7. The Oxidation of Amines by Bacteria

By Ernest Frederick Gale,* *From the Biochemical Laboratory, Cambridge*

(Received 19 December 1941)

The conditions under which various amines are produced by bacteria, and the properties of the amino-acid decarboxylases involved have been studied in a series of papers by Gale [1940; 1941]. In these studies the production of histamine by *Bact. coli*, *Cl. welchii* and *Cl. bifermentans*, of tyramine by *S. faecalis* and *Cl. aerofetidum*, of putrescine by *Bact. coli*, *Pr. vulgaris* and *Cl. septicum*, of cadaverine by *Bact. coli* and *Bact. friedländeri*, and of agmatine by *Bact. coli* have been reported; in addition, Arai [1921] has reported the production of isoamylamine from leucine by *Bact. proteus* and *B. subtilis*, and Berthelot & Bertrand [1912] the decarboxylation of tryptophan to tryptamine by *B. aminophilus intestinalis*. The amino-acid decarboxylases are only formed when the organisms are grown under acid conditions and are active at acid pH values, the optimum activity-pH falling below pH 5 in all cases studied. Any attempt to assess the significance of the bacterial production of amines must also take into account any further breakdown of these substances by the organisms in question, and the present communication deals with the oxidative breakdown of certain amines by selected facultative anaerobes. Den Dooren de Jong [1926] found that few organisms are able to utilize amines as sources of carbon for growth, but that some amines can be utilized as a source of nitrogen in a medium containing NH_3 -free salts and glucose by organisms such as *Myobact. phlei*, *Bact. coli*, *Bact. aerogenes*, *Bact. herbicola* etc.

Animal tissues contain two enzymes responsible for the oxidation of amines: the amine oxidase which is specific for monoamines and is active in the presence of semicarbazide, splitting off NH_3 from the amine with the uptake of 1 atom of oxygen per molecule to form the corresponding aldehyde [Pugh & Quastel, 1937; Richter, 1937; Kohn, 1937]; and the diamine oxidase which is specific for diamines, splitting off NH_3 and taking up oxygen. The diamine oxidase is inhibited by semicarbazide and other carbonyl-fixatives so that the primary product of the oxidation cannot be easily identified but is presumed to be the corresponding aldehyde [Best & McHenry, 1930; Edlbacher & Zeller, 1937; Zeller, 1938].

Methods and organisms used

Organisms. The strains of *Ps. pyocyanea* were freshly isolated from faeces and given to me by Dr H. L. Carruthers of the E.P.H.L.S.; they were all good pyocyanine producers, but when tested under the usual experimental conditions [Gale, 1940] did not possess the power to decarboxylate amino-acids with the formation of amines. Most of the other organisms have been mentioned and histories given in previous papers. The organisms were grown overnight at 37° on the surface of agar containing tryptic digest of casein and such other additions as are outlined below. In the morning they were harvested, centrifuged, washed (100 ml. water to 100 mg. organism) and made up into washed suspension in distilled water. The strength of the suspension was determined by use of a photoelectric turbidimeter previously calibrated for the organisms concerned.

Oxygen uptake. The O_2 consumption of the washed suspensions was followed in Warburg manometers shaken in a bath at 30°. Manometer cup contents: 1 ml. washed suspension; 1.5 ml. *M*/15 phosphate buffer pH 7.5; 0.5 ml. *M*/50 amine solution (side bulb) or water in controls; 0.2 ml. 10 % NaOH in centre cup. All amine solutions were prepared from the products of Hoffmann la Roche with the exception of tyramine where the products of British Drug Houses and Burroughs Wellcome Ltd. were also tested.

* Beit Memorial Fellow and Fellow of St John's College, Cambridge.

CO₂ production. This was also estimated manometrically. As the oxidative breakdown of some of the amines may involve NH₃ and acid formation combined with an O₂ uptake, the usual method employing bicarbonate buffer with an O₂-CO₂ gas mixture can only be used as a check, and the actual CO₂ production must be estimated by the indirect 'acid-tip' method described by Woods & Clifton [1937].

NH₃ formation. In the case of non-volatile amines, NH₃ formation was estimated by withdrawal of 1 ml. from the Warburg cup, distillation in Conway vessels and nesslerization. No NH₃ has been obtained from volatile amines.

