 4th day, when a maximum about 33% higher than that in red light is reached.

Catalase activity is high, initially, in the etiolated seedlings, and, as has been shown earlier (1) in the case of etiolated seedlings exposed to white light, there is an initial drop in activity. We find here that in plants grown in red light the decrease is small, roughly about 15% and is reversed after 24 to 48 hours. In plants grown in blue light the decrease is large, about 70%, and reaches a minimum activity in 4 to 5 days of irradiation. It should be noted that under both types of irradiation the minimum in catalase activity coincides with the most rapid chlorophyll synthesis.
and catalase activity begins to increase again only after chlorophyll has reached its maximum.

The surprisingly high catalase activity of the red grown plants and the low value to which catalase drops in the blue grown plants prompted us to investigate whether these quantitative relationships could be reversed. An experiment was set up, therefore, to investigate this possibility.

Two lots of 5-day-old etiolated barley seedlings were placed in the chambers under the red and the blue light respectively. A third lot was continued in the dark. After four days several beakers of red

![Cross section of light chamber.](image)

**Fig. 2.** Cross section of light chamber. Outside dimensions 24" × 24" × 25" high. The space for plants is 14" × 24" × 18" high. A—Exhaust fan, capacity 35 ft³/min. B—Walls of chamber made of ¾" marine plywood. C—Light panels which are interchangeable (1—Light shield to prevent light from reaching slots in the mounting board, 2—Fluorescent tubes). D—Perforated ¾" copper tubes running the length of the chamber, infrequently used to blow in air. E—Air intake from the outside. F—Adjustable slot to permit the incoming air to go through the inner chamber. The outlets for this air are located in front and back of the frame holding the upper filter. G—¾" slots for glass filter.

irradiated plants were placed in the dark and several in blue light. Also, plants which had been irradiated for four days in the blue chamber were likewise placed in red and in darkness respectively. The results of this experiment are shown in figure 5. It is apparent from the figure that the effect of light quality on catalase activity is rapidly reversible. Catalase activity is rapidly increased in red light and equally rapidly decreased by blue light. It is interesting that the blue plants placed in the dark, while increasing somewhat in catalase activity, did not reach the same high value as those placed in the red. This is contrary to the

![Graphs](image)

**Fig. 3.** Plants were grown for 5 days in dark room then placed in the red light chamber.

![Graphs](image)

**Fig. 4.** Plant grown for 5 days in dark room and placed in blue light chamber.

notion that plants grown in red light are similar to plants grown in the complete absence of light.

We next attempted to determine whether the previously described light effects were purely photochemical and therefore independent of temperature. By working in a room maintained at 0° C, we were able to keep the temperature inside the light chambers at 0° to 2° C. Plants kept in the dark were outside the chambers at a temperature that varied little from 0° C. Six-day-old etiolated seedlings were placed in the chambers and irradiated for four days. Catalase activity at this time was as follows: plants grown in dark 1.8 units/gm of fresh weight, plants grown in red light 2.0 units, plants grown in blue light 1.7 units. Comparable plants irradiated at 24° C for four days gave activities as follows: dark 2.2 units, red 2.3 units and blue 0.5 units. It is apparent that temperature does influence the light effects.

Talaricio (14) in 1909 reported that crude liver
catalase when exposed to blue light suffered a loss in activity, while when exposed to red light it either remained constant or showed a slight increase in activity. Murakami (8) studied the effect of monochromatic light on a number of enzymes, including catalase, in autolyzed yeast and found an increase in activity of catalase in the blue light greater than in red light. We thought, therefore, it was desirable to check the effect of the light on the tissue homogenates. We exposed homogenates of barley leaves and also of avocado fruit tissue, which is very high in catalase, to red and blue light for periods varying from one hour to four days. No change in the catalase activity was produced as compared to samples kept for the same period of time in complete darkness. Since our experimental results do not agree with those of Talario and Murakami, we must conclude that our observed photochemical reactions required organization of the cell and are probably part of a complex system in which other living processes are involved.

