CXIII. SOME OBSERVATIONS ON THE OXIDATION OF TYRAMINE IN THE LIVER

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THE oxidation of tyramine in the liver has been investigated *in vivo* by Ewins & Laidlaw [1910] and *in vitro* by Hare [1928] and Bernheim [1931]. The former workers found that in feeding and liver perfusion experiments tyramine disappeared and *p*-hydroxyphenylacetic acid could be recovered from the urine and perfusates. Hare and Bernheim, using the Barcroft and Warburg manometric techniques, studied the oxidation of tyramine by liver extracts and "suspensions".

In the course of recent work I have made further observations which seemed to be of interest concerning the oxidation of tyramine and the general question of the investigation of tissue oxidations.

TECHNIQUE

Measurement of oxygen uptake. The usual Barcroft technique was employed. All experiments were performed at 38°. The tyramine was added to the other reagents after temperature equilibration. When using tissue slices the tyramine was added direct and allowance was made, in considering the results, for oxidation occurring during the period of equilibration. The manometers and bottles were evacuated and filled with oxygen when slices were used. All experiments were done at least in duplicate.

Liver preparations. The following types of liver preparations were made.

(1) Slices were cut with a razor as soon as possible after removal of the liver from the animal and floated on Ringer's solution. Just before beginning the experiment they were drained on filter paper, weighed on a torsion balance and quickly placed in phosphate buffer in the Barcroft bottles. About 100 mg. wet weight of slices were used in each bottle.

(2) The rest of the liver was either minced and ground with sand in Ringer's solution or was frozen solid under Ringer's solution and then pounded up. This second method was always used latterly. The resulting pulp was then squeezed through muslin and centrifuged. The precipitate was suspended in phosphate buffer or was further washed with Ringer on the centrifuge. The supernatant fluid was generally discarded.

(3) The pulp which came through the muslin (see (2) above) was treated with an equal volume of saturated $(NH_4)_2SO_4$ and the precipitate filtered off and dried *in vacuo*. When dry this could be very finely powdered.

(4) Minced liver was treated several times with acetone, sucked dry on a Büchner funnel, dried further *in vacuo* and finely powdered.

Phosphate buffer. *M*/15 phosphate buffer of *p*H 7·4 was used throughout.

Tyramine. Solutions were made up containing 20 mg. of free base per ml.

Ammonia was estimated according to Hare [1928]. Toluene was used to prevent frothing (octyl alcohol affects Nessler's reagent). The solutions were estimated colorimetrically against a standard solution of ammonium chloride.

Experimental

Activity of the enzyme. The activity of any preparation was defined as the O_2 uptake in μl. per 30 min. per mg. total N. The N was determined by the micro-Kjeldahl method on samples taken from the same preparations. It can be seen from Table I that the enzyme does not go into solution and that the activity of the washed debris is comparable with that of the slices.

Table I. *Activities of different fractions*

Preparation	Activity
Whole pulp	9·0
Precipitate (after centrifuging × 1)	34·5
Supernatant (after centrifuging × 1)	6·0
Slices	34·0

Activity = oxygen uptake in μl. per 30 min. per mg. N (total). These figures are the average of determinations on five livers.

Oxygen uptake. Using apparently similar liver "suspensions" Bernheim found that under different defined conditions the oxygen equivalent of a given

