# *Some Problems of Tyrosine Metabolism.*

A LECTURE DELIVERED BEFORE THE CHEMICAL SOCIETY ON MARCH 4TH, 1937.

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TYROSINE is one of the amino-acids that has been longest known. It was first isolated in 1846 by Liebig, as a product of the fusion of cheese with caustic potash. Erlenmeyer and Lipp established its constitution and synthesised it in 1882. In proteins which form part of our diet it is not very abundant, and caseinogen, which contains about 6%, is the richest of the food proteins in this amino-acid. In an ordinary mixed diet probably not more than 3 g. are taken daily by the average adult. This is normally readily oxidised. Much larger amounts can be dealt with by the body, for Abderhalden, after taking 150 g., records that no intermediate products of its oxidation were secreted in the urine.

Attempts have been made to find out whether tyrosine should be included in the group of "essential" amino-acids. By this is meant amino-acids which must be supplied in food proteins because they cannot be synthesised in the body. In dogs, Abderhalden failed to get maintenance of body weight when the protein of the diet was replaced by an amino-acid mixture produced by the hydrolysis of caseinogen from which the tyrosine had been largely removed by crystallisation. On the other hand, recent experiments by Alcock have shown that rats will grow satisfactorily on such an amino-acid mixture even when the tyrosine has been removed completely by oxidation with the enzyme tyrosinase. Since there is satisfactory evidence that tyrosine may be produced in the body from another common constituent of proteins, namely, phenylalanine, there seems little reason to doubt that a deficiency of tyrosine in the diet may be made good in this way. The conversion of phenylalanine into tyrosine involves oxidation of the benzene nucleus in the para-position. So far no oxidation system in the body has been found which will bring about this change, though it can be achieved *in vitro* by means of Fenton's reagent.

The general mode of oxidation of amino-acids in the body was first indicated by Otto Neubauer, who, by making use of a non-natural amino-acid (phenylglycine), was able to show that it was converted into phenylglyoxylic acid, which was excreted in the urine:

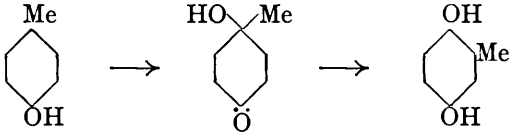
$$C_6H_5{\cdot}CH(NH_2){\cdot}CO_2H \longrightarrow C_6H_5{\cdot}CO{\cdot}CO_2H$$

This oxidative deamination of α-amino-acids with formation of the corresponding α-keto-acid has also been demonstrated to occur in the same type of experiment with other amino-acids which do not readily undergo oxidation, *e.g.*, α-amino-γ-phenylbutyric acid, *m*-chlorophenylalanine, and *m*-hydroxyphenylalanine. Enzymes which will bring about this oxidation have since been shown to be present in various organs, notably the kidney, by Kisch and Krebs. More recently an interesting example of this change has been found in certain cases of amentia. In these subjects phenylalanine is not oxidised beyond this first stage and they therefore regularly excrete phenylpyruvic acid in the urine.

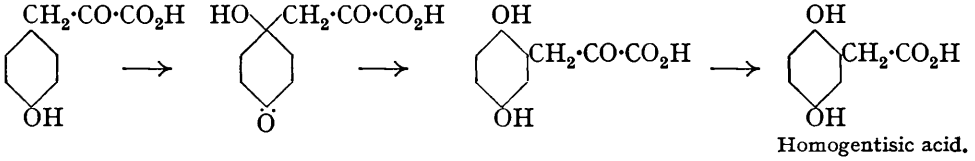
There is good evidence that tyrosine may undergo oxidative deamination in the body. A few years ago a case of abnormal tyrosine metabolism was discovered in Minneapolis by Miss Medes. This subject was admitted into hospital for the treatment of a disease of the muscles, *myasthenia gravis*. It was then discovered by accident that he excreted in the urine daily 2—3 g. of *p*-hydroxyphenylpyruvic acid. Administration of tyrosine caused an increase in the production of this substance, thus clearly indicating its origin. No other example of this metabolic abnormality has been discovered, and it appears to bear no relation to the disease for which the man was originally admitted to hospital. Normal subjects are able to oxidise *p*-hydroxyphenylpyruvic acid with ease, so in this particular case, although oxidative deamination could be effected normally, the mechanism for the next stage of oxidation was defective. This abnormality, which has been named tyrosinosis, has other points of interest in connection with tyrosine metabolism. When phenylalanine was administered to this patient, the excretion in the urine of both tyrosine and *p*-hydroxyphenylpyruvic acid was observed, thus indicating that phenylalanine can be converted into tyrosine in the body. Furthermore, when large amounts of tyrosine

were administered, a small amount of *l*-3 : 4-dihydroxyphenylalanine was found in the urine. This gives proof that nuclear oxidation of tyrosine may occur as well as the more easily demonstrated side-chain oxidation.