Amine estimations. Aliphatic amines were estimated by the method of Van Slyke [1929], tyramine and histamine by the colorimetric methods of Koessler & Hanke [1919; 1922], and aromatic amines by a diazotization method: the nitrous-acid-sulphanilic-acid reagent was prepared as before and 2 ml. added to standard solutions of amine made up in 5 ml. 5% NaHCO₃. A colour which can be used for estimation purposes develops on standing in the cases of aniline and of α - and β -naphthylamine; satisfactory standards are obtained in most cases with a range of 100–500 μ g. amine.

Preliminary investigations

At first the O₂ consumption of thick washed suspensions (10–15 mg./ml.) of *Bact. coli* and *Ps. pyocyanea* in the presence of various amines was studied. It became apparent that in the case of *Ps. pyocyanea*, which has a high 'blank' O₂ consumption of the order $Q_{O_2} = 10$ –15, that all the amines tested gave rise to an increased rate of O₂ uptake when first added to the washed suspension. This increased O₂ consumption appears to have two causes, depending on the amine concerned, as the substrates fell into two groups: group A in which the O₂ consumption is strictly proportional to the amount of amine added, and group B in which the increase in O₂ uptake induced by the addition of the amine is not constant and not proportional to the amount of substrate. In the case of *Ps. pyocyanea* the groups have the following composition:

Group A: tyramine, histamine, putrescine, cadaverine, agmatine and benzylamine.

Group B: ethylamine, isoamylamine, phenylethylamine, tryptamine, aniline, α - and β -naphthylamine.

Other organisms show the same effects except that the group B effect is less marked in most other cases as its magnitude appears to be correlated with the 'blank respiration' and is most marked with freshly isolated strains. Some amines falling into group A with *Ps. pyocyanea* come into group B with other organisms. It is shown below that the substrates classed in Group A undergo a true oxidative degradation, whereas it is probable that the action of those in group B is to catalyse the oxidation of some unidentified substance whose oxidation constitutes part of the 'blank respiration'.

Rates of oxidation of amines by various organisms

Table 1 gives the Q_{O_2} values ($= \mu$ l. O₂ taken up due to substrate oxidation/hr./mg. dry-weight bacteria) obtained with various amines on addition to washed suspensions of certain facultative anaerobes. The Q_{O_2} figures are only given in cases where a true oxidative degradation is obtained, group B effects being omitted. The organisms were grown in every case on the surface of agar containing tryptic digest of casein with the addition of $M/500$ tyramine and $M/500$ histamine for reasons explained below.

From Table 1 the following facts appear: (1) the power to oxidize amines is limited in distribution, and in few cases is the oxidation carried out at any significant rate compared with the rates at which amines can be produced by bacteria (Fig. 2); (2) none of the selected organisms can oxidize monoamines other than tyramine; it will be shown below that this is not due to a need for substrate adaptation; (3) of the organisms tested, *Ps. pyocyanea* has the widest range of oxidative attack towards amines.

Table 1. *Oxidation of amines by bacteria*

Washed suspensions, prepared from organisms grown on tryptic-digest agar + *M*/500 tyramine + *M*/500 histamine, shaken at 30° with 0.5 ml. *M*/50 amine in presence of phosphate buffer pH 7.5. Activities expressed as Q_{O_2} (substrate).

Organism	Strain	Tyr-amine	Hist-amine	Putres-cine	Cada-verine	Agma-tine	Ethyl-amine	iso-Amyl-amine	Phenyl-ethyl-amine	Aniline
<i>Ps. pyocyanea</i>	A	17	17	23	12	10	—	—	—	—
<i>Ps. pyocyanea</i>	268	21	4	9	5	11	—	—	—	—
<i>Ps. pyocyanea</i>	265	17	5	12	7	11	—	—	—	—
<i>Bact. coli</i>	Esch.	83	—	—	—	—	—	—	—	—
<i>Bact. coli</i>	216	—	—	—	—	—	—	—	—	—
<i>Bact. coli</i>	217	—	—	—	—	—	—	—	—	—
<i>Bact. coli</i>	Stock	—	—	7	6	1	—	—	—	—
<i>Bact. coli</i>	Sheff.	—	—	?	11	1	—	—	—	—
<i>Bact. coli</i>	88	—	—	10	—	—	—	—	—	—
<i>Pr. vulgaris</i>	882	—	—	—	—	—	—	—	—	—
<i>Pr. morgani</i>	Carr.	—	—	10	12	2	—	—	—	—
<i>Paracolon</i>	AO	32	—	—	—	—	—	—	—	—
<i>Staph. aureus</i>	I	—	—	—	12	—	—	—	—	—

Other amines giving negative results throughout are α - and β -naphthylamines.