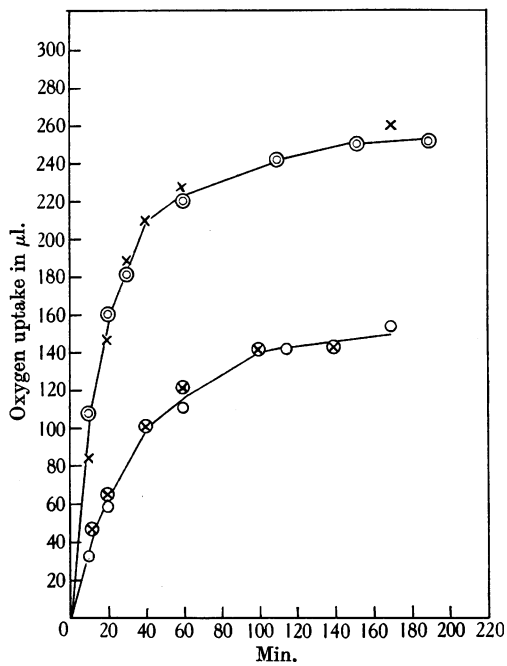

Fig. 1. Oxygen uptake curves of slices and "suspensions" of livers, in the presence of 2 mg. tyramine. ◎—◎, ×—× suspensions; o—o, ⊗—⊗ slices.

amount of tyramine (completely oxidized) could be 1, 2 or 4. I was not able to repeat these observations, possibly owing to the difficulty of rigidly defining

the conditions when the concentration and age of a liver suspension are important factors.

On the other hand I found that on all occasions the oxygen uptake, in presence of tyramine, of my "suspensions" was twice that of liver slices and they always approached 2 and 1 equivalents respectively. Thus the oxygen uptake was for slices one atom O_2 per mol. tyramine, and for "suspensions" two atoms O_2 per mol. tyramine.

The oxygen uptake, in the presence of 1–4 mg. tyramine, was followed to completion over a period of 3–4 hr., readings being taken every 10 min. at first and then at longer intervals. Typical curves are shown in Fig. 1. The oxygen uptake of the controls without added tyramine has been subtracted in each case.

End product. Following the method described by Bernheim I isolated a small amount of *p*-hydroxyphenylacetic acid, after incubating liver "suspension" with tyramine. I also showed, in the following way, that during tyramine oxidation a compound having a carbonyl group (probably *p*-hydroxyphenylacetaldehyde) is formed. When oxidation was complete the contents of the Barcroft bottles were washed out, the protein precipitated with trichloroacetic acid and the filtrate tested with 2 : 4-dinitrophenylhydrazine. Both slices and suspensions gave a much more bulky precipitate after incubation with tyramine than did the controls.

Deamination. Ammonia estimations were made on the contents of Barcroft bottles when oxidation was complete. 80–90 % of the ammonia from the added tyramine was recovered from slices and suspensions alike.These results are shown in Table II.

Table II. *Deamination of tyramine*

Tyramine added	0 mg. control	1 mg. Total	1 mg. Total – control	2 mg. Total	2 mg. Total – control	3 mg. Total	3 mg. Total – control	Preparation
NH_3 found	0·116	—	—	0·294	0·178	0·397	0·278	Suspension 15
	0·040	0·119	0·079	0·207	0·167	—	—	,, 16
	0·060	—	—	0·179	0·119	0·317	0·257	,, II
	0·048	0·141	0·093	0·236	0·188	—	—	,, IV
	0·036	0·116	0·080	0·200	0·164	—	—	Slices 17
	0·015	0·080	0·065	0·180	0·165	—	—	,, 17
	0·026	0·117	0·091	0·179	0·153	—	—	,, V
	0·020	0·106	0·086	0·173	0·153	—	—	,, VI
Average (suspensions)	—	—	0·085	—	0·163	—	0·269	—
% of theoretical	—	—	83 %	—	80 %	—	88 %	—
Average (slices)	—	—	0·081	—	0·159	—	—	—
% of theoretical	—	—	79·4 %	—	78 %	—	—	—

Ammonia is expressed as mg. of ammonia-N.

Xanthine oxidase. In view of the presence of an aldehyde as an oxidation product I decided to test whether xanthine oxidase was concerned in its further oxidation to the corresponding acid. If the xanthine oxidase were for some reason able only to act in the suspensions and not in the slices, the different oxygen uptakes would be explained. I therefore added excess uric acid to both slices and suspensions in order to inhibit the xanthine oxidase [Dixon & Thurlow, 1924]. The uric acid itself was strongly oxidized by the uricase present in liver. When this was allowed for I found that uric acid produced approximately 50 % inhibition of the oxidation of tyramine by both slices and suspensions (see Fig. 2).

