TYROSINASE AND PLANT RESPIRATION*

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It appears reasonable to assume that plant oxidases, such as tyrosinase[1] take part in the respiratory process in plants. This view, however, appears not to be accepted by all workers in this field. Thus Szent-Györgyi and Vietorisz (1) have suggested that possibly the oxidase occurs dormant in the plant tissue and only becomes active when the tissue is injured. At such a time, it brings about the oxidation of phenolic bodies, present, in the plant, to quinones, and the latter in turn not only act in a bactericidal capacity, but also combine with protein, forming an insoluble coating over the injured tissue.

Recently Boswell and Whiting (2) have attempted to show that the oxidase in potato tubers really does play the rôle of a respiratory enzyme. They studied by means of the Warburg respirometer (3) the rates of oxygen uptake and evolution of carbon dioxide when thin potato slices were permitted to respire in the presence of water buffered with phosphate (pH about 5.5). In this way they found that the rates of oxygen uptake and evolution of carbon dioxide remained practically constant for several hours, and the respiratory quotient was close to unity. On the addition of catechol, there was a sudden marked rise in the rate of oxygen uptake. This increased rate, however, was only of short duration and was followed by a gradual drop, culminating finally in a value of about one-third of the rate shown by the slices respiring in presence of phosphate only.

Adding more catechol to the reaction mixture, after this final low respiration rate had been reached, gave no further new increase in the rate of oxygen uptake, showing that all the oxidase had become exhausted or inactivated. They, therefore, attributed this lowering of the respiration rate to one-third of the normal rate in phosphate alone, to the inactivation of the oxidase, and concluded that two-thirds of the respiration of the potato slices was dependent on the oxidase present in the slices.

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[1] There is a difference of opinion as to the name for this oxidase, usually prepared from potato tubers and from certain varieties of mushrooms. Many workers use the terms polyphenol oxidase and catechol oxidase. The present authors prefer to adhere to the original name, tyrosinase, proposed by Bertrand (Sur une nouvelle oxydase, on ferment soluble oxydant, d'origine végétale, *Compt. rend. Acad. sc.*, 1896, **122,** 1215) due to the fact that this enzyme differs from other oxidases, in that besides catalyzing the aerobic oxidation of polyhydric phenols, it also catalyzes the oxidation of certain monohydric phenols. The terms polyphenol oxidase and catechol oxidase fail to emphasize this characteristic activity of this oxidase towards monohydric phenols.

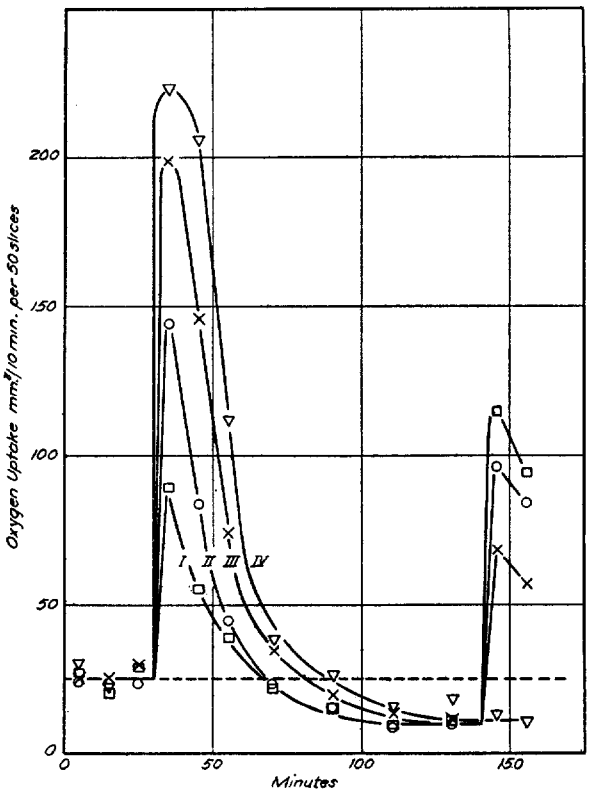

FIG. 1. Showing the eventual lowering of the rate of respiration of washed potato slices in the presence of added catechol. Reaction mixtures contained in the Warburg flasks consisted of 50 slices and 5 cc. of 0.04 M phosphate buffer solution. Center well contained filter paper moistened with 0.2 cc. of a 20 per cent KOH solution. After the elapse of 30 minutes 1 cc. of an aqueous solution containing various amounts of catechol, as specified below, was added from the side arm. Total volumes of the final reaction mixtures were 10.2 cc. pH 5.7, temperature 25°C.