This consideration prompted us to investigate other physiological and biochemical changes that occur in plants when they are irradiated with red and blue light. We do not wish to imply that the changes reported below are necessarily, either directly or indirectly, related to the changes in catalase activity. The values for catalase given along with the other data are simply to indicate that the irradiation was effective in producing some change.

Nitrogen: There are some inferences, in the literature dealing with morphological effects of irradiation, that the blue end of the spectrum accelerates protein synthesis in plants. Voskresenskaya (15) in a recent paper concludes that the blue-violet end of the spectrum favors protein synthesis, while the longer wave lengths favor the synthesis of carbohydrates. In this it was of interest to see whether a relatively short period of irradiation with red or blue light produces a detectable effect on the protein content of barley seedlings.

Representative samples of 10-day-old barley leaves which had been exposed for 4 days to either red or blue light were subjected to a nitrogen fractionation by the method of Bathurst (3). The results given in Table I indicate that the blue irradiated leaves have a smaller amount of free amino acids than the red irradiated leaves but a larger amount of protein. The higher total nitrogen in the dark grown leaves does not indicate a higher nitrogen per leaf but a lower fresh weight per leaf. It appears that red light makes for greater growth in length, and blue light for greater protein synthesis. Admittedly this study by itself is inadequate to establish this observation. However, this view gains support from many total nitrogen determinations (Kjeldahl) on a per plant basis which show that blue irradiated leaves have a consistently higher total nitrogen than leaves of the same age irradiated with red light or grown in the dark. The data on pea epicotyls given in Table VI lend additional support.

Ascorbic Acid: It is well known that reducing substances such as glutathione, cystein and ascorbic acid have an inhibitory effect on catalase activity in vitro (5). It is also known that accumulation of ascorbic acid in seedlings is affected by light (11). It was thought desirable to determine whether there are differences in ascorbic acid in red and blue irradiated seedlings. Ascorbic acid (AA) was determined by the method of Ramsey and Colichman (10). The ascorbic plus the dehydroascorbic acid was determined by the

Table I

<table>
<thead>
<tr>
<th>Nitrogen Fraction</th>
<th>Blue</th>
<th>Red</th>
<th>Dark control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amino acids and amides</td>
<td>0.43</td>
<td>0.54</td>
<td>1.07</td>
</tr>
<tr>
<td>Polypeptides</td>
<td>0.19</td>
<td>0.19</td>
<td>0.14</td>
</tr>
<tr>
<td>Proteins</td>
<td>2.90</td>
<td>2.62</td>
<td>2.81</td>
</tr>
<tr>
<td>Total N</td>
<td>3.52</td>
<td>3.35</td>
<td>4.02</td>
</tr>
<tr>
<td>Catalase units/gm fresh wt</td>
<td>1.08</td>
<td>2.22</td>
<td>2.27</td>
</tr>
</tbody>
</table>

Table II

<table>
<thead>
<tr>
<th>Light</th>
<th>Ascorbic Acid (mg/gm fresh wt)</th>
<th>Catalase units/mg N</th>
<th>Catalase units/gm fresh wt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AA + DAA</td>
<td></td>
</tr>
<tr>
<td>Blue</td>
<td>0.19</td>
<td>0.27</td>
<td>0.27</td>
</tr>
<tr>
<td>Red</td>
<td>0.06</td>
<td>0.11</td>
<td>0.61</td>
</tr>
<tr>
<td>Dark</td>
<td>0.10</td>
<td>0.17</td>
<td>0.49</td>
</tr>
</tbody>
</table>
same method on an aliquot of leaf homogenate through which \( \text{H}_2\text{S} \) had been bubbled. The results of a typical determination are given in table II. There is definitely a higher ascorbic acid content in blue irradiated leaves as compared to either dark or red. However, adding varying concentrations of AA to the culture solution of red irradiated seedlings or spraying the leaves with dilute solutions of AA did not influence the catalase activity of the leaves.

