CCXII. TYRAMINE OXIDASE

By HENRY IRVING KOHN

From the Molteno Institute, University of Cambridge

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We owe our present knowledge of tyramine oxidase to M. L. C. Bernheim. In her first paper [Hare, 1928], she reported that cell-free extracts of the livers of the rat, sheep, pig, ox, and dog oxidize tyramine (*p*-hydroxyphenylethylamine). A detailed study on the rabbit's liver showed that one atom of oxygen is consumed and half a molecule of ammonia is released. The oxidase is not inhibited by cyanide and cannot reduce methylene blue, and qualitative evidence was presented that hydrogen peroxide is formed during the reaction. In a second paper, using guinea pig liver, Bernheim [1931] reported that the number of atoms of oxygen consumed varies from one to four, depending upon the *p*H of the medium and the age and concentration of the extract. In the presence of cyanide only one atom is used.

On the other hand, Ewins & Laidlaw [1910] showed that orally administered tyramine can be partially recovered in the urine as the corresponding aromatic acetic acid (dog, 25 %) and that about 70 % equivalent acid can be recovered in the perfusion of the liver (cat and rabbit), although they failed to obtain an acid production with chopped liver. Bernheim [1931], however, showed in the case of pig liver that this was due to a lack of aeration.

From the foregoing it is certain that tyramine is converted into *p*-hydroxyphenylacetic acid by the liver, and that this involves an oxidase system. However, the exact nature of the reaction catalysed by tyramine oxidase is not quite clear. In this paper we shall show that there is a system which brings about the reaction:

$$OH.C_6H_4.CH_2.CH_2.NH_2 + H_2O + O_2 \rightarrow OH.C_6H_4.CH_2.CHO + H_2O_2 + NH_3$$

and for the present we shall specifically reserve the term *tyramine oxidase* for this system, although perhaps amine oxidase would be more descriptive, as will be seen later. No doubt the further oxidation of the above aldehyde, possibly by Schardinger enzyme or aldehyde mutase, gives rise to the acid in question.

It will be observed that this reaction is a perfect example of the Wieland type of oxidation, involving hydration and peroxide formation, although a suitable acceptor to replace oxygen is not yet known. Furthermore, it is analogous to the reaction of *d*-amino-acid deaminase in which the non-terminal amino-group is removed and the corresponding ketone formed [Krebs, 1933; 1935]. However, Keilin & Hartree [1936, 1] have shown that the dimethylamino-group is not attacked by the deaminase, while tyramine oxidase does oxidize at least one tertiary amine. The relationship of "adrenaline oxidase" to tyramine oxidase will be considered later.

Concerning the physiological significance of tyramine oxidase, nothing can be said at present. It is of interest, however, that Heinsen [1936] reports the formation of tyramine from tyrosine by the pancreas. Also, of the substrates tested, tyramine seems to be the most "suitable" for the enzyme.

Preparation

Tyramine oxidase is insoluble (or is associated with insoluble material), but it can form a rather stable suspension in the range of *p*H 7–8. The suspension is easily precipitated by making the reaction acid to methyl red. These facts allow the enzyme to be concentrated and, to a certain extent, freed from extraneous material.

Attempts were made to employ other precipitating agents, such as ammonium sulphate and alcohol, to extract fat etc., but these were unsuccessful. As the washing proceeds, and particularly once the enzyme has been "released" by grinding with sand, it becomes very much more fragile. Thus the final product is more or less completely inactivated by precipitation with ammonium sulphate, alcohol, or acetone, or by precipitation resulting from dialysis against tap water. Also, drying the precipitate completely inactivates it, as does several minutes of boiling.

Preparation. 450 g. of pig liver were minced in a Latapie mincer, suspended in 1·5 times their volume of tap water,[1] and squeezed through muslin. The resulting suspension was made up to 4 times its volume with tap water and allowed to stand for about three-quarters of an hour during which the insoluble material settled out. The top layer of liquid was sucked off, and the insoluble material centrifuged out of the bottom layer (*c.* 800 ml.). The precipitate thus collected was washed (suspended, then centrifuged out) once with 800 ml. of tap water, twice with 800 ml. of 0·9 % NaCl, and once with 800 ml. of 0·1 *M*, *p*H 7·7, phosphate buffer. The final weight was 85 g. These were ground in a mortar with an equal volume of sand for about 5 min., then extracted with 800 ml. of 0·03 *M*, *p*H 7·7, phosphate buffer. After centrifuging, the extract was precipitated by gradually adding normal acetic acid until the reaction was on the acid side of methyl red (colour, reddish orange). After centrifuging briskly, 27·5 g. of precipitate (dry weight = 8·5 %) were obtained; these were quickly made up to 100 ml. in 0·3 *M*, *p*H 7·7, phosphate buffer. Generally the Q_{O_2} (μl. O_2 per hr. per mg. dry weight) of such a preparation, when saturated with tyramine is between 8 and 14 at 39°. The dry weight may be as low as 5 %. The enzyme solution will keep for 4–7 days if stored in the ice chest. At first the blank is negligible, but it increases with time, and sometimes, when it is large, its subtraction from the experimental rate leads to a negative instead of a zero rate at the end of the reaction.