All curves, I, II, III, and IV, up to the time the experiments had been in progress for 30 minutes, represent the rates of oxygen uptake by 50 slices respiring in the presence of phosphate buffer only. During the time between 30 and 140 minutes curves I, II, III, and IV show respectively the influences on the rate of oxygen uptake of 0.63, 1.25, 2.5, and 5 mg. of added catechol. In each instance the final rate of oxygen uptake, due to the influence of the catechol, fell below the rate shown by the slices respiring in phosphate alone, and to the same extent irrespective of the amount of catechol added.

To decide whether or not the tyrosinase had been inactivated, during the 140 minutes in which the experiments had been in progress, the Warburg flasks were removed from the thermostat and 1 cc. of a solution containing 2.5 mg. of catechol was added from the side arm of each flask. The flasks were then returned to the thermostat,

Repeating the experiments of Boswell and Whiting, using conditions similar to theirs, it was found that this drop in rate of oxygen uptake to a value lower than that shown by the slices in phosphate alone cannot be attributed entirely to the removal of the oxidase activity by inactivation (see Fig. 1). For example, employing an amount of catechol, insufficient to inactivate all of the oxidase in the slices, as shown by subsequent addition of more catechol, the rate of oxygen uptake still continued to drop, after the initial rise, to a value considerably below the rate shown by the slices respiring in the phosphate alone. In other words, the rate of oxygen uptake fell to practically the same low value as that observed by Boswell and Whiting, even though considerable active oxidase still remained in the slices.

That the inactivation of the oxidase cannot account for this drop in the rate of oxygen uptake below the rate when the slices respire in the presence of phosphate only, can be shown even in a more striking manner by using 4-tertiary butyl catechol. This substance, judging from observations (unpublished) made by Roth and Dawson, in these laboratories in their study of tyrosinase from the common mushroom, hardly inactivates the enzyme at all. Yet as can be seen from the data shown in Fig. 2, even though only slight inactivation of the oxidase occurred, still the drop in the rate of oxygen uptake was greater than in the case of the catechol experiment (see Fig. 1).

This drop in the rate of oxygen uptake to a value lower than that when the slices respired in phosphate alone therefore cannot be accounted for by the inactivation of the oxidase, but must be due to some other cause. Hence, the claim made by Boswell and Whiting that two-thirds of the respiration of the slices is dependent on the oxidase loses its main support, and leaves the question still unanswered as to whether or not the oxidase takes part in plant respiration. The present authors, however, feel that it is possible, by the procedure described below to show that tyrosinase does play the rôle of a respiratory enzyme in potato tubers.

The activity of potato tyrosinase towards protocatechuic acid (4-carboxy-

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and after thermol equilibrium had been attained, the rates of oxygen uptake again noted. Only in the case of the experiment corresponding to curve IV did the rate of oxygen uptake fail to respond to the addition of the second quantity of catechol. In this instance (IV) the tyrosinase, due to the large amount of catechol added, had apparently been completely inactivated. The renewed increase in the rates of oxygen uptake, shown by the curves I, II, and III, on the addition of the second quantity of catechol shows that considerable tyrosinase activity still remained in the slices.