**Hill Reaction:** The photolysis of water by isolated chloroplasts is known to be cyanide insensitive. It, therefore, has been assumed that catalase does not directly participate in it. There remained, however, the possibility that the chloroplasts of plants irradiated with red and blue light may have other changes produced in them which do affect their Hill reaction capacity.

The method we used for both chloroplast preparation and measurement of their capacity for photoreduction was essentially that of Spikes (13). The Hill reagent used was 0.005 M potassium ferricyanide. Red light of approximately 1500 \( \mu \) was supplied from a 300-watt reflector spot lamp transmitted through a double thickness of cellophane. The reaction temperature was maintained at 10°C and a Beckman model G pH meter was used as a potentiometer.

Admittedly our setup was crude, to the very elegant apparatus of Spikes. The results, however, were consistent and reproducible. Typical Hill reaction results are summarized in table III. In all the plants studied the chloroplast materials from red irradiated plants are only 60 to 70% as efficient in photoreduction of ferricyanide as are their blue counterparts. The following additional information on the plant material used in Hill reaction studies given in table III should be noted. The barley plants varied in age from 8 to 14 days and had been exposed to red and blue light for 4 days. The corn was a dwarf variety 10 days from seed when used and had had 4 days of red or blue irradiation. The tobacco plants were grown in soil in a glasshouse for about 6 weeks, and subsequently irradiated in the light chambers for 6 days prior to their use; only the young leaves which had expanded in the light chamber were used. Experiment 5, table III was performed as follows: Two sets of 6-day-old plants were placed in each of the two chambers. After 4 days one set from each chamber was removed and placed in the other chamber. Thus B→R designates a plant that was in the blue chamber 4 days and was then shifted to the red chamber and similarly for R→B. After another 4 days the plants were harvested and used for Hill effect determinations. It appears that the exposure of a plant to blue light produces an irreversible effect with respect to the Hill reaction. This effect is not changed by exposing the plant to red light either prior or subsequent to its exposure to blue light. The catalase activity values given in this table are just to indicate the degree to which the plant has reacted to the irradiation.

**Experiments on Barley Coleoptiles:** So far we have considered the effect of red and blue light on leaves. Because of the general interest in coleoptiles as a light receptive tissue, it was thought desirable to determine how their catalase activity is affected by similar irradiation.

Barley seedlings were grown in the dark room as previously described. Four to six days after planting, between 50 and 100 coleoptiles were detached from the seedlings in the dark and placed in light-proof aluminum weighing cans, quickly weighed and transferred to Petri dishes on moist filter paper. The coleoptiles, in the covered dishes, were placed in the red and blue light chambers or in darkness for predetermined periods of time. The coleoptiles from each dish were homogenized and the various determinations made on the homogenate. In a number of experiments the intact seedlings were irradiated and the coleoptiles detached after the period of irradiation. These are referred to in table IV as “attached.” It is apparent from the results that catalase activity in the