Tyrosinosis gives no information about the stages of oxidation subsequent to the production of *p*-hydroxyphenylpyruvic acid. For this we must go to another abnormality of tyrosine metabolism, namely, alcaptonuria. As far back as the 16th century, observations were recorded of patients who passed urine which turned black. In 1858 Boedeker detected a reducing substance in cases of this character, and in 1891 Wolkow and Baumann showed that this reducing substance was homogentisic acid. They also showed that it owed its origin to the tyrosine and phenylalanine of dietary proteins. Subsequently, it has been shown in these cases that homogentisic acid is also produced when *p*-hydroxyphenylpyruvic acid is administered. These subjects have therefore no difficulty in bringing about the first, and some of the subsequent stages in the oxidation of tyrosine. This abnormality of tyrosine metabolism raises two problems of considerable interest. First, how is homogentisic acid produced from *p*-hydroxyphenylpyruvic acid? Secondly, is homogentisic acid on the normal path of tyrosine metabolism, and is alcaptonuria therefore a condition in which homogentisic acid cannot be oxidised because of some deficiency in the normal mechanism for dealing with it, or is the production of this acid a pathological phenomenon, indicating a departure from the normal process of oxidation? The first problem arises because homogentisic acid is 2 : 5-dihydroxyphenylacetic acid, whereas its parent acid, *p*-hydroxyphenylpyruvic acid, is substituted in the 4-position. A reduction of the *p*-hydroxyl group in the latter and re-oxidation in the 2 : 5-position is unlikely, because the reduction of a nuclear hydroxyl group has not yet been demonstrated in the animal body. Further, *o*- or *m*-hydroxyphenylalanine would be expected to give rise to homogentisic acid by oxidation if this mode of formation were possible. But they produce no increase of homogentisic acid excretion in alcaptonuria. The most likely method of formation of this acid is by molecular rearrangement following oxidation. It has been known for many years that when *p*-cresol is oxidised with potassium persulphate, " toluquinol " is produced and this undergoes rearrangement to yield 2-methylquinol :




A similar change may possibly take place in the body when *p*-hydroxyphenylpyruvic acid is oxidised, yielding the corresponding 2 : 5-dihydroxy-acid. This by subsequent oxidation of the side chain—a change that has been demonstrated to occur in alcaptonuria—would give homogentisic acid :




Homogentisic acid.

It should be made clear, however, that, although this type of change has been shown to occur *in vitro* with *p*-cresol, no direct experimental proof of its occurrence with *p*-hydroxyphenylpyruvic acid has been obtainable in the laboratory.

Whether homogentisic acid is a product of tyrosine metabolism in normal human subjects is not easy to determine. They oxidise it readily when given by the mouth, and no evidence of its production when excessive amounts of tyrosine are ingested has been produced except a single instance reported by Abderhalden in which a laboratory assistant excreted a small amount following the administration of 50 g. of tyrosine. If the tyrosine molecule is altered so that the quinonoid rearrangement described above cannot take place, then homogentisic acid is not produced; *e.g.*, *p*-methoxyphenylalanine

is oxidised by the alcaptonuric individual without increase in homogentisic acid excretion. It is chiefly for this reason that it has been suggested that homogentisic acid production is a perversion of normal tyrosine metabolism. This experiment certainly indicates that an alternative path for the oxidation of the benzene nucleus is available even in alcaptonuria, but it does not give conclusive evidence that this path is the one which tyrosine always follows in the normal subject.