Taken together, the four curves show that the lowering of the rate of oxygen uptake on the addition of catechol to a value below that in the presence of phosphate alone cannot be attributed to inactivation of the tyrosinase but must be due to the oxidation of catechol exerting an injurious influence on some other part of the respiratory system of the slices.

catechol) is very much less than it is towards catechol or tertiary butyl catechol. In Fig. 3 is shown the influence of this acid on the rates of oxygen uptake and carbon dioxide given off by the respiring slices. In contrast to the influences of catechol and tertiary butyl catechol (Figs. 1 and 2) it will be noticed that there is a marked rise not only in the rate of oxygen uptake but also a corresponding rise in the rate of carbon dioxide given off, so that the R.Q. still remains, as in the case of the slices in phosphate alone, close to unity. In other words, when protocatechuic acid was added to the slices, there was not just a

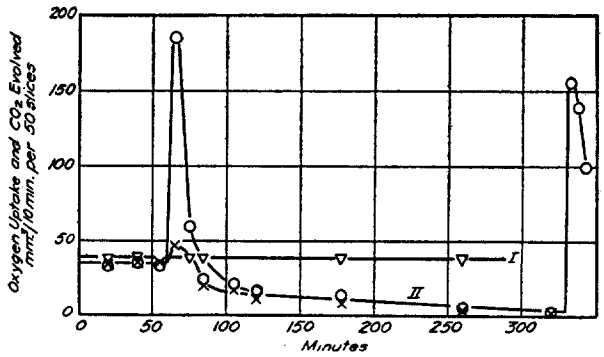

FIG. 2. Showing the influence of 4-tertiary butyl catechol on the eventual rate of respiration of potato slices. Reaction mixtures and conditions same as those described in the legend for Fig. 1, except 4-tertiary butyl catechol was used in place of catechol. Curve I (control) represents the rate of oxygen uptake of the slices in presence of phosphate buffer only. Curve II. 60 minutes after the slices had respired in phosphate buffer only, 2.5 mg. of 4-tertiary butyl catechol were added from the side arm. Circles represent oxygen uptake and the crosses the CO_2 values. Just as in the case of catechol (Fig. 1) a drop in the rate of oxygen uptake occurred, which extends below (approaching zero value) that in the presence of phosphate alone, even though the tyrosinase was not completely inactivated. That the tyrosinase was still active at the expiration of 330 minutes, is shown by the rise in oxygen uptake when more catechol was added.

large rise in the rate of oxygen uptake, as was the case when catechol was used, but a large rise in the rate of respiration, and instead of the rate dropping below the normal rate of the slices in phosphate alone, it remained large and comparatively steady during the 6 hours in which the experiment was in progress. Furthermore, in contrast to the catechol and tertiary butyl catechol experiments, when protocatechuic acid was used the reaction mixture only became slightly colored showing thereby very little accumulation of quinone. The calculated volume of oxygen required to convert the 2 mg. of protocatechuic acid, used in the experiment, to quinone is 195 μl. The volume taken up during the 240 minutes after the addition of the acid to the slices, deducting

the volume which would have been consumed by the slices if they had respired in phosphate alone, was 813 μl. The fact that this large volume of oxygen was used up and the corresponding volume of carbon dioxide given off taken together with the fact that hardly any quinone accumulated, shows that the protocatechuic acid must have acted as a shuttle or hydrogen carrier. First it was oxidized to quinone by means of the tyrosinase, and then the quinone in turn was reduced by a hydrogen donor, adjacent to it, in the respiratory chain. Knowing that potato tyrosinase catalyzes the aerobic oxidation of protocate-

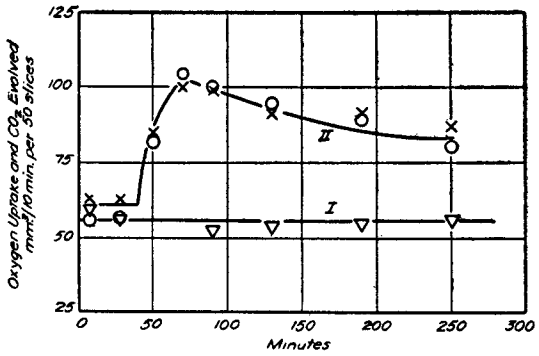

FIG. 3. Showing the influence of protocatechuic acid on the rate of respiration of potato slices. Reaction mixtures and conditions the same as those described in the legend for Fig. 1, except protocatechuic acid was used in the place of catechol. Curve I (control) shows the rate of oxygen uptake for 50 slices respiring in phosphate alone. Curve II. 40 minutes after the slices had respired in the phosphate alone 2 mg. of protocatechuic acid were added from the side arm. Circles represent oxygen uptake and the crosses the CO_2 values. Since the oxygen values approach closely the carbon dioxide values the R. Q. is close to unity. Curve II shows that not only was the rate of oxygen uptake increased by the protocatechuic acid, but that this increased rate continues for more than 250 minutes. This is very different from the influence of catechol on the rate of oxygen uptake shown in Fig. 1.