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**Table III**

**Hill Reaction Determinations on Chloroplasts from Comparable Blue and Red Irradiated Plants**

<table>
<thead>
<tr>
<th>Expt. No</th>
<th>Plant and Color of Light</th>
<th>Chlorophyll in Sample, mg</th>
<th>Microequivalents of Fe**+** Reduced/mg Chlorophyll Per Minute</th>
<th>Catalase Units in Plant Tissue Susp/0.10 mg Chlorophyll</th>
<th>Catalase Units in Leaf/0.10 N</th>
<th>Relative Hill Reaction R/B</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Barley B</td>
<td>1.75</td>
<td>0.974</td>
<td>0.124</td>
<td>...</td>
<td>0.59</td>
</tr>
<tr>
<td></td>
<td>Barley R</td>
<td>1.82</td>
<td>0.577</td>
<td>0.038</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>2</td>
<td>Barley B</td>
<td>1.60</td>
<td>0.938</td>
<td>0.165</td>
<td>...</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>Barley R</td>
<td>1.98</td>
<td>0.606</td>
<td>0.766</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>3</td>
<td>Corn B</td>
<td>2.66</td>
<td>0.624</td>
<td>...</td>
<td>0.09</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>Corn R</td>
<td>2.36</td>
<td>0.435</td>
<td>...</td>
<td>0.18</td>
<td>0.69</td>
</tr>
<tr>
<td>4</td>
<td>Tobacco B</td>
<td>1.25</td>
<td>1.40</td>
<td>...</td>
<td>0.23</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>Tobacco R</td>
<td>0.68</td>
<td>0.85</td>
<td>...</td>
<td>0.66</td>
<td>0.60</td>
</tr>
<tr>
<td>5</td>
<td>Barley B</td>
<td>2.60</td>
<td>1.44</td>
<td>0.18</td>
<td>0.55</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>Barley R</td>
<td>2.00</td>
<td>0.98</td>
<td>0.63</td>
<td>0.72</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>Barley B→R</td>
<td>2.22</td>
<td>1.39</td>
<td>0.58</td>
<td>0.64</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>Barley R→B</td>
<td>2.58</td>
<td>1.34</td>
<td>0.21</td>
<td>0.43</td>
<td>...</td>
</tr>
</tbody>
</table>
dark grown coleoptiles is reduced to approximately half when exposed to blue light for four hours but is very slightly influenced by exposure to red light. The data in table IV are average results of four experiments in our regular light chambers. In addition we performed many experiments with detached coleoptiles using high intensity incandescent lights and Corning glass filters to obtain the desired light intensity but a higher degree of spectral purity. The primary purpose of these experiments was to ascertain whether the effect of blue light on catalase was not due to the small amount of “far-red” (7400 Å) which the blue fluorescent tubes emit. To eliminate the far-red, blue filter No. 42 from a Klett-Summerson photo-colorimeter was used. It transmits very little light of the far-red region. To obtain the far-red, Corning filter No. 5574, which transmits the far-red and also a band in the violet, was used. The results of these experiments have satisfactorily shown that the observed effect on catalase by the use of blue fluorescent tubes is due to the blue light and not to the small amounts of far-red.

It is worth pointing out the advantages of using the barley coleoptile for this type of research. The complication of the presence or formation of chlorophyll is eliminated, since the quantity involved is insignificant. The time required to produce a change in catalase is relatively short. Also, the experimental manipulation is much simpler than with whole seedlings.

Effects of Red and Blue Light on Plants Other Than Barley: For most of our work barley seedlings were the experimental material. We have, however, made many determinations on other plants. Some of these we feel are worth recording for the information of those who may wish to work with other plants. In general, all the grain seedlings we used (oats, corn, wheat) behave like the barley. With respect to the dicotyledonous plants, table V shows catalase data on several species from two families. Since seedlings of dicotyledonous plants do not form leaves when etiolated, the plants were grown in a glasshouse until they developed several normal mature leaves. They were then placed in the light chambers for 3 to 6 days and catalase determinations were subsequently made on the leaves. It is evident that the catalase activity in the two species of Solanaceae is affected by the monochromatic light in the same way as the grains. The Leguminosae, on the other hand, react in the opposite manner.

In view of these observations, we set up an experiment on pea seedlings. Alaska peas were soaked for 4 hours, then planted in moist vermiculite in 400-ml beakers. The seedlings were left to grow in complete darkness for four days. By this time they were 5 to 6 cm high. Part of them were now removed from the dark room and placed in the red and blue chambers. After 2 days of irradiation the seedlings were cut at the surface of the vermiculite and subjected to the regular analyses for catalase and nitrogen. Subsequently other samples were taken after 4 days’ irradiation. The results given in table VI indicate some striking differences from the barley seedlings. The catalase activity and the total nitrogen in the dark grown seedlings are very much lower than in either the red or the blue irradiated seedlings. Also the catalase activity differences between plants grown in red and blue are much smaller and appear to be decreasing with continued irradiation and age. Actually the leaves of the young plants exposed to blue light have a higher catalase activity than those exposed to red. It seems evident that the catalase picture in pea epicotyls is more complex and subject to more rapid changes than that of barley. The whole subject of catalase in legumes shows promise of an interesting study.