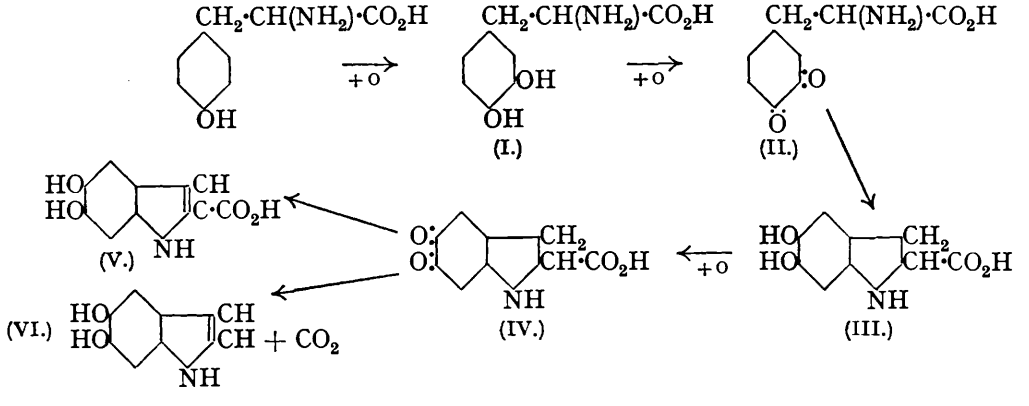
It seems unlikely that further progress in this field will be made until it is possible to isolate enzyme systems which oxidise tyrosine and thus enable a more detailed analysis of the separate stages to be undertaken.

*Pigment Formation from Tyrosine.*

Although the greater part of the tyrosine ingested and not utilised for tissue structure is probably oxidised by one or other of the mechanisms discussed above, the metabolism of some of it may proceed on other lines. One of these results in the production of a black pigment, melanin, which is found principally in hair, skin and the pigment layer of the eyeball.

An enzyme which oxidises tyrosine with the formation of melanin was discovered by Bourquelot and Bertrand in 1895. It was first found by them in certain fungi, but has since been shown to have a wide distribution not only in the vegetable but also in the animal kingdom. Its occurrence in mammals has been demonstrated by Onslow and Pugh, but it is much more commonly found in the invertebrates. The action of the enzyme on tyrosine is not specific. It will act on other monohydric phenols. When tyrosine is the substrate, a series of colour changes is produced. The first visible colour is red and from this various stages of brown are passed through until finally the black, insoluble melanin is deposited.

By modifying the $p_H$ of the reacting system it is possible to demonstrate three separate stages in the oxidation. Tyrosine gives rise to the red pigment in the first stage; in the second this pigment changes into a colourless substance and in the third stage the colourless substance is oxidised to melanin. The second stage is not oxidative, since it will take place in an atmosphere of nitrogen. The first stage is the only one which requires the presence of the enzyme, since the other two will take place in its absence. When the second stage has been completed, the presence of three substances derived from tyrosine can be demonstrated. They are 3 : 4-dihydroxyphenylalanine, 5 : 6-dihydroxyindole, and 5 : 6-dihydroxyindole-2-carboxylic acid. The following scheme has been put forward to explain the production of these three substances from tyrosine:




It involves the production of 3 : 4-dihydroxyphenylalanine ("Dopa") (I), and then of its quinone (II) by oxidation. The quinone next undergoes an intramolecular change with the production of 5 : 6-dihydroxy-2 : 3-dihydroindole-2-carboxylic acid (III). This in turn is oxidised to its quinone (IV), which is the red pigment appearing in the first of the three reaction stages described above. This pigment undergoes further change on

