XI. THE TYROSINASE-TYROSINE REACTION.

IV. NOTE ON THE IDENTITY OF TYROSINASE FROM DIFFERENT SOURCES.

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In 1923 Raper and Wormall described three stages which may be sharply distinguished in the reaction between potato tyrosinase and tyrosine [1923]. The first of the stages is the production of a red substance; in the second stage the red substance changes spontaneously into a colourless substance, this being best observed in an inert gas or *in vacuo*; finally, on exposure to oxygen the colourless substance blackens to form melanin, this being the third stage. It was shown that the enzyme is necessary only for the first stage of the reaction, *i.e.* the oxidation of the tyrosine to the red substance. If at this stage the enzyme be removed with "colloidal iron" the red substance still changes to the colourless one and the subsequent oxidation of this to melanin also takes place. These observations were made solely with tyrosinase derived from potatoes. Gortner [1910, 1924], using an animal tyrosinase prepared from the mealworm (*Tenebrio molitor*), was unable to observe the change from the red substance into the colourless one, even after many days. This made it appear that the enzyme from mealworms was different from that present in potatoes, in that the red substance produced in the two cases had different properties. We have therefore repeated Gortner's experiment adhering exactly to his directions for the preparation of the enzyme and the carrying out of the experiment, but we have failed completely to note any difference in the behaviour of the red substance whether it is produced by the potato or mealworm enzyme. Thinking that the p_H of the solution might influence the reaction, the effect of this on the rate of change of the red substance into the colourless one has been investigated. At p_H 8 the change is rapid and it becomes considerably slower at p_H 6 but even at this p_H the change is complete at the ordinary temperature in 24 hours. This therefore could not explain the difference between Gortner's experiment and our own, so that we must conclude that some condition, not described by Gortner, which is essential for stabilising the red substance, has been overlooked in his description. As a further proof of the identity of the two

enzymes, the limits of p_H at which the mealworm enzyme acts have been determined and they are substantially the same as for the potato enzyme. In addition no difference has been detected between the soluble and insoluble tyrosinase of the mealworm, although Gortner [1910] states that the latter has no action on quinol. In order to determine whether tyrosinase prepared from a fungus displayed the same properties as the enzyme from the potato and mealworm, *Agaricus dryophilus* has been used as a source of the enzyme. The red substance produced from tyrosine by this preparation displayed exactly the same properties as that obtained by the action of the other two enzymes. The p_H on the acid side of the neutral point at which the action of the *Agaricus* enzyme was inhibited was, however, lower than with either the potato or mealworm enzyme. Since the last two preparations contain protein which is precipitated along with the tyrosinase at p_H 5, whereas no precipitate forms in the *Agaricus* preparation at this p_H, we attribute the difference in the limits of reaction at which the enzymes display activity to this fact. When tested on a series of phenols in buffered solutions at p_H 7, no differences were found between the action of the mealworm and potato enzymes. Incidentally, the rapid change of the red substance into the colourless one at p_H 8 explains why melanin formation takes place most rapidly at this degree of reaction when tyrosinase acts on tyrosine. At p_H 8 the colourless substance, rapidly produced from the initial red pigment, is just as rapidly oxidised at this p_H into melanin, whereas at p_H 6 both processes are much slower.

Experimental.

The insoluble enzyme was prepared from 35 g. *Tenebrio molitor* according to the directions of Gortner [1910], and emulsified in 200 cc. chloroform water.

Exp. 1. To 50 cc. of a saturated solution of tyrosine in a phosphate buffer mixture at p_H 6, 10 cc. of the enzyme suspension were added. The solution was shaken at intervals and had become deep red in 5 hours. It was filtered, a few drops of 10 % acetic acid added and boiled. The filtrate became colourless on boiling and blackened almost immediately on making slightly alkaline with sodium carbonate. This behaviour is identical with that described by Raper and Wormall [1923] using potato tyrosinase.

Exp. 2. *p_H range.* Portions of 25 cc. each of saturated, buffered tyrosine solution, ranging from p_H 4 to 9·5, were set up in each of a series of flasks in two sets. To the flasks of one set, 2 cc. of the mealworm enzyme were added and to the other, 2 cc. of freshly dialysed potato juice. Examined after 24 hours there was no qualitative difference between the two sets. The maximum coloration was produced at p_H 7·8 and at p_H 4 and 9·5 there was no action.