Experimental conditions: Group A oxidations

pH-activity curves. Fig. 1 shows the variation of Q_{O_2} with reaction pH for the oxidation of various amines by *Ps. pyocyanea* and the *paracolon* organism. The estimations were carried out with suspensions of suitable strength and 0.5 ml. *M*/50 amine: as the oxidation

Fig. 1.

Fig. 2.

Fig. 1. Variation of activity (Q_{O_2}) with reaction pH; oxidation of amines by *Ps. pyocyanea* and *Paracolon* AO. 1, tyramine (AO). 2, histamine (*pyo.*). 3, putrescine (*pyo.*). 4, cadaverine (*pyo.*). 5, agmatine (*pyo.*).

Fig. 2. Effect of pH on formation and oxidation of amines.

Amino-acid decarboxylase activities (Q_{O_2})

1. $\blacktriangle$ Arginine decarboxylase (*Bact. coli* Esch.)
2. $\bullet$ Tyrosine decarboxylase (*S. faecalis*)
3. $\times$ Histidine decarboxylase (*Bact. coli* Esch.)
4. $\blacksquare$ Ornithine decarboxylase (*Bact. coli* Esch.)

Amine oxidation (Q_{O_2})

- 1a. $\triangle$ Agmatine oxidation (*Ps. pyocyanea*)
- 2a. $\circ$ Tyramine oxidation (*Bact. coli* Esch.)
- 3a. $\times$ Histamine oxidation (*Ps. pyocyanea*)
- 4a. $\square$ Putrescine oxidation (*B. pyocyanea*)

exceeds that corresponding to the uptake of 1 atom oxygen and the elimination of 1 mol. NH_3 , the rates quoted do not represent the rate of any one enzyme system. The optimum pH falls in every case on the alkaline side of neutrality, the activities falling off rapidly on

the acid side. Fig. 2 shows the formation and destruction of amines by various organisms and the variation of these activities with reaction *pH*. It can be seen that the conditions suitable for optimum amine formation are those in which negligible amine destruction occurs and *vice versa*.

Growth conditions. Table 2 shows the Q_{O_2} values obtained with tyramine, histamine, putrescine, cadaverine and agmatine for washed suspensions of *Ps. pyocyanea* and *Bact. coli* Esch. which have been grown in the presence of the various amines as substrate. In the case of *Ps. pyocyanea* the enzymes responsible for the oxidation of putrescine, cadaverine and agmatine are constitutive in the sense used by Karström [1938], while those involved in the initial oxidation of tyramine and histamine appear to be adaptive. *Bact. coli* Esch. does not possess the enzymes necessary for the degradation of histamine, putrescine, cadaverine or agmatine whether it is grown in the presence of these substances or not. Neither organism can be made to attack ethylamine or isoamylamine by inclusion of these amines in high concentration (1%) in the growth medium. The case of tyramine involves a special type of adaptation discussed below.

Table 2. *Effect of growth substrate conditions upon oxidation of amines by Ps. pyocyanea and Bact. coli Esch.*

Q_{O_2} values estimated at 30° and *pH* 7.5. Broth = tryptic digest of casein.

Organism and growth medium	Tyr- amine	Hist- amine	Putres- cine	Cada- verine	Agma- tine	Ethyl- amine	iso- Amyl- amine
<i>Ps. pyocyanea</i>							
Stephenson's inorganic medium 1% lactate	(—)	—	22	12	5	—	—
Broth-agar	(—)	—	23	10	10	—	—
Broth-agar + <i>M</i> /500 tyramine	17	—	25	12	9	—	—
Broth-agar + <i>M</i> /500 histamine	(—)	17	20	15	7	—	—
Broth-agar + <i>M</i> /500 putrescine	(—)	—	22	10	17	—	—
Broth-agar + <i>M</i> /500 cadaverine	(—)	—	21	11	15	—	—
Broth-agar + <i>M</i> /500 isoamylamine	(—)	—	—	—	—	—	—
Broth-agar + 1% isoamylamine	(—)	—	—	—	—	—	—
Broth-agar + <i>M</i> /500 phenylethylamine	(—)	—	—	—	—	—	—
<i>Bact. coli</i> Esch.							
Broth-agar	(—)	—	—	—	—	—	—
Broth-agar + <i>M</i> /500 tyramine	83	—	—	—	—	—	—
Broth-agar + <i>M</i> /500 histamine	(—)	—	—	—	—	—	—
Broth-agar + <i>M</i> /500 putrescine, cadaverine, agmatine, ethylamine etc.	(—)	—	—	—	—	—	—
Broth-agar + $\begin{cases} 1\% \text{ ethylamine} \\ 1\% \text{ isoamylamine} \end{cases}$	(—)	—	—	—	—	—	—

