LXIII. OXIDISING ENZYMES.

VIII. THE OXIDATION OF CERTAIN PARAHYDROXY-COMPOUNDS BY PLANT ENZYMES AND ITS CONNECTION WITH "TYROSINASE."

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IT has been shown by one of us [Onslow, M. W., 1920] that the oxidases of the higher plants may be represented as a system consisting of two enzymes (oxygenase and peroxidase) and a substance having the orthodihydroxy-grouping of catechol. The oxygenase catalyses the autoxidation of catechol with formation of peroxide; the peroxidase catalyses the transference of active oxygen to guaiacum. Substances having the catechol grouping, for instance, catechol, protocatechuic acid, caffeic acid, hydrocaffeic acid and chlorogenic acid [Gorter, 1909] have been detected by various workers in a number of plants, all of which (in cases where tested) have been oxidase plants.

It was also shown [Onslow, M. W., 1920] that, in addition to substances with the catechol grouping, the widely distributed oxygenase will catalyse the oxidation of *p*-cresol with the resultant blueing of guaiacum. With this exception, however, the oxygenase was unable to act on various phenols with groupings other than catechol, for instance, phloroglucinol, resorcinol, etc. It was suggested [Onslow, M. W., 1923] that this behaviour of *p*-cresol might be explained by the presence of traces of a dihydroxy-derivative formed either by autoxidation or by traces of active oxygen in the enzyme preparation.

The present communication records the fact that when an enzyme preparation from the potato tuber acts on either *p*-cresol, tyrosol (*p*-hydroxy-phenylethyl alcohol) or phenol a dihydroxy-derivative is formed which gives rise to peroxide and subsequent blueing of guaiacum.

The potato tuber, as previously shown, contains oxygenase, peroxidase and a substance with the catechol grouping. It also contains "tyrosinase" and free tyrosine or polypeptides of tyrosine, or both.

An enzyme preparation of the tuber was made by thoroughly extracting with cold alcohol, and then extracting the residue with water. When added to *p*-cresol solution, the mixture rapidly turned orange-pink. On addition of guaiacum to a portion, a deep blue colour was produced. On addition, to

another portion, of a few drops of a weak solution of ferrous sulphate or ferrous ammonium sulphate, the pink colour turned green and subsequently with 1-2 % sodium carbonate blue, violet and red, a reaction characteristic of the catechol grouping. When the enzyme was added to tyrosol, a pink colour was rapidly developed. The solution then gave the same reactions as those of *p*-cresol with guaiacum and with ferrous sulphate and sodium carbonate. In the early (red) stage of the action of the enzyme on tyrosine the iron solution appeared to give the catechol reaction, but it was not normal.

The above results indicate a new type of oxidation. The solutions of *p*-cresol and tyrosol may contain traces of a dihydroxy-compound formed by autoxidation, too small in amount to be detected by the iron reaction¹. Should this be so, the enzyme may be regarded as catalysing this autoxidation by means of active oxygen. An alternative suggestion is that the enzyme preparation is never quite free from traces of active oxygen which may produce traces of the dihydroxy-compound [Dakin and Herter, 1907] thus enabling the reaction to proceed. A third suggestion is that an enzyme exists which has a specific action on parahydroxy-compounds, and the same distribution as oxygenase.

An enzyme preparation from the tuber was also added to a solution of dihydroxyphenylalanine (prepared from *Vicia faba*). A red colour was first formed which eventually became black as in the case of tyrosine. Addition of guaiacum at the red stage did not produce more than a trace of blue colour on the surface of the liquid. It is well known, however, that the products of "tyrosinase" on tyrosine do not blue guaiacum.

It has been shown collectively by Chodat and Schweizer [1913], Happold and Raper [1925] and Robinson and McCance [1925] that *p*-cresol, catechol and phenol, in presence of an enzyme preparation from the potato tuber, will oxidise glycine, alanine and leucine.