The "soluble" tyrosinase prepared from mealworm by Gortner [1910] was also examined as to its p_H range. The preparation obtained by us had

only a feeble action on tyrosine but this was most clearly marked around p_H 8. It should be noted that the filtrate from the "insoluble" enzyme prepared according to Gortner is at first turbid. It was our practice to return this to the funnel until a clear solution came through and not to precipitate straight away with ammonium sulphate. If the first runnings are included in the "soluble" enzyme solution it is obviously contaminated with the "insoluble" enzyme. This difference in procedure no doubt explains our lack of success in obtaining very active preparations of the "soluble" enzyme. As a matter of fact, the "insoluble" enzyme is quite soluble if it be suspended in water rendered faintly alkaline with ammonia and it only owes its insolubility when prepared according to Gortner's method to the fact that on standing it becomes slightly acid. We suggest therefore that there is no real difference between Gortner's "soluble" and "insoluble" tyrosinase from the mealworm.

Exp. 3. A 1 % solution of quinol buffered at p_H 7·4 was divided into three portions. To these 3 cc. of potato tyrosinase, mealworm tyrosinase (soluble) and mealworm tyrosinase (insoluble) were added respectively. After 24 hours they were all brownish red in colour. Gortner describes the insoluble enzyme as having no action on quinol in 72 hours, but since the p_H of the solution was not adjusted this is a sufficient explanation of the discrepancy between our result and his. A similar experiment to that just described was carried out with the "insoluble" enzyme and the potato enzyme, using phenol, *p*-cresol, resorcinol and catechol as substrates, but no qualitative difference could be detected between the action of the two preparations. Both were without action on resorcinol.

Exp. 4. *Influence of p_H on decolorisation of the red compound.* Two 50 cc. portions, A and B, of a saturated tyrosine solution, buffered at p_H 6, were taken. To A, 3 cc. of freshly dialysed potato juice were added and to B, 3 cc. of the "insoluble" *Tenebrio* suspension. When a marked red colour had developed in both flasks, the contents were filtered through fine paper and paper pulp until the filtrates were clear. A little "dialysed iron" was added to the A filtrate and it was again filtered. Three samples of 5 cc. each from the two filtrates were now placed in Thunberg tubes and the p_H adjusted as follows:

(*a*) 5 cc. filtrate + 1·03 cc. water; p_H 6.

(*b*) 5 cc. filtrate + 0·43 cc. water + 0·6 cc. *N*/5 NaOH; p_H 7.

(*c*) 5 cc. filtrate + 1·03 cc. *N*/5 NaOH; p_H 8.

The tubes were immediately evacuated by a water-pump and then placed in a water-bath at 37° in which the colour changes were followed. In 4 minutes both the tubes at p_H 8 were colourless. In 13 minutes the tubes at p_H 7 were colourless whilst the tubes at p_H 6 were still pale pink. In the last two the colour faded much more slowly than in the tubes at p_H 7 and 8. After 20 minutes the tubes were taken out of the bath at 37° and left at the ordinary temperature. In 18 hours the tubes at p_H 6 were also colourless. Air was

now admitted to all the tubes and those at p_H 6 and 7 were adjusted to 8. At the end of 48 hours all the tubes showed an equally black pigmentation.

Exp. 5. 3 cc. of the insoluble enzyme preparation were added to 50 cc. of saturated tyrosine solution at p_H 6. A well-marked red colour had developed in 4½ hours. The solution was filtered through fine paper [cf. Gortner, 1924] and gave a clear red filtrate. This was placed in the dark at room temperature for 24 hours when all traces of red colour had disappeared, the solution being now blackish brown. This experiment has been repeated several times always with the same result. In the similar experiment described by Gortner the red colour persisted for 18 days. The difference is not due to the use of a phosphate buffer since the same result is obtained when no buffer is used. It is apparently due to some unknown factor which we have been unable to discover.

Exp. 6. Several specimens of *Agaricus dryophilus* were ground up with chloroform water and allowed to macerate for 3 days. The solution contained an active tyrosinase. A portion was filtered and an experiment similar to number 4 was carried out using this as the tyrosinase preparation. The results were identical with those obtained with the potato and *Tenebrio* enzymes. This enzyme preparation when tested as to its p_H range, unlike the *Tenebrio* and potato enzymes, showed some action at p_H 4, but the maximum was around p_H 8 as with the other tyrosinases. It was not precipitated at p_H 4 or 5.

Summary.

(1) Preparations of tyrosinase from the potato, the mealworm (*Tenebrio molitor*) and *Agaricus dryophilus* produce a red compound from tyrosine which has the same properties in each case. There is thus no reason to believe that different tyrosinases have different actions on tyrosine.

(2) No differences could be detected between the actions of the mealworm and potato enzymes on quinol, phenol, *p*-cresol, resorcinol or catechol nor in the p_H range at which they act.

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