chuic acid, coupled with the fact that the acid can take part in the respiration of the slices, it follows that potato tyrosinase can take part in the respiratory system of the slices.

That tyrosinase remains in the potato slices, after they have been subjected to being washed in running water for over 2 days, can be shown by the oxidase in the slices still retaining the characteristic ability to catalyze the oxidation of monohydroxy phenols. In Fig. 4 is shown the increase in oxygen uptake when *p*-cresol was added to the respiring slices. The retarding influence on the respiration of the slices by substances known to act as inhibitors towards tyrosinase action also indicates that tyrosinase is the active oxidase in the slices. Potassium cyanide, which is known to inhibit or retard the action of

most metal-bearing enzymes, was found to reduce the rate of respiration of the slices over 85 per cent. This, therefore, argues that a metal-bearing enzyme, such as tyrosinase, is involved in the respiratory process. 4-Nitrocatechol is known to exert a strong inhibiting action on the activity of tyrosinase (4). When this inhibitor was added to the respiring slices, it was found, as shown in Fig. 5, that not only was the rate of oxygen uptake greatly decreased, but also the rate of carbon dioxide given off. In other words, retarding the tyrosinase action also retarded the respiration of the slices. The fact that both of the above retardants lowered the rate of oxygen uptake about 85 per cent

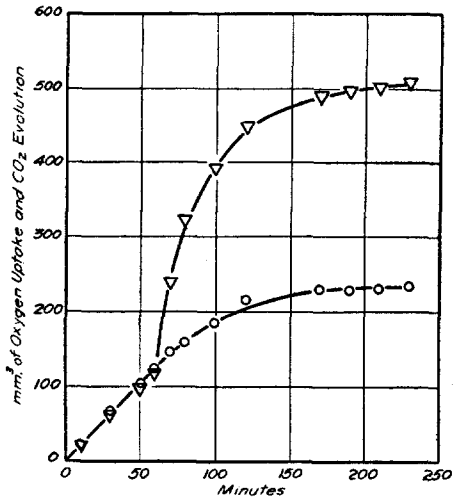

FIG. 4. Showing the presence of tyrosinase in the washed potato slices. Reaction mixture and conditions the same as those described in the legend of Fig. 1. Triangles represent mm.^{3} of oxygen and circles mm.^{3} of CO_2. After the elapse of 60 minutes 1 mg. of *p*-cresol was added to the Warburg flasks containing the 50 slices and phosphate buffer solution.

constitutes strong evidence that the respiratory process of the slices is chiefly dependent on tyrosinase as the terminal oxidase.

The aerobic oxidation of various ortho-dihydroxy phenolic compounds not only is catalyzed at widely different rates by tyrosinase, but as has been mentioned above (Roth and Dawson), these compounds vary in their tendency to inactivate the enzyme. Catechol and tertiary butyl catechol are oxidized very fast when they are added to the respiring slices and the reaction mixtures in the Warburg flasks soon became highly colored due to the accumulation of quinones. The latter are known to be chemically very reactive, and therefore might easily exert a harmful or retarding influence on some intermediate link in the respiratory chain operating in the slices. These two catechols therefore are not suited to serve as hydrogen carriers in the respiratory system in potato

slices. On the other hand, protocatechuic acid which is oxidized more slowly, yields quinone at such a slow rate that the rest of the respiratory system is able to reduce the quinone as rapidly as it is formed, thereby enabling the acid to serve as a hydrogen carrier.