Table 3. *Effect of growth conditions on oxidation of tyramine by Bact. coli Esch. grown in presence of M/500 tyramine*

Activities expressed as Q_{O_2} (tyramine) at 30° and *pH* 7.5.

Growth conditions	Initial Q_{O_2}	Growth conditions	Initial Q_{O_2}
Surface of broth-agar	75	Broth adjusted to: <i>pH</i> 5.2 in flask	75
Broth adjusted to: <i>pH</i> 8.5 in flask	132	<i>pH</i> 4.7 in flask	9
<i>pH</i> 7.4 in flask	108	<i>pH</i> 7.4 strictly anaerobic	6
<i>pH</i> 6.0 in flask	94	Broth + 2% glucose (final <i>pH</i> = 5.2)	6

Other growth conditions have been investigated in the case of *Bact. coli* Esch. grown in the presence of *M*/500 tyramine. Table 3 shows that the enzymes involved in tyramine oxidation are formed as well during growth in broth in a flask as on the surface of broth-agar but that strictly anaerobic growth conditions inhibit the formation of the enzymes necessary for the immediate oxidation of tyramine, although, as is shown below, the organism still possesses the power to attack this amine. The enzymes are formed best under alkaline or neutral growth conditions, but a growth *pH* of less than 5.0 is necessary

to cause marked inhibition of their formation. Growth in 2% glucose-broth results in organisms having little activity, which effect is probably accounted for by the anaerobiosis produced by fermentation gas combined with the low pH produced by fermentation acids.

Fig. 3 shows the course of the oxidation of tyramine by washed suspensions of *Ps. pyocyanea* grown on the surface of agar with (curve 2) and without (curve 3) the addition of $M/500$ tyramine, and by *Bact. coli* Esch. grown aerobically (curve 1) and anaerobically (curve 4) in the presence of tyramine. When the organism is grown aerobically in the

Fig. 3. Oxidation of tyramine by *Ps. pyocyanea* (●—●) and *Bact. coli* Esch. (○—○). 1 and 2, organism grown on surface of broth-agar + $M/500$ tyramine. 3, 5, 6, organism grown on surface of broth-agar without tyramine. 4, organism grown anaerobically in broth + $M/500$ tyramine.

Substrate concentration = $2 \mu M$. in 1, 2, 3 and 4; $6 \mu M$. in 5; $10 \mu M$. in 6.

Dry weight of bacteria = 10–12 mg. in all cases.

presence of tyramine then oxidation begins as soon as the substrate is added to the washed suspension (curves 1, 2) and the oxidation continues until 13–14 atoms oxygen have been taken up. If the organism is grown aerobically in the absence of tyramine, it can still attack this amine but oxidation does not start until some time after the addition of tyramine to the suspension; the time elapsing before the oxidation starts is proportional to the amount of substrate added. Thus when 0.1 ml. $M/50$ tyramine is added, oxidation starts after approx. 1 hr. (curve 3); after approx. 3 hr. when 0.3 ml. is added (curve 5), while 0.5 ml. delays the onset of oxidation for nearly 4 hr. In these cases the oxidation ceases at an O_2 uptake corresponding to 10–12 atoms per molecule tyramine. When *Bact. coli* Esch. is grown anaerobically in the presence of tyramine, then the course of the O_2 consumption is apparently similar to that obtained with the organism grown aerobically in the absence of tyramine (curve 4).

A similar latent period was found by Willstätter *et al.* [1927] when working on the hydrolysis of ethyl mandelate by liver esterase; this was shown to be due to the presence

of traces of benzoylformic ester in the substrate, the impurity having 5000 times the affinity for the enzyme that mandelate has but a low velocity of breakdown [Baumann & Schmeller, 1930]. Thus the enzyme was not free to attack the ethyl mandelate until the impurity was hydrolysed.