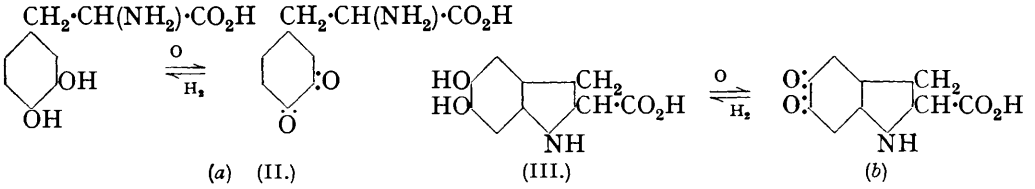
standing, with or without loss of carbon dioxide according to the conditions, and yields 5 : 6-dihydroxyindole (VI) or 5 : 6-dihydroxyindole-2-carboxylic acid (V) respectively. Both are oxidisable in the air, especially in alkaline solution, and yield a black pigment. Apart from the isolation of (I) as its lead salt and (V) and (VI) by methylation, there is other evidence in favour of this scheme. Dopa is very readily oxidised by the enzyme and gives the same end-products. When monohydric phenols are oxidised by the enzyme, they also yield the corresponding *o*-quinones, indicating the likelihood of dopa proceeding next to its quinone (II). Dopa, when oxidised with silver oxide, yields the same products as with the enzyme. The bases tyramine, β-3 : 4-dihydroxyphenylethylamine, and its *N*-methyl derivative all yield the corresponding dihydroxyindoles when oxidised by the enzyme. The red pigment (IV) has been found in the worm *Halla parthenopoea* by Mazza and Stolfi and undergoes a conversion into (V) under the same conditions as in the enzyme action. It may also be reduced catalytically to its dihydro-derivative (III), which will yield (IV) again on shaking in air. The worm *Halla parthenopoea* contains the enzyme tyrosinase, which is no doubt responsible for the production of the pigment. By using β-3 : 4-dihydroxyphenylethylmethylamine as substrate for the oxidation, the red pigment obtained from this may be separated as a crystalline monophenylhydrazone. Similarly, the red pigment from *Halla parthenopoea* (hallachrome) may be obtained in the form of a *p*-bromophenylhydrazone. There is thus a considerable body of evidence in support of the oxidation scheme put forward above.

The processes taking place in the last stage of the reaction, which consists in the conversion of 5 : 6-dihydroxyindole and its 2-carboxylic acid into melanin, are at present obscure. Two atoms of oxygen per molecule of indole derivative are utilised, but whether they both remain in the melanin molecule or only one of them is fixed, is undecided. Analyses of animal melanins have yielded different results according to their source. They are amorphous and difficult to purify. At present there is no satisfactory criterion of their purity. Beyond the statement that, in the formation of this pigment from the dihydroxyindoles, quinone formation is a probable first step, it is not possible to go.

Two additional points of interest about the tyrosinase reaction may be referred to. One of these is the stability of hallachrome and the other the accumulation of dopa. Hallachrome made by the action of tyrosinase on tyrosine or dopa is relatively unstable. It undergoes reduction very readily and in the quinone form may serve as a hydrogen acceptor in biological systems. Friedhein has demonstrated that in this way it may facilitate oxidation in the red blood corpuscles of the rabbit. As ordinarily produced in the enzyme action it persists for only a short time, since it changes with moderate rapidity into its corresponding dihydroxyindole. This change is difficult to prevent and this fact makes its isolation from the products of the enzyme action very difficult. On the other hand, as it occurs in the worm *Halla parthenopoea*, it is more stable and may be obtained by suitable means in crystalline form. This difference in stability is at present unexplained. It may be due to adsorption on the components of the epidermis of *Halla*, but trial of other adsorbents in the laboratory has not revealed any with similar stabilising properties.

The accumulation of dopa in the enzyme action is paradoxical. Dopa is much more rapidly attacked by the enzyme than tyrosine and should, therefore, never attain a concentration which would enable it to be isolated. Such, however, is not the case. The point is of interest because its explanation may help us to picture how catechol compounds may be produced under natural conditions. Recent work on this subject by W. C. Evans at Manchester has indicated what is the most likely cause of the accumulation. Two possibilities seemed likely. One was that dehydrase systems occurring in the crude enzyme were using the quinone of dopa as a hydrogen acceptor and so maintaining a certain concentration of dopa in the reacting system. The other was that some later product of the tyrosinase reaction was reducing the dopa quinone and itself being oxidised. Investigation of the ratio, dopa produced : tyrosinase oxidised under different conditions, has shown that the ratio depends on (1) the source of the enzyme, not because of the presence of dehydrases but rather of peroxidase. The latter enzyme diminishes the ratio.

And (2) the presence of reduced hallachrome, which increases the ratio. There appear to be two reversible reactions, (*a*) and (*b*) :



(*a*) (II.) (III.) (*b*)

Dopa quinone (II) is reduced to dopa by the dihydroindole derivative (III), into which it is normally transformed [reaction (*a*)], whilst the dihydroindole derivative is oxidised to hallachrome.