Discussion

From the experimental results presented it is clear that the loss of catalase activity in etiolated barley seedlings, when exposed to visible light, is due to the light of the blue end of the spectrum. Also this decrease in activity may be rapidly restored by irradia-
tion with light of the red end of the spectrum. Neither the reduction of activity by blue light nor the restoration, or actual increase, in activity by red light takes place in the tissue homogenate. This indicates that grinding the tissue produces changes either in the receptor pigment or in the thermal components of the system. At present no attempt has been made to identify the receptor pigment. It certainly is not chlorophyll, since non-chlorophyllous tissues such as barley coleoptiles and *Prototoca zopha* (author's unpublished work) are equally affected. It may possibly be catalase or some other heme compound. We do not think it likely that leaves of leguminous plants have a different receptor pigment than the other plants studied. Their different reaction to red and blue light we are inclined to ascribe to the thermal rather than the photochemical phase of the reaction.

The other effects of red and blue light, such as the effect on the Hill reaction, indicate that other enzymes may also be changed by light from these regions of the spectrum. This presents an interesting experimental approach to other plant physiological problems.

The initial drop in catalase on irradiation of etiolated barley seedlings with light of any part of the spectrum, we think, is significant. The initial decrease of catalase in the red grown plants is relatively small. We have, therefore, checked it many times, and we are convinced that the decrease is indeed real. The maximum drop in catalase under all light conditions used coincides with the period of most active chlorophyll synthesis. This is in agreement with other instances known where active synthetic activity in an organism or tissue is accomplished or preceded by a decrease in catalase activity. It is well known, for example, that the liver and kidney catalase of a tumor host is greatly reduced during the period of most rapid tumor growth and is very rapidly restored on excision of the tumor. Similarly, there is a drop in catalase activity in rats during pregnancy. Also, the embryonic liver of rats is very much lower in catalase than the adult liver. Pope (9) studied catalase in barley throughout the life cycle for three successive years. He concludes, "Catalase activities are roughly proportional to the reciprocal of growth rate, being, in general, lowest during the stages of most active growth, as measured by length and deposition of dry matter." Unfortunately most of the work on catalase in plants that one finds in the literature is not accompanied by sufficient data on the physiological state of the tissue or organism to permit further deductions.

It is not known, at present, what actually happens to the catalase in the living cell, when a determination of catalase activity on the cell homogenate indicates a decrease. Does it represent a major breakdown of the whole molecule or a small, reversible change, perhaps, concerned with the linkage of the prosthetic group to the protein? Such information would be very helpful in explaining some of our observations. But, whatever the change in activity may be due to, it is reasonable to assume that a change in catalase activity is reflected in an opposite change in the H$_2$O$_2$ concentration in the cell. The hydrogen peroxide, because of its capacity to initiate free radical formation, may play an important role in biosynthetic processes. In this regard our data are in harmony with the speculations of Rondoni and Cudkowicz (12) on the indirect function of catalase in protein synthesis. Another aspect of growth over which catalase may have an indirect control, is illustrated in the scheme for indole-acetic acid oxidase as proposed by Galston and co-workers (6). We appreciate the fact that catalase controls only the decomposition of H$_2$O$_2$ and that factors involved in the formation of peroxide must also be taken into consideration. We simply wish to suggest that catalase, because of its control on H$_2$O$_2$ concentration in the cell, may be exerting a powerful control over several physiological processes.