The latent period displayed in the oxidation of tyramine by the unadapted organism has been investigated as follows: solutions were tested that had been made from tyramine hydrochloride of B.D.H. Analar and of Hoffmann la Roche; from tyramine acid phosphate of Burroughs Wellcome Ltd.; from recrystallized tyramine acid phosphate; and from tyramine prepared by the decarboxylation of recrystallized tyrosine with washed suspensions of *S. faecalis*, isolated as dibenzoyltyramine [Gale, 1940], hydrolysed and thrown out of dry ethereal solution as the hydrochloride. The oxidation started after a latent period of the same order of time in all cases for a given amount of substrate. The addition of small amounts of tyrosine does not increase the length of the latent period. The addition of acid immediately the latent period is over shows that no significant NH_3 formation has occurred and that the greater part, at least 70 %, of the tyramine appears to be un-attacked. The addition of further amounts of tyramine once the oxidation of the initial substrate is under way does not alter the course of the oxidation which continues unchecked until the *whole* of the added substrate has been oxidized. The latent period must thus remain unexplained for the present.

It would appear that the organisms can attack tyramine in at least two ways: the enzyme responsible for the immediate initial oxidative attack, under the experimental conditions described, being formed only when the organism is grown in the presence of both the specific substrate and oxygen. The organism so 'adapted' to attack tyramine is unable to attack phenylethylamine and the presence of this latter substance in the growth medium does not bring about the adaptation to tyramine. It is shown below that if the adapted organism oxidizes tyramine in the presence of semicarbazide, then *p*-hydroxy-phenylacetaldehyde can be isolated from the reaction products; this is not the case with the non-adapted organism as the presence of semicarbazide inhibits the occurrence of oxidation in this case.

From the facts established so far it appears that the organisms studied have not the power to attack amines possessing a single active chemical grouping in the molecule. Thus the monoamines are un-attacked unless they possess some active group other than the $-\text{NH}_2$, such as the $-\text{OH}$ in tyramine, the $-\text{NH}$ in histamine or the *para*-H in benzylamine, while the diamines are attacked by several organisms.

Temperature. Fig. 4 shows the variation with temperature of initial Q_{O_2} for various amines using washed suspensions of *Ps. pyocyanea*. The experimental temperature adopted was 30° so that the activity figures could be compared with those formerly determined for amino-acid decarboxylases. The temperature coefficients (θ) for a rise of temperature from 25 to 35° are given below the figure; the activities of the enzymes studied fall off at temperatures above 40° .

Fig. 4. Variation of oxidation activity (Q_{O_2}) with temperature: *Ps. pyocyanea*. 1, tyramine: temp. coeff. $\theta_{25}^{35} = 2.0$. 2, putrescine, $\theta = 4.5$. 3, agmatine, $\theta = 3.2$. 4, cadaverine, $\theta = 3.8$. 5, histamine, $\theta = 3.7$.

Course of the oxidative breakdown of amines

Fig. 5 shows the course of the O_2 uptake (blank O_2 consumption subtracted in all cases) in the presence of various amines with washed suspensions of *Ps. pyocyanea*. The organ-

Fig. 5. Oxidation of amines ($2 \mu M$) by washed suspensions of *Ps. pyocyanea* at 30° and pH 7.5. 1, cadaverine. 2, putrescine. 3, agmatine. 4, histamine. 5, benzylamine.

Table 4. Action of washed suspensions of *Ps. pyocyanea* on amines:
*O*₂ consumption, CO₂ and NH₃ formation

Dry weight of organism = 10–15 mg./ml. washed suspension. Amount of substrate = 0.1 ml. $M/50$ amine (neutral) in all cases. Temperature = 30° . Experimental pH 7.5 or 8.0. 1 atom oxygen = $22.4 \mu l$. 1 mol. CO₂ = $44.8 \mu l$. 1 mol. NH₃ = $28 \mu g$.

Substrate	O ₂ uptake		CO ₂ formation		NH ₃ formation	
	μl .	Atoms	μl .	mol.	μg .	mol.
Putrescine	258	11.5	160	3.6	61	2.2
	247	11.0	171	3.8	63	2.2
Cadaverine	331	14.8	241	5.4	66	2.4
	322	14.4	222	4.9	57	2.0
Agmatine	254	11.3	220	4.9	105	3.8
	235	10.5	212	4.75	89	3.2
Benzylamine	389	17.4	323	7.2	25	0.9
	378	17.0	316	7.05	27	1.0
Histamine	159	7.0	175	3.9	41	1.5
	195	8.75	230	5.1	42	1.5
	173	7.7	173	3.9	41	1.5
	135	6.5	149	3.3	44	1.6
Tyramine: Adapted	295	13.2	229	5.0	19	0.7
	291	13.1	240	5.3	17	0.6
	332	14.8	254	5.6	23	0.8
	290	13.0	223	5.0	21	0.75
Non-adapted	271	12.1	183	4.1	13	0.47
	259	11.6	167	3.7	10	0.34

isms were grown on the surface of broth-agar with the addition of $M/500$ substrate in the case of tyramine, histamine and benzylamine oxidation.