Brei may also be used to demonstrate tyramine oxidase. That prepared by mincing liver with the Latapie, when mixed with buffer, consists of a suspension of cells or small clumps of cells. In 2 ml. of Ringer-phosphate, at *c.* *p*H 7·3, $T = 39°$, 0·5 g. has a Q_{O_2} of about 4·5.

We shall define the number of enzyme units E.U. as equal to grams dry weight multiplied by Q_{O_2} (39°). According to the brei experiments, which most likely set the lower limit, an ordinary pig liver weighing about 3 lb. contains something like 1800 E.U. The preparation already described in detail yielded 2·3 g. dry weight of Q_{O_2} 10, or 23 E.U. from 1 lb., a yield of about 5 %. Two sources of loss in the method of preparation are (1) squeezing through muslin eliminates a considerable amount of coarse material, (2) precipitation with acetic acid reduces the activity by about 30 %.

Properties of tyramine oxidase

(*a*) *Method*

Unless otherwise stated, all experiments detailed below were performed at 38·5–39° in air, using enzyme solution prepared as described above. The gas measurements were made with the Barcroft differential manometer, the total volume of material in each vessel being equal. The right-hand vessel contained 2·5 ml. of enzyme solution and 0·3 ml. of 15 % KOH in the inset. Tyramine

[1] It is preferable, though not necessary, that cold (5–10°) solutions be used throughout.

(1–3 mg.) dissolved in about 0·2 ml. of water was added at zero time from a Keilin dangling cup. The left-hand vessel contained an equivalent amount of buffer and KOH. A separate manometer was employed to determine the blank (oxygen consumption in absence of tyramine) which was subtracted from the consumption of the tyramine-containing sample.

(*b*) *Oxygen consumption*

In view of Bernheim's statements concerning the effect of age, concentration, and *p*H on the number of equivalents of oxygen consumed, the preparation was tested with respect to these factors, and the oxygen consumption was found to be largely independent of them and equal to 1 atom per molecule of tyramine. Curves illustrating the one atom uptake may be seen in Figs. 3 and 4. Sometimes the total consumption exceeds the theoretical, but in all such cases the course of oxygen uptake can be divided into two parts, an initial rapid phase involving about one atom, and a second very slow phase involving the extra amount. On the other hand, if too much tyramine per enzyme is present, an uptake of less than the theoretical will result. This most likely is due to the inhibitory effect of the reaction product [Hare, 1928].

As the *p*H is decreased, the rate of tyramine oxidation decreases. The theoretical yield can still be obtained at *p*H 6·3, the rate being only about 40 % of that at *p*H 7·7 (when stored overnight). The results at *p*H 5·3 are obviously of a different nature. Tyramine at this *p*H is oxidized, but initially at only about 5 % of the standard rate. Furthermore, the course of the oxidation is different, practically stopping at about one-third of an equivalent. The blank respiration behaves in just the opposite way, increasing fairly rapidly on the acid side of neutrality, the rate at *p*H 5·3 being about three times that at *p*H 7·7; this indicates the fundamental difference between the blank and the tyramine systems.

(*c*) *Inhibitors and carriers*

Cyanide has no effect upon the oxidation of tyramine, even at a concentration of 0·01 *M* (neutralized) but it may be used to reduce the blank and to inhibit the extra oxidation which makes the oxygen-tyramine ratio too high. Also, neither hydroxylamine nor iodoacetic acid (0·01 *M* neutralized) inhibits the reaction, but 0·01 *M* hydrazine hydrate (neutralized) inhibits about 30 % as does 4 % ethyl urethane.

As already noted, the oxidase system exhibits the properties of a thermolabile protein. Furthermore, dialysis (which should be made against buffer) does not decrease its activity, so that it probably comprises only a protein component. This does not react with methylene blue.

