XXX. THE TYROSINASE-TYROSINE REACTION.

VII. THE ACTION OF TYROSINASE ON CERTAIN SUBSTANCES RELATED TO TYROSINE.

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IN Part VI of this series [Raper, 1927] the identification of 5 : 6-dihydroxyindole and 5 : 6-dihydroxyindole-2-carboxylic acid as products of the action of tyrosinase on tyrosine was described. It seemed of interest to find out whether certain substances closely related to tyrosine when oxidised by means of tyrosinase would also give rise to indole derivatives, and with this object the following substances have been submitted to the action of the enzyme: tyramine, 3 : 4-dihydroxyphenylethylamine, 3 : 4-dihydroxyphenylethylmethylamine (epinine) and *N*-methyltyrosine. Using the procedure that has been previously described [Raper, 1927] it was possible to isolate 5 : 6-dimethoxyindole from the products of oxidation both of tyramine and of 3 : 4-dihydroxyphenylethylamine. From epinine, a dimethoxy-derivative was obtained which is presumably 5 : 6-dimethoxy-*N*-methylindole, though this substance has not yet been synthesised for comparison. With *N*-methyltyrosine evidence of the production of an indole derivative has been obtained but it was not isolated in crystalline form. These experiments indicate therefore that the series of reactions undergone by tyrosine to give rise eventually to 5 : 6-dihydroxyindole and its 2-carboxylic acid is a general one provided that a 3 : 4-quinone is produced in the initial stages of the oxidation process. It has already been shown by Pugh and Raper [1927] that the production of orthoquinones from those monohydric phenols on which tyrosinase acts is characteristic of this enzyme and that they are also produced by it from *o*-dihydric phenols. It was not surprising therefore that the above-mentioned substances related to tyrosine, which contain a hydroxyl group in the *para*-position or have hydroxyl groups in the 3 : 4 positions, should undergo similar changes.

Of the series of reactions which takes place when tyrosine is acted upon by tyrosinase the only specific one is the production of 3 : 4-dihydroxyphenylalanine. The subsequent oxidation of this to the corresponding orthoquinone and the further transformation of this in the manner previously described [Raper, 1927] should be capable of being brought about by any oxidising

agent that produces orthoquinones from catechol derivatives. It was decided therefore to find out whether evidence supporting this could be obtained by oxidising 3 : 4-dihydroxyphenylalanine and epinine with a mild oxidising agent. Both these substances were in fact found to be readily oxidised when shaken with freshly prepared moist silver oxide and as products of the oxidation 5 : 6-dihydroxyindole-2-carboxylic acid and 5 : 6-dihydroxy-*N*-methylindole, respectively, were detected by the isolation of their corresponding methoxy-derivatives.

In the oxidation of tyramine, 3 : 4-dihydroxyphenylethylamine, epinine and *N*-methyltyrosine with tyrosinase certain minor differences as compared with the behaviour of tyrosine were observed. The *o*-dihydroxy-compounds are much more rapidly oxidised than tyrosine to give red substances and the latter (presumably the 5 : 6-quinones of the corresponding dihydro-indole derivatives) are more stable if separated from the enzyme than the red substance produced from tyrosine and 3 : 4-dihydroxyphenylalanine. *N*-methyltyrosine is oxidised more slowly than tyrosine and also gives rise to a somewhat more stable red substance. These results indicate therefore that the velocity of the internal oxidation-reduction process by which the 5 : 6-quinone of dihydroxyindole-2-carboxylic acid is converted into a 5 : 6-dihydroxyindole derivative is increased by the presence of the carboxyl group and that methylation of the nitrogen atom of the indole has a tendency to diminish the speed of this change. The oxidation of *N*-methyltyrosine is of particular interest because of the close relationship that this substance bears to adrenaline. It is conceivable that *N*-methyltyrosine might give rise to adrenaline by oxidation and loss of CO₂ as follows:

The production of the 3 : 4-quinone of phenyl-*N*-methylalanine (I) by the action of tyrosinase is in full accord with what we already know of the action of this enzyme. In the scheme represented above the conversion of this quinone into adrenaline with the evolution of CO₂ is represented as an internal oxidation-reduction reaction. With tyrosine, the quinone corresponding to (I) changes at once into a dihydroxyindole derivative and although this same type of change has also been demonstrated to take place readily with the quinone corresponding to epinine, it appears to take place less readily with the quinone produced from *N*-methyltyrosine, judged by the yield of indole

derivative obtained. It is possible therefore that the quinone (I) represented above undergoes, in part, some change different from the usual one and the production of adrenaline from it, in the manner suggested, is a possibility to be borne in mind. A few experiments which have been carried out show that a small amount of a pressor substance is produced when the red solution produced by the action of tyrosinase on *N*-methyltyrosine is allowed to decolorise *in vacuo*. Whether this pressor substance is adrenaline cannot yet be stated and much more evidence is necessary before this can be settled.

All the four substances related to tyrosine mentioned above finally give rise to melanin when the action of the enzyme is sufficiently prolonged and carried out at about p_H 8.0. At p_H 6.0 melanin production is much slower and this is especially so with tyramine, and *N*-methyltyrosine. The fact, however, that the *N*-methyl-derivatives give rise to melanin suggests that the nitrogen atom of 5:6-dihydroxyindole is not further oxidised in the formation of melanin from tyrosine.

In order to get more precise information regarding the oxidative changes by which melanin is produced from 5:6-dihydroxyindole the oxidation of tyrosine and several of the related substances already mentioned has been studied, using a respirometer to measure the oxygen uptake. This method gives, in addition, quantitative evidence of the extent to which tyrosine undergoes the series of changes already described in Parts V and VI of this series when it is oxidised by tyrosinase. The evidence for these changes has been obtained by isolation of 3:4-dihydroxyphenylalanine and the methoxyindole derivatives. This is by no means a quantitative method and it seemed desirable to determine whether the oxygen uptake per atom of tyrosine corresponds with that theoretically deduced. The following scheme represents the changes which have been postulated [Raper, 1927] to explain the conversion of tyrosine into 5:6-dihydroxyindole:

It will be observed that 3 atoms of oxygen are required to convert 1 molecule of tyrosine into 5:6-dihydroxyindole. These 3 atoms have been taken up by the time the red substance has been formed so that the final

change of this to dihydroxyindole will take place in an inert atmosphere. Melanin formed from tyrosine has been found to contain 8.65 % of nitrogen [Raper and Wormall, 1925]. Dihydroxyindole, $C_8H_7O_2N$, contains 9.39 % of nitrogen. By the loss of 2 atoms of hydrogen and the addition of 1 atom of oxygen it would yield a substance $C_8H_5O_3N$ which contains 8.59 % nitrogen. If this be the empirical formula of melanin then its formation from 5 : 6-dihydroxyindole would entail the utilisation of 2 atoms of oxygen. Starting from tyrosine therefore, 5 atoms of oxygen in all would be required to produce melanin. The mean of several experiments yielded the figure 5.23 atoms per molecule of tyrosine. With 3 : 4-dihydroxyphenylalanine 4 atoms of oxygen should be required to produce melanin. Experiment in this instance gave the figure 4.12. Tyramine and epinine both show a much slower rate of conversion of the red substance to dihydroxyindole or dihydroxy-*N*-methylindole respectively, and the figures 4.75 and 3.92 were obtained instead of the theoretical 5 and 4 respectively. These results suggest therefore that 2 atoms of oxygen are required for the conversion of each molecule of dihydroxyindole or its *N*-methyl-derivative into melanin and they confirm the explanation already given of the various stages by which tyrosine is converted into 5 : 6-dihydroxyindole and its 2-carboxylic acid. *N*-methyltyrosine and adrenaline have also been examined by the respirometric method. The former took up 4.92 atoms of oxygen and this corresponds fairly well with the 5 atoms required theoretically for the production of melanin. It has already been pointed out above, however, that the course of oxidation of this substance may be more complex than that of tyrosine so that the interpretation of this result is uncertain. Adrenaline was expected to take up only 3 atoms of oxygen but took up 5.3. It is, however, unlikely that the stages in the oxidation of adrenaline are the same as those for the other substances described above. When oxidised by tyrosinase it yields a black precipitate and a deep reddish-brown solution, differing in this respect from tyrosine, which is completely converted into melanin.