In fact, the influence of the protocatechuic acid on the respiring slices appears to be quite similar to that observed by Boswell and Whiting when they added a substance[2] extracted from potato tubers. They found that when this substance was added to the respiring potato slices an increase in the rate of oxygen uptake took place, and just as in the case of the addition of the protocatechuic acid, there was only a slight tendency toward a decrease in this higher rate during the 6 hours in which the experiment was conducted. The fact that this substance influenced the respiration of potato slices in much the same way as

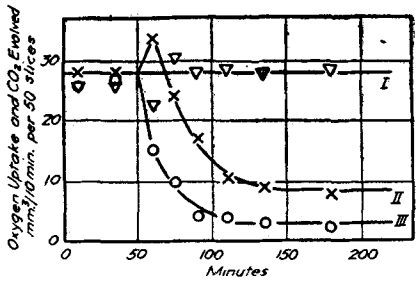

FIG. 5. Showing the inhibiting action of 4-nitrocatechol on the rate of respiration o potato slices. Reaction mixtures and conditions the same as those described in the legend for Fig. 1. Curve I (control) represents the rate of respiration of the potato slices in phosphate alone. Curve II. 50 minutes after the slices had been respiring in phosphate alone 2 mg. of 4-nitrocatechol were added. Circles—oxygen uptake, and crosses, CO_2 given off.

the protocatechuic acid, shows that it too functions as a hydrogen carrier. And since it was isolated from potato tubers, it probably is the natural substrate for potato tyrosinase, or at least closely related to it.

The claim made by Boswell and Whiting that one-third of the respiration of the slices is due to some other respiratory system than the one dependent on the oxidase in the slices loses weight in the light of the objection pointed out against their proof that two-thirds is dependent on the oxidase. Lowering the rate of respiration of the slices over 85 per cent by the above mentioned retardants, potassium cyanide and 4-nitrocatechol, is more in line with the view that probably all of the respiration is dependent on the oxidase, tyrosinase.

[2] Boswell and Whiting extracted a substance from potato tubers which possessed many of the properties common to *o*-dihydroxy phenolic compounds. It was soluble in water, in alcohol, precipitated by lead acetate, and gave a green color reaction with ferric chloride. They, however, did not identify the substance any further.

The study described above, together with that of Boswell and Whiting, on the respiration of potato tubers establishes experimentally in living tissue another terminal oxidase besides the cytochrome C oxidase.

EXPERIMENTAL DETAILS

The potatoes used were of no particular variety. They were bought in the open market and were in good firm condition showing no tendency towards sprouting at the time. Since most of this work was done between December and May, 2 or more months had elapsed since the potatoes had been harvested.

The tubers were cut into slices of about 1 $cm.^2$ and 400 μ thick. The slices were placed in running tap water and left in this running water from 20 to 100 hours. The length of time in washing the slices did not appear to change the rate of respiration. Slices from different potatoes, however, did show differences in rate of respiration. The temperature of the tap water varied from 10 to 15°C. In comparable experiments the slices were always prepared from the same potato. The dry weight of 50 slices was about 250 mg.

A Warburg respirometer (3) was used for following the amount of oxygen uptake and the carbon dioxide given off. The temperature at which the respiration experiments were run was 25°C. The reaction flasks of the respirometer were of 50 cc. capacity. Fifty wet slices, weighing about 4.5 gm. together with 5 cc. 0.04 M phosphate buffer were placed in the reaction flask. To this mixture was added, usually from the side arm, water or solutions of other substances such as catechol (see legends for the figures in the text) making the final volume in the flasks 10.2 cc., and the pH = 5.7. The carbon dioxide was determined by the direct method of Warburg (3), using filter paper moistened with 0.2 cc. of 20 per cent aqueous KOH for absorbing the carbon dioxide as it was formed. The rate of shaking the Warburg apparatus was 120 complete oscillations per minute. The rate of respiration was independent of the rate of shaking.

SUMMARY

The evidence presented in this paper supports the conclusion that at least 85 per cent of the oxygen uptake of the respiring tissue of potato tuber enters the chemistry of the cell by way of a tyrosinase-catalyzed oxidation.

The qualitative aspects of this conclusion are in agreement with the claim made by Boswell and Whiting. However, the evidence offered by them in support of this conclusion is shown to be inadequate.

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