(*d*) *Oxygen tension*

The rate of oxidation is proportional to the oxygen tension (Fig. 1, dotted circles), the increase in going from air to oxygen being generally 200–250 %. This behaviour is in marked contrast to that of the Warburg-Keilin system which *in vivo* is certainly saturated below 10 %.

For comparison, some experiments were made with xanthine oxidase prepared from milk by precipitation with ammonium sulphate [Dixon & Kodama, 1926]. The right-hand flasks contained 2·5 ml. of 8 % enzyme solution (*p*H 7·7) to which there was added 0·1 ml. of strong catalase solution. The catalase protects the oxidase from the peroxide formed during the course of the reaction. The Keilin cup contained 1 mg. of hypoxanthine dissolved in 0·3 ml. of 0·02 *M*

soda. The left-hand vessel contained only enzyme and catalase. The temperature was 38·5°. The series shown in Fig. 1 (solid circles) indicates that the enzyme is saturated at about 20% oxygen, thus placing it between tyramine oxidase and

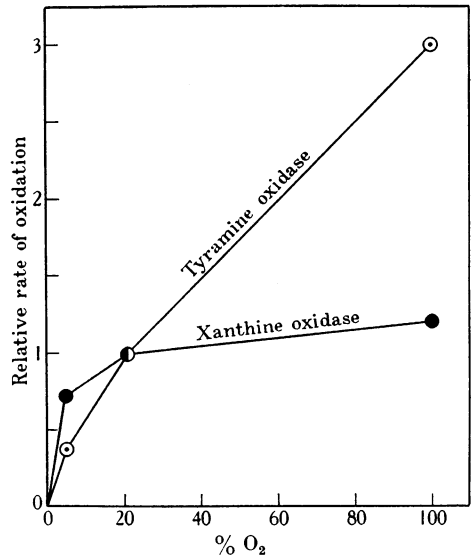

Fig. 1. Relative rate of oxidation as a function of oxygen tension. Dotted circles are for tyramine oxidase, solid for xanthine oxidase.

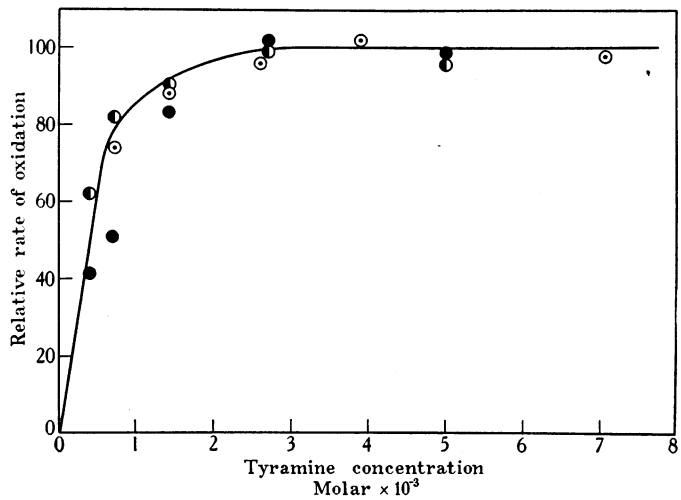

Fig. 2. Relative rate of oxidation as a function of the concentration of tyramine.

the Warburg-Keilin system. If methylene blue (1×10^{-5} M) is present, the rate of oxidation in air is increased by 35%, and in 1·5% oxygen by 60%. In the latter case the methylene blue remains in the reduced state, indicating that the factor limiting the increase in rate is the velocity of methylene blue oxidation

(e) *Tyramine concentration*

Fig. 2 shows the relationship between rate of oxygen consumption and tyramine concentration, based on three separate experiments. For each experiment a smooth curve was drawn through the graphed data in order to determine the limiting value of the rate. This was then set equal to 100 and the values of the rates at lower concentrations recalculated relative to it. The basic data for any particular concentration were obtained as follows. At zero minutes the Keilin cup was dislodged, and thereafter the course of the oxidation was followed as closely as possible. The rate, of course, decreases with time, but from the data the initial maximum rate can be estimated. This can be done accurately at high concentrations, but when the concentration is below 0·001 *M* the determination is rather inaccurate, since the total oxidation is small and the rate is changing very rapidly. At the lowest concentrations it was necessary to use 5 or 7 ml. of solution in the vessels.

In Fig. 2 the two lowest points (solid circles) are probably too low. Nevertheless, although the points in this region scatter somewhat, they fix the concentration for 50 % of maximum rate between 0·4 and $0{\cdot}7 \times 10^{-3}$ *M*, and the smooth curve places it as $0{\cdot}5 \times 10^{-3}$ *M*. As for the saturating concentrations, these clearly lie at and above $2{\cdot}5 \times 10^{-3}$ *M*. The 50 % concentration for amino-acid oxidase (tested with *dl*-alanine) is 5×10^{-3} *M* [Keilin & Hartree, 1936, 1].