EXPERIMENTAL.

The enzyme used in the following experiments was prepared from mealworms by the method already described [Raper, 1926].

Action of tyrosinase on tyramine.

Tyramine hydrochloride was prepared by the method of Johnson and Deschavsky [1925]. 1 g. of the hydrochloride in 1 litre of water was warmed to 25–30° and 100 cc. enzyme solution were added. The reaction was rapidly adjusted to p_H 6–6.5 by addition of very dilute ammonia or acetic acid as required, the solution saturated with oxygen in a stoppered bottle and well shaken. Oxygen was bubbled through at half-hour intervals and the shaking repeated frequently. A red colour developed after about 5 minutes and gradually deepened. After 3 hours the enzyme had precipitated. 10 cc. of 1 % acetic acid were added, the solution was filtered and 30 cc. of a saturated solution

of SO_2 added. After standing in a stoppered bottle for 24 hours the solution was distilled under reduced pressure in a stream of hydrogen until it was reduced to about 20 cc. The reaction products were now methylated in an atmosphere of hydrogen, using 13 cc. of 20 % NaOH and 3 cc. of dimethyl sulphate. The whole of the alkali was added at once, the solution warmed slightly and the dimethyl sulphate added in three portions with vigorous shaking. After warming for an hour on the water-bath the solution was cooled and shaken out three times with its own volume of ether. The combined ether extracts were washed with a little 2 % sulphuric acid, then with water and evaporated. A crystalline residue was left. A trace of this dissolved in alcohol gave a good indole reaction with *p*-dimethylaminobenzaldehyde and concentrated HCl. It also gave a reddish-violet colour with sodium nitroprusside and caustic soda, which was turned to blue on acidifying with acetic acid. The residue crystallised twice from alcohol melted at $154-5^\circ$ and when mixed with 5 : 6-dimethoxyindole the m.p. remained unchanged. An alternative method is to allow the red solution obtained as described above to decolorise in an atmosphere of hydrogen. The process is hastened by the addition of 2 cc. 10 % NaOH. This should be introduced after evacuation of the flask containing the solution, shaking to get rid of oxygen, and then admitting hydrogen. The disappearance of the red colour takes about 3 days at 25° . The evaporation in a stream of hydrogen and the subsequent methylation may then be carried out as described above. The ether extract on evaporation yields 5 : 6-dimethoxyindole.

Action of tyrosinase on 3 : 4-dihydroxyphenylethylamine.

We are indebted to Prof. R. Robinson for the 3 : 4-dihydroxyphenylethylamine used in this experiment. 1 g. of the hydrochloride was dissolved in 2 litres of water warmed to 25° and 100 cc. of the enzyme solution were added. The p_{H} was quickly adjusted to 6-6.5. On saturation with oxygen and shaking a deep red colour rapidly developed and within a few minutes the enzyme had precipitated. This is in marked contrast with the period of about 3 hours required to reach the same stage with tyramine. After half an hour the solution was filtered and placed in a flask, which was then evacuated and filled with hydrogen. 2 cc. 10 % NaOH were added and the solution was kept under hydrogen for 3 days, when its colour had become a pale brown. It was evaporated to small bulk *in vacuo* in a stream of hydrogen and methylated as described in the experiments with tyramine. The ether extract yielded 0.103 g. of a crystalline residue which on recrystallisation from absolute alcohol melted at $154-5^\circ$; mixed m.p. with 5 : 6-dimethoxyindole, $154-5^\circ$. The substance gave good indole reactions with *p*-dimethylaminobenzaldehyde and with sodium nitroprusside.