One of us (M. E. R.) has also found that potato enzyme and tyrosol will oxidise glycine. Tyrosine and dihydroxyphenylalanine will not, however, effect the oxidation of glycine.

The conclusions which we draw from the above facts are as follows:

The oxygenase (or some other enzyme) oxidises *p*-cresol and tyrosol to 3,4-dihydroxytoluene and 3,4-dihydroxyphenylethyl alcohol respectively. These then give rise to active oxygen in the same way as catechol.

The active oxygen is the agent in the deamination of glycine, alanine and leucine.

In the case of dihydroxyphenylalanine, active oxygen formed may be used in deamination of the side chain. Hence there is no blueing of guaiacum. Dihydroxyphenylacetaldehyde and ammonia formed may take part in melanin formation.

¹ Against this view in the case of *p*-cresol is the fact that a solution of this substance, on standing in air, does not give a blue colour with horse-radish peroxidase and guaiacum as does a solution of catechol kept under similar conditions.

Tyrosine may be oxidised (in the same way as tyrosol and *p*-cresol) to dihydroxyphenylalanine, the reaction proceeding subsequently as above.

On the other hand, Raper and Wormall [1925] maintain that deamination of tyrosine does not take place, since no ammonia can be detected during oxidation. In addition, they have stated that the enzyme has no action on *p*-hydroxyphenylpyruvic acid in the presence of ammonia.

It has been shown by Bach [1914] that the preliminary red colour formed in the oxidation of tyrosine by "tyrosinase" disappears if the products are placed under anaerobic conditions. Hence Bach postulated the presence in the potato of an oxido-reductase (deaminase) which forms products which are eventually oxidised to melanin either spontaneously or through the accelerating influence of a phenolase.

It has been suggested by one of us [Onslow, 1923] that the oxido-reductase may act as an intermediary between the active oxygen and the amino-acid.

Raper and Wormall [1923] doubt the existence of an oxido-reductase for the following reasons. Potato "tyrosinase" (dialysed juice) was allowed to act on tyrosine until a red colour was produced. The solution was divided into two portions. From one portion the enzymes were removed by colloidal "iron," leaving a red solution. Both portions were then placed in an incubator at 38°, and they decolorised equally well.

We noted, as did these authors, that the red oxidation product of "tyrosinase" on tyrosine was decolorised on heating. So also, to a lesser extent, was the red-brown product formed by the action of oxygenase on catechol. The same was true for the blue oxidation product of guaiacum. A similar experiment was made by us as follows. An enzyme preparation of the potato tuber was prepared by reprecipitation with alcohol so as to remove amino-acids as far as possible. This was allowed to act on catechol until a red-brown product was formed. Half the solution was treated with colloidal "iron." The two solutions, with and without enzyme, were then evacuated in Thunberg tubes and left at room temperature. The tube containing enzyme decolorised in 3 hours. The control tube remained coloured for one or more days. We do not necessarily conclude that an oxido-reductase is present, since the enzyme preparation would certainly contain proteolytic enzymes, and oxidised catechol is undoubtedly decolorised by products of autolysis.

Further work is in progress to determine, if possible, whether or no an oxido-reductase is present which catalyses the oxidation of the amino-acids present.

We conclude from the evidence available that the so-called action of "tyrosinase" may be as follows. Oxygenase (or some other enzyme) introduces a second hydroxy-grouping forming dihydroxyphenylalanine. Oxygenase catalyses the oxidation of this substance to a peroxide. Active oxygen may oxidise the amino grouping directly or may act as an acceptor for the hydrogen produced by an oxido-reductase, the amino-acid acting as the acceptor for the oxygen.

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Note added at proof reading, May 21st, 1925. We have found that *m*-cresol (both Merck and Kahlbaum samples) is oxidised by the enzyme preparation to a compound giving the catechol reaction. A 1–2 % solution of *p*-hydroxybenzoic acid, neutralised with sodium hydroxide to litmus, is also slowly oxidised by the enzyme, and the solution, after 24 hours, gives a slight but distinct catechol reaction.