The reaction products

(a) *Ammonia*

That the tyramine is deaminated, one molecule of ammonia being released for one equivalent of oxygen consumed, is shown by the following experiment.

Three manometers were used, and at zero time their respective enzyme solutions received (*a*) zero, (*b*) 1·77, (*c*) 3·00 mg. of tyramine. The net tyramine oxygen consumption was at 20 min. (*b*) 147 μl., (*c*) 154 μl. and at 65 min. (*b*) 156 μl., (*c*) 248 μl. Just after 65 min. the reactions in (*a*), (*b*) and (*c*) were stopped by the addition of 1 ml. of 20 % trichloroacetic acid and the solutions removed from the vessels, which were washed out with 2 ml. of distilled water. The solutions were then neutralized by adding 1·1 ml. of *N* NaOH and filtered. A sample (about 5 ml.) of the clear filtrate was distilled under reduced pressure at room temperature by steam in the Parnas apparatus. The distillate, received in enough HCl, was made up to 25 ml. Samples of the latter were nesslerized, and their colour was compared to standards which contained equivalent amounts of the blank. The results were as follows. To (*b*) there were added 1·77 mg. of tyramine, which consumed 1·07 atoms of oxygen and formed 1·01 molecules of ammonia per molecule of tyramine. To (*c*) there were added 3 mg. of tyramine, which consumed 1·02 atoms of oxygen and formed 1·00 molecule of ammonia.

(b) *Peroxide*

In order to demonstrate the production of peroxide, enzymic methods involving coupled oxidations were resorted to; it is not possible to eliminate the catalase activity of the tyramine oxidase preparations, so that very fast reactions must be used to catch the peroxide. The most successful one, which gave a theoretical yield of H_2O_2 and which was used by Hare for qualitative detection of H_2O_2, consisted in adding peroxidase.

In Fig. 3 there is shown the influence of adding various amounts of horseradish peroxidase. The theory of the experiment is as follows. If peroxide is

formed, the catalase present will decompose it into water and oxygen. However, in the presence of peroxidase and a suitable substrate, the peroxide will be used in an oxidation; hence, its oxygen will not be released and the net oxygen uptake will increase by an amount equivalent to the peroxide formed. The Q_{O_2} of the tyramine oxidase preparation was about 15, and there were 40 mg. (dry weight) of it in 2·5 ml. of solution. For this quantity of enzyme acting on 0·78 mg. of tyramine, something less than 0·1 P.U.[1] is sufficient to double the oxygen uptake.

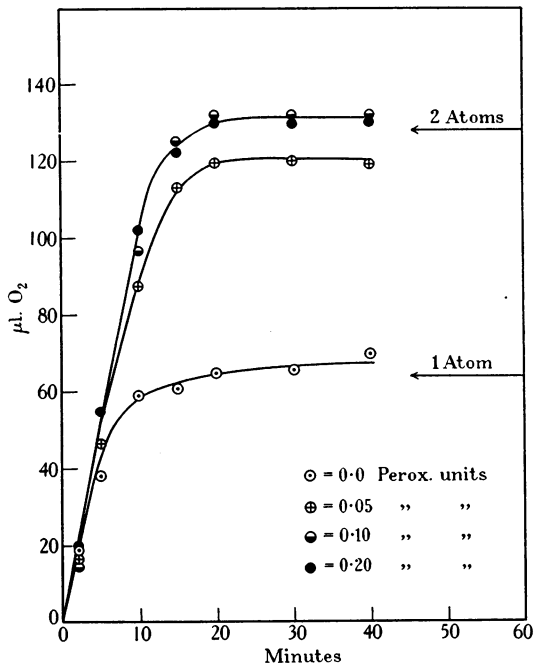

Fig. 3. Increase in total oxygen consumption due to presence of peroxidase, indicating peroxide formation.

We are therefore led to the conclusion that peroxide is formed. This signifies that actually one molecule of oxygen is used per molecule of tyramine oxidized, but that owing to the decomposition of a product (peroxide) it appears as if only 1 atom of oxygen is necessary.