Action of tyrosinase on 3 : 4-dihydroxyphenylethylmethylethylamine.

The 3 : 4-dihydroxyphenylethylmethylethylamine hydrochloride (epinine) was obtained from Messrs. Burroughs, Wellcome and Co. The procedure used was

exactly as described above for tyramine. The epinine was rapidly attacked by tyrosinase and in 10 minutes yielded a deep red solution with accompanying precipitation of the enzyme. The red substance was allowed to undergo conversion into the dihydroxyindole derivative either by keeping under hydrogen or by adding sulphurous acid as described with tyramine. After concentration of the resulting pale brown solution and methylation the ether extract yielded a crystalline solid. From 1.9 g. of epinine, 0.40 g. was obtained. This was recrystallised from absolute alcohol until its m.p. was constant at 138–9°.

Micro-analysis: found, H, 6.7 and 6.45 %; C, 68.85 and 68.98 %. Calculated for $C_{11}H_{13}NO_2$, H, 6.8 %; C, 69.1 %.

A solution in alcohol gave a strong indole reaction with *p*-dimethylamino-benzaldehyde and a drop of conc. HCl. It did not give a colour reaction with sodium nitroprusside and NaOH, thus differing from 5 : 6-dimethoxyindole. A trace of the substance dissolved in glacial acetic acid gave a yellow colour changing to brown on adding a drop of conc. HNO_3 and warming; no trace of orange or red colour was produced. From analogy with the behaviour of 3 : 4-dihydroxyphenylethylamine with tyrosinase the substance obtained as above from epinine should be 5 : 6-dimethoxy-*N*-methylindole, the stages in the reaction being represented as follows:

A satisfactory method for the synthesis of 5 : 6-dimethoxy-*N*-methylindole has not yet been found, but from the known changes undergone by tyramine and 3 : 4-dihydroxyphenylethylamine when oxidised by tyrosinase there can be little doubt that the substance obtained from epinine is the corresponding *N*-methylindole. It will be shown presently that epinine when oxidised with silver oxide yields the same substance.

Action of tyrosinase on N-methyltyrosine.

The *N*-methyltyrosine was kindly prepared for us by Dr J. F. Wilkinson by the method of Johnson and Nicolet [1913].

1 g. of the amino-acid was dissolved in 750 cc. boiling water, diluted to 2 litres and cooled to 30°. 80 cc. of the enzyme solution were added, the p_H adjusted to 6.5 and the liquid saturated with oxygen and shaken vigorously. The saturation with oxygen was repeated half-hourly, and the solution was shaken at frequent intervals. A red colour was soon produced, and after 2½ hours the enzyme had precipitated. 10 cc. of 1 % acetic acid were added, the solution filtered, and the red filtrate after evacuating and shaking to