Table 4 shows representative results of analysis of the oxidation: the amount of CO_2 formed and of NH_3 liberated. The amount of substrate used in each case corresponded to 0.1 ml. $M/50$ amine solution so that the complete oxidation could be followed manometrically. The oxidation of putrescine, cadaverine, agmatine and benzylamine appears to be complete so that the breakdown of these molecules corresponds to the equations:

The oxidation of benzylamine is very slow ($Q_{\text{O}_2} = 3-5$) but the oxidation of the other substrates proceeds fairly rapidly until 60–70 % of the total O_2 consumption has been taken up when the rate falls to a low value which is maintained until completion.

The oxidation of tyramine and histamine is not complete. The variation of the O_2 consumption obtained for tyramine with the growth conditions has been discussed above. The adapted organism oxidizes tyramine with the uptake of 13–14 atoms of oxygen per molecule, the formation of some NH_3 and 5 mol. of CO_2 . The NH_3 formation is not theoretical but always amounts to 60–70 % of the original amino-N. Estimation of residual tyramine shows that this is insignificant so that the oxidation breaks down the whole of the tyramine with the production of NH_3 , CO_2 and unidentified products. Investigation of the deproteinized solution obtained at the completion of oxidation shows that the molecule has lost its phenolic and ring structure (Millon and xanthoproteic tests negative) and will not give an insoluble product on benzoylation. Clifton [1937] has shown that the oxidation of many substrates by bacteria ceases at 60–70 % completion, the remainder of the substrate being assimilated by the cells, and that this 'oxidative assimilation' can be inhibited by suitable concentrations of azide or dinitrophenol. The effect of varying concentrations of these inhibitors was tested; $M/3000$ dinitrophenol appeared to increase the O_2 uptake slightly in the presence of tyramine and histamine so that the number of oxygen atoms taken up per molecule substrate was increased to 15 and 9 respectively. Azide was without effect. Thus it is probable that the incomplete oxidation is due to the formation of non-oxidizable products rather than to 'oxidative assimilation' and this hypothesis is supported by the abrupt nature of the cessation of O_2 consumption as shown in Figs. 3 and 5 for these two substrates. The course of the oxidation of tyramine appears to be the same for *Bact. coli* Esch., *Paracolon* AO and *Ps. pyocyanea*. It is shown below that if the oxidation is carried out in the presence of semicarbazide, then a small yield of *p*-hydroxyphenylacetaldehyde is formed from tyramine.

The oxidation of histamine by *Ps. pyocyanea* is also incomplete and again the NH_3 formation accounts for only 50 % of the total available N of the histamine molecule. Treatment of the resulting solution with diazobenzenesulphonic acid fails to give the colour usually associated with iminazole compounds; benzoylation fails to give an insoluble product and no precipitate is formed on the addition of 2:4-dinitrophenylhydrazine.

Action of cyanide and semicarbazide (Table 5). The oxidation of putrescine, cadaverine, agmatine and histamine by *Ps. pyocyanea* is completely inhibited by $M/100$ semicarbazide or $M/10,000$ cyanide; $M/1000$ semicarbazide inhibits the rate of oxidation some 90 %. The diamine oxidase of animal tissues is completely inhibited by semicarbazide and other carbonyl-fixatives so that it is suggested [Zeller, 1938] that the active group of the enzyme is of ketonic structure.

The oxidation of tyramine by *Bact. coli* Esch. is completely inhibited by $M/100$ CN, 66 % inhibited by $M/1000$ CN and 5 % by 10^{-4} M CN. Semicarbazide is less effective as an

Table 5. *Actions of semicarbazide and cyanide on amine oxidation*Activities expressed as Q_{O_2} at 30° and pH 7.5.