The question arises as to what is the substrate of the coupled peroxidase reaction; the evidence suggests that it is tyramine[2] or its initial oxidation product. (1) Dialysis of the enzyme preparation does not prevent the reaction. (2) When tyramine is added to peroxidase and peroxide, the solution turns yellow owing to oxidation of the tyramine. The addition of peroxidase and peroxide to the enzyme solution causes no colour change. At the end of the coupled oxidation, however, the enzyme solution is darker.

Keilin & Hartree [1936, 2] have shown that alcohol is oxidized to acetaldehyde by the hydrogen peroxide produced during the course of certain enzyme

[1] The *Purpurogallinzahl* or P.Z. is equal to the number of mg. of purpurogallin formed in 5 min. at 20° by 1 mg. of dry enzyme. The number of *peroxidase units* or P.U. = g. dry weight × P.Z.

[2] Probably the ring portion of the molecule.

reactions. Since the tyramine oxidase preparation contains catalase, only the addition of alcohol should be necessary to test for peroxide formation. Fig. 4 shows that adding 8 mg. of alcohol almost doubles the oxygen consumption. At the end of this experiment the potash papers of the 8 mg. and 2 mg. vessels were definitely yellow, indicating, as Keilin & Hartree [1936, 2] had found, that the acetaldehyde formed had distilled over and had undergone a condensation. The 0·35 mg. paper was only tinged, while the 0·0 mg. paper was colourless.

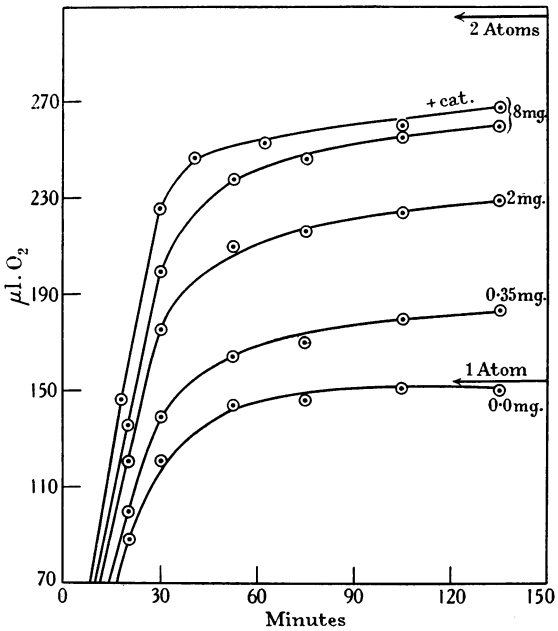

Fig. 4. Increase in total oxygen consumption due to the addition of 0·0, 0·35, 2·0 and 8·0 mg. of alcohol, and of 8 mg. of alcohol plus 28 γ (haemin content) of catalase.

The uppermost curve in Fig. 4 shows that the addition of an amount of catalase whose haemin content was 28γ does not further increase the extra oxygen consumption due to 8 mg. of alcohol. The potash paper turned yellow in this experiment. In other experiments it was found that the addition of catalase by itself would increase the oxygen uptake, 30γ (haemin content) causing an increase of 70%. In these cases the potash papers never became coloured, showing that contamination of the catalase by alcohol (used in its preparation) was not the cause. This also showed how thoroughly the catalase had been dialysed. The substrate for this reaction is therefore in the tyramine oxidase solution and may be the tyramine or its oxidation product. As a matter of fact, Raper [1932] has shown that tyramine is oxidized by peroxide in the presence of ferrous sulphate to a catechol compound.

(*c*) *Aldehyde*

There are two lines of evidence that lead to the conclusion that an aldehyde is formed when tyramine is oxidized. In the first place, aldehyde formation can be detected manometrically on adding Schardinger enzyme which increases the oxygen consumption up to 75%. Secondly, a precipitate was obtained when the reaction mixture, cleared of protein with trichloroacetic acid, was poured into a

cold saturated solution of 2:4-dinitrophenylhydrazine in 2*N* HCl and allowed to stand on ice for 1 hr. After washing with 2*N* HCl, water and several drops of alcohol and ether, a 20 % yield was obtained.

(*d*) *Discussion*

The fact that Bernheim [1931] obtained an oxygen consumption of more than one equivalent in certain cases is in no way contradictory of the present results. Her preparations, consisting of liver ground with sand, must contain, in addition to substrates, Schardinger enzyme, aldehyde mutase and other enzymes. Furthermore, haemoglobin is present which is oxidized to methaemoglobin by nascent peroxide [Bernheim & Michel, 1937], as well as the intracellular haematins which can function like peroxidases.