**Summary**

Changes in catalase activity in plants, caused by irradiation for short periods of time with red or blue light, were studied. It was found that:

1. In etiolated barley seedlings and other grains, catalase activity is greatly depressed by blue light and elevated by red light. This is also true for young tomato and tobacco plants grown in normal daylight and illuminated with colored light for 3 to 6 days.

2. Catalase in legumes (beans, peas and clover) was increased by irradiation with blue light and decreased by red light.

3. Quantitative changes, due to red or blue light, in the Hill reaction and in ascorbic acid and protein content are reported.

4. The probable significance of catalase in biosynthetic processes is discussed.

**Literature Cited**


Mitochondria prepared from broccoli buds by the usual procedure, using 0.5 M sucrose as the homogenizing medium, showed poor activity. The rate of oxidation of succinate rapidly declined with time and there was virtually no oxidation of a-ketoglutarate. Several workers, with both plant (10, 16) and animal (3, 14) material, reported that disodium EDTA (disodium ethylene-diamine tetra-acetic acid) added to the preparative medium activated and stabilized mitochondrial oxidations. This type of activation was obtained with mitochondria from broccoli buds. The object of the present study was to determine the effectiveness of EDTA on broccoli mitochondria and to obtain clues as to the mechanism of activation.

**Materials and Methods**

With some modifications the method outlined by Laties (9) was used to prepare the mitochondria. In the preparative procedures all manipulations were carried out at 0° C. The broccoli buds were cut off at the flower stalks and 12 gm were ground in a mortar with 20 gm of fine acid-washed sand and 25 ml of either 0.5 M sucrose or 0.5 M sucrose containing 0.01 M EDTA. Additional 10-ml portions of preparative medium were added to aid in homogenizing the tissue. Finally the homogenate was diluted with the same medium to make a total volume of 90 ml containing the 12 gm of tissue.

The resulting homogenate was squeezed through four layers of cheesecloth and the filtrate centrifuged at 1000 × g for 5 minutes to precipitate pollen grains, unbroken cells, nuclei, cell debris and sand. The supernatant was then centrifuged at 17,000 × g for 15 minutes. The second precipitate, which yielded the mitochondrial pellet, is described as "once washed". A "twice washed" preparation was one in which the pellet was resuspended in 20 ml of 0.5 M sucrose and homogenized in the centrifuge tube with a snug-fitting lucite pestle. The resuspended particles were spun down at 17,000 × g for 15 minutes. This procedure was repeated once more to give a "thrice washed" preparation. After all washes the final mitochondrial pellet was suspended in 4 ml of 0.5 M sucrose and homogenized with a motor-driven Teflon pestle.

The oxidative capacity of the mitochondria was determined by their ability to oxidize a-ketoglutarate or succinate. This was assayed at 25° C by standard Warburg manometric techniques. Details as to the content of the reaction mixtures are given in the legend for each figure.

Each experiment was replicated at least 3 or 4 times and the data are averages of these experiments. The data are reported in microliters oxygen per milligram nitrogen per hour designated as QO2 (N).

**Results**

**Activation and Stabilization Effect of EDTA:** The effect of isolating mitochondria in 0.5 M sucrose containing 0.01 M disodium EDTA and in 0.5 M sucrose is shown in figures 1 and 2. With a-ketoglutarate as substrate the preparation without EDTA showed very low activity (QO2 (N) of approximately 40). The preparation with EDTA exhibited a fourfold increase in oxidation (QO2 (N) of approximately 160), which was maintained for at least 2 hours. A similar activation was shown with succinate as a substrate (fig 2). The most striking effect, especially with succinate, was the stabilization of activity for at least a 2-hour period.

**Effect of Washing on the Activation Effect of EDTA:** The purpose of the washing experiments was to reveal whether the activation affected by preparation in EDTA could be reversed by removal of the EDTA. In these experiments the activity of EDTA-prepared mitochondria washed once was compared with preparations washed twice and thrice with 0.5 M sucrose. Removal of EDTA by washing did not.

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