remove oxygen was kept under hydrogen until the red colour had given place to a pale brown. The solution was distilled under reduced pressure in a stream of hydrogen until about 20 cc. were left. Methylation was carried out in the usual way, using 3 cc. dimethyl sulphate and 12 cc. of 20 % NaOH. The alkaline solution was extracted three times with its own volume of ether, and the ether extracts were washed with a little water. On distilling off the ether about 50 mg. of a clear resinous mass was obtained which partially crystallised on dissolving in alcohol and evaporating. It gave a strong indole reaction with *p*-dimethylaminobenzaldehyde. After ether-extraction of the alkaline solution resulting from the methylation, it was acidified to Congo red and again extracted with ether, but only a trace of ether-soluble substance was obtained. No greater success was obtained when the red substance obtained by acting on *N*-methyltyrosine with tyrosinase was allowed to decolorise in presence of sulphurous acid. After methylation only a trace of ether-soluble substance was obtained from the acidified solution. Judged by previous experiments with tyrosine it was expected that a reasonable amount of either 5 : 6-dimethoxy-*N*-methylindole or its 2-carboxylic acid should have been obtained according to the procedure used in the decoloration of the red substance. As pointed out before [Raper, 1927], the use of sulphurous acid favours the formation of the indolecarboxylic acid. The above results show that with *N*-methyltyrosine the internal oxidation-reduction process by which the 5 : 6-quinone of dihydroxyindole-2-carboxylic acid is converted into the corresponding 5 : 6-dihydroxyindole-2-carboxylic acid is more difficult when the nitrogen of the dihydroindole ring is methylated. It is possible that the oxidation of *N*-methyltyrosine by tyrosinase does not proceed entirely on the same lines as that of tyrosine. In the introduction to this communication it has been pointed out that by simple oxidative changes and loss of CO₂ adrenaline might be produced. In order to determine whether any pressor substance could be detected amongst the oxidation products a solution of 0.025 g. *N*-methyltyrosine in 50 cc. of water was treated with 3 cc. of the enzyme, the *p*_H being adjusted to 6.5. After 3 hours, when a good red colour had been produced and the enzyme had precipitated, a few drops of 0.1 % acetic acid were added and the solution filtered. A portion of the filtrate was kept *in vacuo* in a Thunberg tube for 3 days, when it had almost lost its colour. This solution was tested for pressor activity by our colleague, Dr A. D. Macdonald, to whom our best thanks are due. In the decerebrate cat it produced a rise in blood-pressure judged to be equivalent to that given by 1 in 300,000 adrenaline. When the red solution obtained by oxidation was decolorised in presence of sulphurous acid, the pressor activity was less. Controls with the enzyme solution alone had no action.

Oxidation of 3:4-dihydroxyphenylalanine and epinine with silver oxide.

Tyrosine is not attacked when shaken with freshly prepared silver oxide. It is well known, however, that catechol derivatives easily reduce ammoniacal silver solutions and it was therefore expected that both 3:4-dihydroxyphenylalanine and epinine would be oxidised by silver oxide suspensions. Preliminary experiments showed that this was so and a more detailed investigation was carried out.

3:4-Dihydroxyphenylalanine. 1 g. of the amino-acid was dissolved in 400 cc. hot water and cooled to room temperature. To this a suspension of 2.4 g. freshly prepared silver oxide in about 100 cc. water were added and the liquid was vigorously shaken for 15 minutes. The solution rapidly became coloured red and later, deep reddish brown. 10 cc. of a 10 % solution of sodium sulphate and 10 cc. of 10 % acetic acid were now added, the solution was shaken for a short time, to assist coagulation of the colloidal silver, and finally allowed to stand for 30 minutes. It was filtered by suction through filter paper pulp, 20 cc. of a saturated solution of SO_2 were added to the filtrate and it was left overnight. After filtering from a small amount of deposit it was concentrated *in vacuo* in a stream of hydrogen to about 20 cc. The products of the reaction were methylated under hydrogen, 8 cc. of 40 % NaOH were added, the solution was warmed slightly and then 3 cc. dimethyl sulphate were added in three portions with vigorous shaking. The alkaline solution was finally heated on a boiling water-bath for an hour. After cooling it was extracted three times with ether but only a trace of ether-soluble substance was removed. It was then acidified with sulphuric acid to Congo red and again extracted three times with its own volume of ether. The combined ether extracts were washed with a little water and the ether distilled off. The crude residue weighed 0.139 g. This was extracted with about 10 cc. boiling water, filtered to remove tarry matter and the filtrate taken to dryness in a vacuum desiccator. At this stage the products of two preparations carried out as above were worked up together. After crystallising four times from benzene containing 5 % of acetone colourless plates were obtained, m.p. $202-3^\circ$ with vigorous evolution of gas. This corresponds with the behaviour of 5:6-dimethoxyindole-2-carboxylic acid which loses CO_2 at its melting point and is converted into 5:6-dimethoxyindole. A mixture with the synthetic acid gave the same m.p. The acid gave a good indole reaction with *p*-dimethylaminobenzaldehyde as also did the residue obtained when the acid was allowed to decompose at its m.p. These results show that silver oxide and tyrosinase can both bring about the same oxidative changes in 3:4-dihydroxyphenylalanine. The yield of dimethoxyindole-2-carboxylic acid obtained with silver oxide is smaller than with tyrosinase. Probably side reactions occur with the former.