Specificity

(*a*) *Hordenine*

Hordenine p-OH.C_6H_4.CH_2.CH_2.N$(CH_3)_2$ is the tertiary amine corresponding to tyramine, and its maximum initial rate of oxidation is about 75 % of that of the latter. However, the stoichiometrical relationships obtained with hordenine are not good; when, for example, 1·85, 4·64, and 11·2 mg. were added to 2·5 ml. samples of oxidase, the number of atoms of oxygen consumed per molecule of hordenine were respectively 1·19, 0·65, 0·36. In general it may be said that small quantities of hordenine consume one equivalent of oxygen, but large ones consume less.

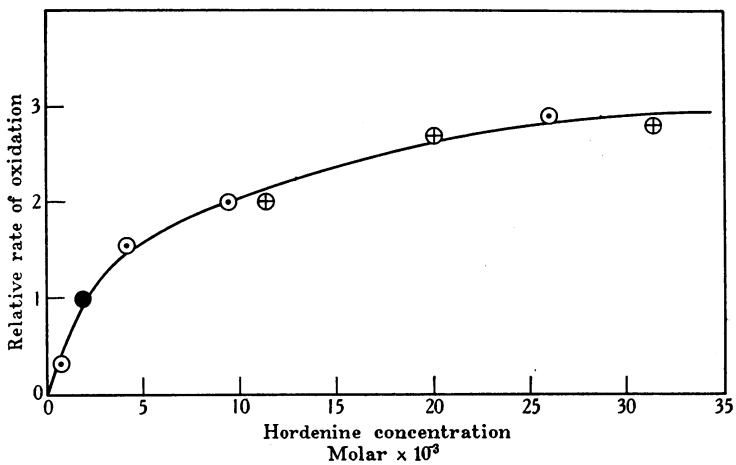

Fig. 5. Relative rate of hordenine oxidation as a function of hordenine concentration.

Fig. 5 shows the relationship between rate of oxidation and concentration, based on two experiments (dotted circles and crossed circles). In each case the rate at $1{\cdot}9 \times 10^{-3}$ *M* (solid circle) was taken for unity, and the individual points were determined as in the case of tyramine. The curve which is rather stretched out places the half-saturating concentration at about 5×10^{-3} *M*. This is ten times that for tyramine, and consequently the affinity constant is only one-tenth as great.

Evidence that tyramine and hordenine are oxidized by the same enzyme can be obtained from competition experiments, as illustrated by Fig. 6. The argument is as follows. If two enzymes are involved, the rate of oxidation of tyramine and hordenine added simultaneously to oxidase solution should equal the sum of their rates of oxidation determined separately. On the other hand, if only one enzyme is involved (and if approximately saturating concentrations of the substrates are used), then the sum of the individual rates should be greater. The theory rests upon the assumption that if two enzyme systems are involved, their reactions can proceed without mutual interference, a condition not always fulfilled [cf. Keilin & Hartree, 1936, 1, p. 130].

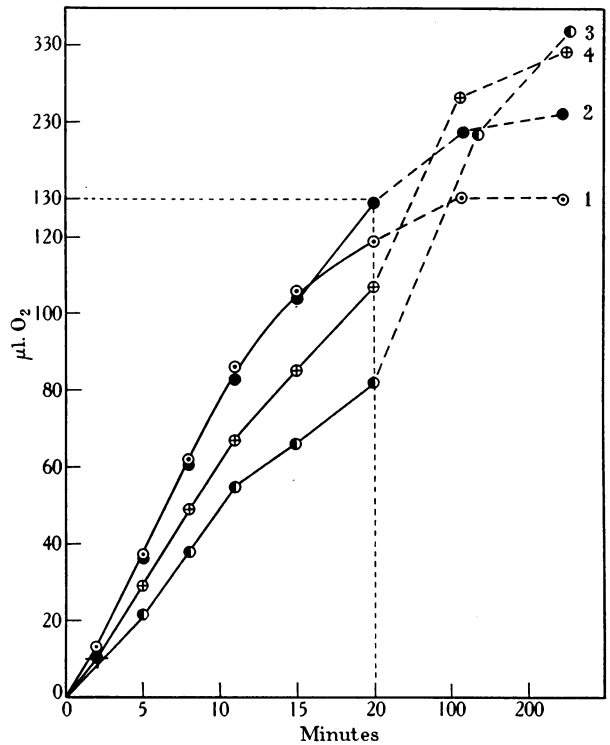

Fig. 6. Competition between tyramine and hordenine for tyramine oxidase.