This accumulation of dopa in the tyrosinase–tyrosine reaction may be increased still further if ascorbic acid is present. This presumably acts also by reducing dopa quinone. In this or in some similar fashion we have a possible explanation of the mode of formation of catechol derivatives in nature.

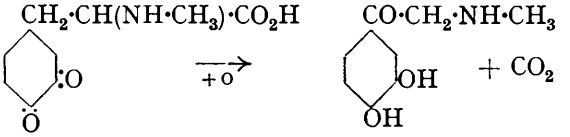
### *Is Melanin of Higher Animals produced from Tyrosine ?*

It is common to find tyrosinase and one of its substrates occurring together in lower animals, *e.g.*, the larvæ of the common blow-fly. This makes it reasonable to assume that the melanin produced in these animals is due to the presence of tyrosinase. In mammals, however, tyrosinase is not regularly found in pigmented regions, and the question has arisen, is pigmentation in the higher animals due to the same chemical mechanism as in the lower? There is some evidence that it is. Bloch has shown that in the pigment-forming cells of the skin an enzyme (dopa oxidase) is present which produces melanin from dopa. It is unlikely that the chemical changes involved are different from those which occur when tyrosinase acts on dopa. Dopa does not occur in food proteins, but Miss Medes has shown that it may be produced in the body from tyrosine, and this adds weight to Bloch's theory of pigment production. Furthermore, in certain cases of malignant disease in which pigmented new growths occur in the body—melanotic sarcomata—the urine may contain melanogens which on exposure to the air give rise to melanin. These melanogens give indole reactions and the injection of 5 : 6-dihydroxyindole into rabbits causes them to secrete urine containing a melanogen or melanogens with similar properties to those found in melanotic sarcoma. On the other hand, Eppinger has described a case of melanotic sarcoma in which the amount of melanogen in the urine was increased when the amino-acid tryptophan was administered, whereas tyrosine gave no increase. The nitroprusside reaction was used for the estimation of melanogen. This is not specific for indole derivatives, so it is possible that some substance containing the pyrrole ring but not indole was concerned. Whether it was a derivative of a precursor of melanin or produced by degradation of melanin in the body, cannot be decided, but it does leave open the question as to whether Bloch's scheme of melanin formation is the only one concerned in higher animals.

### *The Origin of Adrenaline.*

It has often been suggested that tyrosine may be the precursor of adrenaline, because of the close structural relationship between these two substances. Many attempts have been made to demonstrate adrenaline formation from tyrosine by the suprarenal gland, but so far without success. Possible intermediates in the process, *e.g.*, dopa, *N*-methyl dopa, and adrenalone, have all failed to give adrenaline when added to the minced gland or circulated in solution through its bloodvessels. Recently, however, Schuler and Wiedermann have shown that thin slices of the gland when placed in tyramine solution will give a slight formation of adrenaline, so that there is biological evidence that tyrosine may be the amino-acid originally concerned in this synthesis. It has also been demonstrated that the enzyme tyrosinase, acting on *N*-methyl dopa, gives rise to a small amount of adrenalone, although the main reaction, as with dopa itself, is pigment formation.

From what we know of the action of tyrosinase, the adrenalone is probably produced from *N*-methyl dopa quinone :




It is not oxidised by the enzyme and hence accumulates. It seems conceivable, therefore, that some such side reaction might become the principal one, if the process leading to indole formation by ring closure were inhibited. In a methylated tyrosine peptide, for instance, this would be possible, since indole formation would be prevented, but not quinone formation.

*Thyroxine.*

Since the elucidation of the structure of thyroxine as a di-iodophenyl ether of di-iodotyrosine it has been apparent that this hormone is probably derived from tyrosine. This evidence has been strengthened by the isolation of 3 : 5-di-iodotyrosine from the thyroid gland and more recently by the proof that *l*-tyrosine and *l*-thyroxine are related configurationally. So far all attempts to convert di-iodotyrosine into thyroxine by enzyme action have failed, so direct biochemical proof is lacking. The synthesis probably requires conditions that can only be found in the living cell and may require not one enzyme, but several. This, therefore, is also a problem of tyrosine metabolism still awaiting solution.

Although in this lecture the problems of tyrosine metabolism have not been exhausted, perhaps sufficient has been said to indicate that even a single amino-acid, out of the twenty or more that proteins may contain, may provide problems that will only be solved by the close co-operation of the chemist and the biologist.