Epinine. 1 g. epinine in 400 cc. water was treated with 3 g. freshly prepared silver oxide in the way described above for 3:4-dihydroxyphenylalanine. After removal of the silver by filtration sulphurous acid was added to the

solution and it was left overnight. It was reduced to 10 cc. by distillation *in vacuo* in a current of hydrogen and methylation carried out in the usual way, using 3 cc. dimethyl sulphate and 8 cc. of 40 % NaOH. Extraction of the alkaline liquid with ether yielded 0.142 g. of crude methylation product. This was recrystallised twice from alcohol and melted at 138–9°. The M.P. was unchanged on mixing with the similar product obtained by the oxidation of epinine with tyrosinase, which is presumably 5 : 6-dimethoxy-*N*-methylindole.

Respirometer experiments.

These were carried out in a modified form of Haldane's blood gas apparatus [1920]. Instead of the simple flasks used by Haldane, conical flasks with two bent side arms, as illustrated, were employed.

The substrate was placed in the body of the flask, the enzyme solution in the lower side arm and a little 30 % KOH in the other. By tilting the flask, the enzyme may be added to the substrate after equilibration without removing the flask from the thermostat. The KOH absorbs any CO₂ which may be produced. Flasks with ground-glass stoppers were used in the later experiments as they were found to give more consistent results. The experiments lasted several days and during the frequent shaking during this period rubber stoppers may suffer slight displacement which affects the reading.

Solutions used. Tyrosine, 0.05 %; tyramine hydrochloride, 0.1 %; epinine, 0.1 %; 3 : 4-dihydroxyphenylalanine, 0.1 %; adrenaline (free base), 0.1 %; *N*-methyltyrosine, 0.01 %; all in distilled water. In each instance 1 cc. of enzyme solution was used. The reaction flask contained 4 cc. phosphate buffer, of p_H 6 or 8 according to requirements, and 4 cc. of the above tyrosine solution or 2 cc. of the solution of the other substances with the exception of *N*-methyltyrosine, of which 10 cc. were used. Thymol was used as antiseptic. The control flask contained the same amount of enzyme and buffer solutions, but no substrate. The enzyme and KOH solutions were easily introduced into the side arms by means of a curved pipette. The bottles were completely immersed in a thermostat at room temperature and were mechanically shaken. With the taps open to air, 10 minutes' shaking was allowed for equilibration. The taps

were then closed and after a further 15 minutes' shaking a reading was taken. This was repeated, if necessary, to ensure that equilibrium was established before starting the experiment. The enzyme was now added from the side arm of the experimental flask and the shaking continued. Readings were taken periodically until oxygen uptake finally ceased, and for confirmation for at least two days after this. The readings were corrected for barometric pressure and temperature, and are expressed in terms of the number of atoms of oxygen taken up per molecule of substrate used (Table I).

Table I.