The evidence of the competition experiments definitely favours the view that only one enzyme is involved. Fig. 6 illustrates such an experiment; curve 1 is for tyramine alone and curve 3 for a seven times greater concentration of hordenine alone. Curve 4 describes the reaction when the tyramine and hordenine of 1 and 3 are added together; the resulting rate is not the sum but the average of the individual rates. Curve 2 describes the reaction when equimolar amounts of tyramine and hordenine are added together. In this case, the rate is almost exactly that for tyramine alone (curve 1), which is to be expected as a result of the difference in the affinity constants.

(b) Adrenaline

Blaschko & Schlossmann [1936, 1, 2] have reported that adrenaline 3:4-$(OH)_2C_6H_3.CH(OH).CH_2.NH(CH_3)$ is oxidatively inactivated by extracts of rat liver in the presence of 10^{-3} *M* HCN. More recently, together with Richter, they have found "adrenaline oxidase" in mammalian intestine and kidney [1937, 1] and have noted the oxidation of related compounds [1937, 2]. During the course of my work Dr Blaschko informed me that his preparations oxidized tyramine, and in order to learn whether one system was responsible for both oxidations, Dr H. Schlossmann and I performed a number of experiments on my oxidase preparations. These experiments are in agreement with unpublished data and conclusions of Blaschko, Schlossmann & Richter, and the reader is referred to their forthcoming paper for a discussion of them and of related matters of specificity.

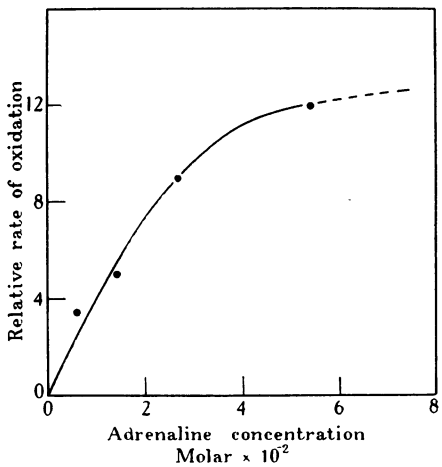

Fig. 7.

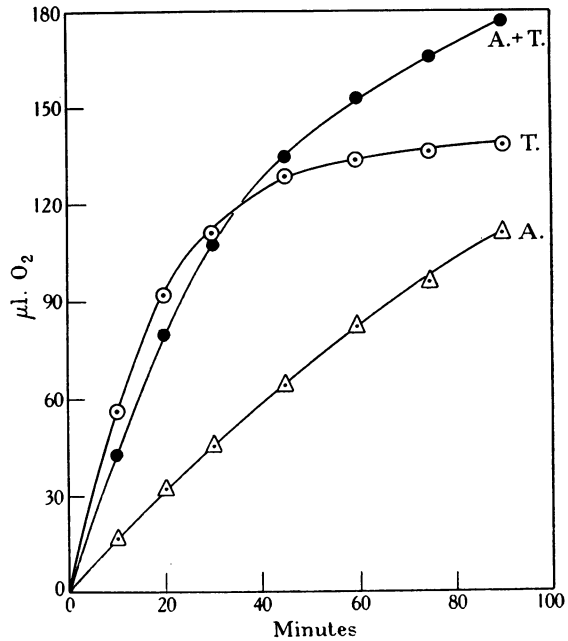

Fig. 8.

Fig. 7. Relative rate of adrenaline oxidation as a function of adrenaline concentration.

Fig. 8. Competition between adrenaline and tyramine for tyramine oxidase. $T = 5 \times 10^{-3}$ *M* tyramine; $A = 5 \times 10^{-2}$ *M* adrenaline.

The experiments were carried out at 38°, *p*H 7·1, in an atmosphere of oxygen. In order to inhibit the autoxidation of adrenaline, 2 mg. of glutathione (in a total reaction volume of 2 ml.) were added as well as cyanide (10^{-3} *M*). The substrates used were neutralized. KOH containing cyanide was present in the inset.

(1) Before attempting an inhibition experiment, it was necessary to determine the relationship between rate and adrenaline concentration. Fig. 7 shows the curve obtained; evidently 5×10^{-2} *M* fails to saturate, and the "50 %"

concentration must lie above $1{\cdot}5 \times 10^{-2}$ *M*. This is about 4 times that of hordenine and 40 times that of tyramine.

(2) Fig. 8 shows the competition for oxidase between tyramine and adrenaline. Curve *T* has an initial Q_{O_2} of 26 and represents the oxidation of 1·4 mg. of tyramine, the initial concentration being 5×10^{-3} *M*. Curve *A* represents the oxidation of 19 mg. of adrenaline, the initial concentration being 5×10^{-2} *M*, and has an initial Q_{O_2} of 9. Curve $A+T$ results from the simultaneous oxidation of 1·4 mg. of tyramine and 19 mg. of adrenaline, the initial Q_{O_2} being 19·5.