Substrate	Organism	Control	Semicarbazide		Cyanide		
			<i>M</i> /10	<i>M</i> /100	<i>M</i> /100	<i>M</i> /10 ³	<i>M</i> /10 ⁴
Putrescine	<i>Ps. pyocyanea</i>	23	0	0	0	0	0
Cadaverine		12	0	0	0	0	0
Agmatine		10	0	0	0	0	0
Histamine		17	0	0	0	0	0
Tyramine	<i>Paracolon</i> AO	32	12	23	0	11	30

inhibitor in this case as *M*/10 causes 60 % inhibition while *M*/100 has only 25 % inhibitory action. The O_2 consumption in the presence of *M*/10 semicarbazide is decreased and amounts to 7–9 atoms oxygen per mol. tyramine compared with 13–14 atoms taken up in the uninhibited control. If the organisms are removed from the resulting solution and 2:4-dinitrophenylhydrazine added, a turbidity is obtained. This has been worked up on a larger scale as follows.

20 ml. *M*/50 tyramine, 40 ml. washed suspension of *Bact. coli* Esch., 10 mg./ml. (grown in presence of *M*/500 tyramine), 40 ml. *M*/15 phosphate buffer, pH 7.5 and 20 ml. 0.6 *M* semicarbazide, were shaken aerobically at 30° until a manometric control experiment indicated that oxidation was complete. The organism was then removed by centrifuging and filtration through a Seitz filter. HCl was added to the filtrate to a concentration of 2 *N* and the theoretical quantity of 2:4-dinitrophenylhydrazine (0.5 % solution in 2 *N* HCl) added. A turbidity formed which on standing overnight settled to an amorphous precipitate. This was filtered off and recrystallized twice from ethyl acetate and ligroin [Clift & Cook, 1932]. Yield 9 mg. m.p. 182–4°. Analysis (Weiler): C, 53.0; H, 4.46; N, 17.75 %. *p*-Hydroxyphenylacetaldehyde 2:4-dinitrophenylhydrazone requires C, 53.2; H, 3.8; N, 17.7 %.

Thus in the presence of high concentrations of semicarbazide some of the tyramine is oxidized to the corresponding aldehyde. Since the yield corresponds to only 6–10 % of the initial tyramine and the O_2 consumption is not greatly reduced by the presence of the semicarbazide, it follows either that the formation of the aldehyde is a side reaction produced by the presence of semicarbazide or that this substance is not an efficient aldehyde-fixative under the experimental conditions employed. Similar results are obtained with *Ps. pyocyanea* and *Paracolon* AO.

Action of amines in group B

All amines tried produce an accelerated O_2 consumption when added to washed suspensions of *Ps. pyocyanea* etc. In some cases this is due to oxidative degradation of the amine as described above; in other cases the effect has the following characteristics.

(1) If the O_2 uptake is followed until the rate in the presence of amine has fallen to that of the control without added substrate and the increased O_2 consumption then calculated in the usual way, it is found that this increase in O_2 consumption, though increasing slightly with substrate concentration, is not proportional to the amount of amine added. This is shown in Table 6 for selected results with ethylamine, isoamylamine, aniline and naphthylamine.

(2) The amount of this increase in O_2 consumption is not constant for any given amount of amine but varies markedly from time to time, being greatest when the organism used is freshly isolated and then slowly falling off until an organism which has been kept for some months on artificial medium no longer gives this effect. Thus a freshly isolated strain of *Ps. pyocyanea* shaken with 0.5 ml. *M*/50 isoamylamine or aniline will give an increased O_2 uptake of 120–140 μ l.; after a few weeks' subcultivation in tryptic digest of casein a similar experiment gives an uptake of 50–60 μ l., and after 12 months the

uptake is insignificant. The figures during this period are so varied that no mean value can be calculated.

At first it was thought that this increased O_2 uptake was due to oxidation of the amine and various possibilities were investigated; as all these proved negative they will only be outlined here.

(1) No evidence could be obtained for the liberation of NH_3 from ethylamine, *iso*-amylamine, phenylethylamine, aniline or naphthylamine and all the usual tests for the formation of aldehydes were completely negative.

(2) No evidence for the production of the corresponding hydroxylamine derivatives could be obtained [Bamberger, 1899; Pucher & Day, 1926].

Table 6. *Effect of addition of certain amines to washed suspensions of Ps. pyocyanea at 30° and pH 7.5*

Gas changes calculated from zero to that time when rate of O_2 consumption in experimental manometer = rate in control without amine. All gas changes vary over a wide range: the following figures being selected cases only.