Substrate and its amount	p_H	No. of exps	cc. O ₂ absorbed at N.T.P.	Average O ₂ uptake cc.	O ₂ uptake. Atoms per molecule of substrate
Tyrosine (2 mg.)	6	1	0.62	0.62	5.00
	8	5	0.656-0.631	0.647	5.23
<i>l</i> -3 : 4-Dihydroxyphenylalanine (2 mg.)	6	2	0.440-0.430	0.435	3.82
	8	5	0.49 -0.447	0.469	4.12
<i>i</i> -3 : 4-Dihydroxyphenylalanine (2 mg.)	8	2	0.466-0.466	0.466	4.09
Epinephrine (2 mg.)	6	1	0.409	0.409	3.71
	8	7	0.456-0.408	0.431	3.92
Tyramine hydrochloride (2 mg.)	6	2	0.538-0.535	0.537	4.17
	8	9	0.646-0.565	0.613	4.75
<i>N</i> -Methyltyrosine (1 mg.)	8	1	0.276	0.276	4.80
	Unbuffered	2	0.308-0.258	0.283	4.92
Adrenaline (2 mg.)	8	4	0.66 -0.63	0.65	5.30

Consideration of these results shows that at p_H 8 the oxygen uptake is always higher than at p_H 6, though the differences are not great. The number of atoms taken up per molecule of substrate is in accordance with the view that tyrosine and tyramine require 3 atoms, and dihydroxyphenylalanine requires 2 atoms, of oxygen to convert them into 5 : 6-dihydroxyindole and a further 2 atoms to convert this into melanin. Epinephrine requires 2 atoms of oxygen to convert it into 5 : 6-dihydroxy-*N*-methylindole and a further 2 atoms to transform this into melanin. It is possible that reactions other than the main one account for the slight divergence from the theoretical values at p_H 8. At p_H 6 the slower rate of action of the enzyme and its precipitation after a time probably account largely for the oxygen uptake being less than the expected value. The red substances produced by tyramine and epinephrine are also much more stable than that from tyrosine and much less rapidly converted into the colourless dihydroxyindole derivatives. For this reason these substrates are probably not completely converted into melanin within the period of time during which the enzyme is still active. The figures for *N*-methyltyrosine suggest that the main reaction proceeding is the same as that with tyrosine, but the possibility of other reactions occurring has already been pointed out.

It may be noted that *i*-3 : 4-dihydroxyphenylalanine, a sample of which

was kindly supplied by Dr C. R. Harington, behaves exactly like the naturally occurring amino-acid. This was to be expected since the enzyme action is not concerned with oxidation of the side chain which contains the asymmetric carbon atom.

If a few drops of 30 % KOH were added to the solution in the experimental flask after all enzyme action had ceased a further oxygen uptake was observed. This occurred outside the limits of p_H at which tyrosinase is active and was probably due to production of oxidation products of melanin itself by atmospheric oxygen.

An experiment was carried out with adrenaline at p_H 8 in the absence of the enzyme. The oxygen uptake observed was 6.14 atoms per molecule of adrenaline as opposed to 5.29 with the enzyme present. It is probable therefore that oxidation of adrenaline in the air, with the production of the well-known pink colour, proceeds on different lines from that produced by the enzyme, but what these are can only be discovered by further investigation.

SUMMARY.

(1) As a result of the action of tyrosinase, tyramine and 3 : 4-dihydroxyphenylethylamine yield 5 : 6-dihydroxyindole, 3 : 4-dihydroxyphenylethylamine yields 5 : 6-dihydroxy-*N*-methylindole, and *N*-methyltyrosine yields an indole derivative not yet identified.

(2) *N*-methyltyrosine on oxidation with tyrosinase also yields a small amount of a pressor substance.

(3) 3 : 4-Dihydroxyphenylalanine and 3 : 4-dihydroxyphenylethylmethylamine on oxidation with silver oxide behave in the same way as when oxidised by tyrosinase and yield 5 : 6-dihydroxyindole-2-carboxylic acid and 5 : 6-dihydroxy-*N*-methylindole respectively.

(4) In the production of melanin from tyrosine and tyramine approximately 5 atoms of oxygen are utilised per molecule of substrate, whereas 4 atoms are required by 3 : 4-dihydroxyphenylalanine and 3 : 4-dihydroxyphenylethylmethylamine. It is probable that 2 atoms of oxygen are needed to convert 5 : 6-dihydroxyindole into melanin.

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