Morgan [1926] showed that there is no xanthine oxidase in rabbit kidney. Experiments on rabbit kidney "suspension" gave just the same type of oxygen uptake as did liver "suspension", showing that the xanthine oxidase is not essential for the second stage of oxidation.

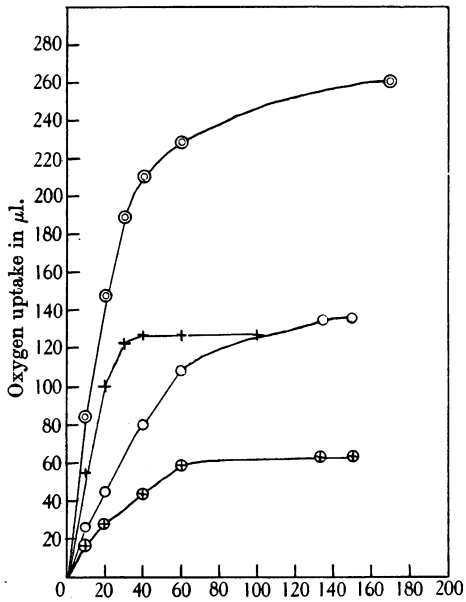

Fig. 2.

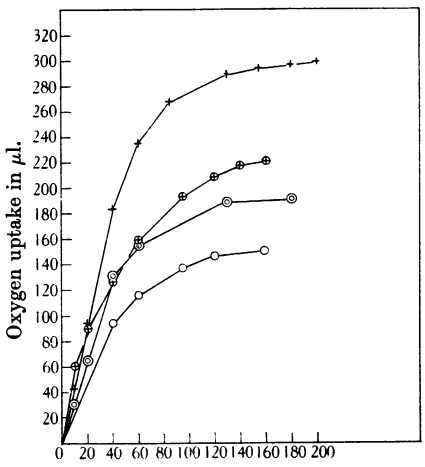

Fig. 3.

Fig. 2. The effect of uric acid on the oxygen uptake of slices and "suspensions" in the presence of 2 mg. tyramine. Uric acid added 10 mg. ◎—◎ suspension; +—+ suspension + uric acid; o—o slices; ⊕—⊕ slices + uric acid.

Fig. 3. The effect of alcohol on the oxygen uptake of slices and "suspensions" in the presence of 2 mg. tyramine. Alcohol added 5 mg. ◎—◎ suspension; +—+ suspension + alcohol; o—o slices; ⊕—⊕ slices + alcohol.

Coupled oxidation. Keilin & Hartree [1936] showed that during oxidation by oxidases H_2O_2 is produced which, in the presence of catalase, can oxidize added alcohol. This process should just double the normal oxygen uptake. Krebs [1936] claims that haemoglobin can also undergo coupled oxidation. All my preparations contained haemoglobin so that it was possible that coupled oxidation, occurring in "suspensions" and not in slices, might account for the difference in oxygen uptake. Added alcohol was, however, oxidized by both preparations only in presence of tyramine, whereas it would have had no effect if coupled oxidation were already occurring to any great extent (see Fig. 3).

KCN inhibits catalase and consequently inhibits coupled oxidation by about 50% when present in a concentration of $M/500$ [Keilin & Hartree, 1936]. Oxidation of tyramine by slices and "suspensions" is not affected by KCN in a concentration of $M/300$.

Coupled oxidation of haemoglobin does take place to some extent in my liver preparations judging from the change in colour of the contents of the bottles with added tyramine during the course of the reaction. The extent of this oxidation is presumably too small seriously to affect the oxygen uptake.