Two conclusions follow from these results. First, the tyramine oxidase can oxidize adrenaline in contradiction to the statement of Hare [1928], but the low value of the affinity constant argues against this reaction being of physiological importance. Thus even assuming as high an adrenaline concentration as 10^{-4} *M*, the enzyme would be working at less than 1 % of its optimum rate at substrate saturation. Furthermore, other compounds are much better substrates for the oxidase. Second, the existence of a separate adrenaline oxidase is rendered unlikely; presumably it is tyramine oxidase.

(*c*) *Other substrates*

Two other substrates were tested. β-Phenylethylamine is oxidized as was also shown by Hare, no doubt to the corresponding aldehyde as indicated by the hyacinth-like smell of the reaction mixture and the uptake of about one atom of oxygen. Low concentrations ($3{\cdot}8 \times 10^{-4}$ *M*) are oxidized more rapidly than higher ones and almost as rapidly as tyramine. *iso*Amylamine is also oxidized; when small amounts at low enough concentrations are used (0·5 mg., $1{\cdot}15 \times 10^{-3}$ *M*) about one equivalent of oxygen is consumed, at a rate equal to perhaps 50 % of the tyramine maximum.

(*d*) *Discussion*

From the foregoing it follows that tyramine oxidase can oxidize primary (tyramine), secondary (adrenaline), and tertiary (hordenine) amines. It also seems likely that the phenolic nature of the ring is not necessary (β-phenylethylamine), or even the ring itself (*iso*amylamine). In this connexion it is of interest that Blaschko *et al.* [1937, 2] have reported that adrenaline oxidase oxidizes primary and secondary amines, and that the ring need not possess OH groups. Also, Pugh & Quastel [1937] have found that *iso*amylamine is oxidized by brain and liver, the oxidation product giving a precipitate when treated with 2:4-dinitrophenylhydrazine.

Summary

1. Details for the preparation of tyramine oxidase from pig's liver are given. No coenzyme is required. It is shown that the enzyme catalyses the following reaction:

$$OH.C_6H_4.CH_2.CH_2.NH_2 + H_2O + O_2 \rightarrow OH.C_6H_4.CH_2.CHO + H_2O_2 + NH_3.$$

2. Methylene blue cannot replace oxygen. Neither cyanide nor iodoacetic acid nor hydroxylamine inhibits the reaction (conc. $= 10^{-2}$ *M*).

3. The relative rates of oxidation in 5, 21 and 100 % oxygen are 0·4, 1 and 3 respectively. Those for xanthine oxidase are 0·7, 1 and 1·2.

4. The specificity of the enzyme seems to be chiefly concerned with the amino-group. Competition experiments show that the one enzyme attacks primary (tyramine), secondary (adrenaline), and tertiary (hordenine) amines. It probably also attacks aliphatic amines (*iso*propylamine).

5. The half-saturating concentrations are: tyramine 5×10^{-4} *M*, hordenine 5×10^{-3} *M* and adrenaline $1{\cdot}5 \times 10^{-2}$ *M*. This indicates that the oxidation of adrenaline is not of physiological importance.

6. From the similarity in their properties, it appears that adrenaline oxidase and tyramine oxidase are the same.

In conclusion, I wish to express my thanks to Prof. D. Keilin, who suggested the problem to me, for his hospitality during my stay at the Molteno Institute.

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Note added 24 September 1937. Since the completion of this paper, Philpot [1937], has reported upon the oxidation of tyramine by liver slices and extracts. The source of the liver was not stated. She found one atom of oxygen per molecule of tyramine with slices, and two atoms with extracts, contrary to Hare-Bernheim. In each case she found 80–90% of the expected ammonia. She observed a large increase in the total oxygen consumed upon adding alcohol, concluding that peroxide is formed. Philpot does not believe xanthine oxidase to be responsible for the consumption of the second atom of oxygen, which is accompanied by the formation of a small amount of *p*-hydroxyphenylacetic acid. She obtained a precipitate with 2:4-dinitrophenylhydrazine after the uptake of either one or two atoms. She also made the important observation that *o*-bromophenolindophenol and *o*-cresolindophenol are reduced only in the presence of tyramine and estimates the potential of the system to lie between $-0{\cdot}046$ and $+0{\cdot}195$ v.