Influence of oxygen pressure. Substitution of oxygen for air in the manometers when using "suspensions" does not affect the final oxygen uptake, though it increases the rate of the reaction. The difference in oxygen pressure cannot, therefore, be responsible for the different types of oxidation of slices and "suspensions".

Redox indicators. Hare found that her liver preparations would not reduce methylene blue or 1-naphthol-2-sulphonate-2:6-dichloroindophenol in presence of tyramine, whilst dyes with a more positive potential were reduced too rapidly by liver alone to permit of any conclusions. I find that methylene blue and toluylene blue strongly inhibit tyramine oxidase; 0·5 ml. of 1/5000 methylene blue produces 80–100 % inhibition of the oxidation. No other dyes that I have tried (*o*-bromophenolindophenol, *o*-cresolindophenol, potassium indigodisulphonate and phenosafranine) produce any inhibition. If the activity of the blank is lowered by washing the "suspension" 3–4 times on the centrifuge, *o*-bromophenolindophenol and *o*-cresolindophenol are rapidly reduced only in the presence of tyramine. Potassium indigodisulphonate and potassium indigotetrasulphonate are not reduced. From this we can conclude that the potential of the tyramine oxidase system lies between −0·046 and +0·195 V.

Conclusions

Although my conditions differ from Bernheim's I have confirmed the existence of two out of the three types of oxidation found by her. Further, I have produced evidence that xanthine oxidase is not concerned with any stage in the normal oxidation of tyramine.

My observations also confirm her opinion that the enzyme is an aerobic oxidase (see classification of Green & Brosteaux [1936]). This is borne out by the demonstration of coupled oxidation and by the fact that redox dyes do not accelerate the aerobic oxidation by the enzyme. Ogston & Green [1935] found that methylene blue usually accelerated the aerobic oxidation by dehydrogenases by about 200 %, whereas the respiration of oxidases was hardly affected.

Although methylene blue is known to inhibit respiration in many normal tissues [Elliot & Baker, 1935; Dickens, 1936], liver is an exception and the inhibition shown here appears to be specific for the oxidation of tyramine.

There are, however, several discrepancies between my own and previous observations. Using liver slices, I have always found an oxygen uptake of one atom of oxygen per mol. tyramine accompanied by almost complete deamination. This would suggest that tyramine has been oxidized to *p*-hydroxyphenylacetaldehyde. Ewins & Laidlaw [1910] however recovered the corresponding acid after perfusing livers with tyramine; with liver "suspensions" and a similar oxygen uptake Hare [1928] obtained only a 50 % yield of ammonia. There is therefore some agreement between perfusion and my "suspensions" with regard to the end product. Which of these treatments more nearly approaches normal conditions is a matter not easily decided. If the first product of oxidation is *p*-hydroxyphenylacetaldehyde, in slices this could dismute with no further oxygen uptake, to give the corresponding acid and alcohol, under the action of aldehyde mutase which is known to be present in liver; whereas when the cell structure is destroyed this reaction might be replaced by a further oxidation of the aldehyde at the expense of molecular oxygen. Without further separation of the enzymes involved it is very hard to see how more light can be thrown on this problem.

Elliot & Schroeder [1934], using kidney cortex, found that mechanical destruction of the tissue structure greatly reduced its power to oxidize added

substrates and remarked on the danger of drawing conclusions from experiments on minced tissues only. In the case of tyramine and the liver, the converse phenomenon occurs, namely mechanical destruction doubles the total oxygen uptake though the initial rate of oxidation is hardly greater than with slices.

SUMMARY

1. The course of oxidation of tyramine in rabbit liver has been reinvestigated and some discrepancies with previous work noticed.
2. The view of previous workers that tyramine oxidase is an aerobic oxidase has been substantiated.
3. The enzyme is strongly inhibited by methylene blue.
4. The redox potential of the system is between $-0{\cdot}046$ and $+0{\cdot}195$ V.

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