	Ethyl-amine		<i>iso</i> -Amylamine		Aniline		β -Naphthyl-amine			α -Naphthyl-amine	
Conc. <i>M</i> /100	0.3	0.2	0.3	0.2	0.3	0.2	0.3	0.2	0.07	0.3	0.07
Apparent O_2 uptake μ l.	111	86	73	65	78	34	152	129	100	107	122
Mol. O_2	0.5	0.6	0.35	0.5	0.35	0.25	0.7	0.95	2.25	0.45	2.5
CO_2 output μ l.	55	46	32	34	42	23	136	—	104	101	92
Mol. CO_2	0.25	0.3	0.18	0.25	0.2	0.2	0.3	—	2.3	0.54	2.1
Amino-N (μ g./ml.):											
Initial	42	28	42	28	—	—	—	—	—	—	—
Final	41	25	38	27	—	—	—	—	—	—	—
Amine (μ g./ml.):											
Initial	—	—	—	—	279	186	430	286	100	430	100
Final	—	—	—	—	280	200	450	300	100	450	100

(3) In the case of aniline, the possibility of the formation of phenol, hydrazobenzene, azobenzene, nitrosobenzene etc., was investigated with negative results.

(4) In the cases of *iso*amylamine and ethylamine with *Ps. pyocyanea*, and putrescine and cadaverine with *Paracolon* AO, estimation of the amino-N before and after the 'oxidation' process shows no decrease during that process.

(5) The product obtained from the 'oxidation' of *iso*amylamine was worked up for the formation of a picrate; red crystals were obtained in good yield which gave the M.P. and mixed M.P. of *iso*-amylamine picrate. Similarly benzylation of the product from aniline gave benzanilide. Thus any oxidation which does occur can affect only a small proportion of the substrate added.

(6) In the cases of aniline and the naphthylamines, the substrate can be estimated colorimetrically at the end of the experiment and Table 6 shows that no disappearance of these amines has occurred despite the O_2 uptake recorded.

(7) Manometric analysis applied to the oxidation process shows that in all cases the increased O_2 consumption is accompanied by an increased CO_2 production.

(8) The effect obtained with β -naphthylamine is more marked than with other simple amines and has consequently been investigated in more detail. Fig. 6 shows the O_2 uptake

Fig. 6. Action of β -naphthylamine on O_2 uptake of washed suspension of *Ps. pyocyanea*. ●—● Control. ○—○ 0.3 ml. *M*/50 β -naphthylamine added at times 0 and 2 hours (arrow).

in manometers containing *Ps. pyocyanea* with and without the addition of β -naphthylamine. The addition of β -naphthylamine produces an accelerated O_2 consumption which falls off after a time but the decreased rate is then less than that of the control, so that the initial increased O_2 uptake is later compensated by a decreased uptake below that of the control. Further, the addition of more β -naphthylamine once the first effect has fallen off does not result in a second acceleration of O_2 uptake. To sum up, the increased O_2 consumption obtained on adding certain amines to washed suspensions of freshly isolated organisms is not strictly proportional to the amount of amine added, is not associated with any decrease in the substrate concentration, does not apparently bring about any change in the amine molecule, is accompanied by an increased CO_2 formation, is followed by a period when the O_2 consumption is less than that of the control and cannot be produced by a second 'stimulus' of added amine. For the present it is tentatively suggested that these facts are best explained by the assumption that the amine acts catalytically on the oxidation of some substance whose oxidation normally comprises part of the 'blank respiration'.

Although the effects have not been investigated in detail, it is probable that this type of action explains results obtained with other amino-compounds such as semicarbazide, urea, phenylurea, phenylhydrazine, guanidine, benzidine, methyl- and dimethyl-aniline etc. These results were obtained only with freshly isolated organisms, while cultures which had been kept on artificial media, with or without the substances in question, for 12 months or more gave insignificant results of this group B type although the true oxidations studied in the first section of this paper remain independent of the time since isolation. A similar pseudo-oxidation was noted by Webster & Bernheim [1936] in certain of their studies on the oxidation of amino-acids by *Ps. pyocyanea*.

SUMMARY

1. Washed suspensions of *Ps. pyocyanea* can oxidize putrescine, cadaverine, agmatine, histamine and tyramine; some strains of *Bact. coli* can oxidize tyramine and/or putrescine and cadaverine; in no case is the activity very marked.

2. The enzymes involved in the oxidation of putrescine, cadaverine and agmatine appear to be constitutive while the primary stages of histamine and tyramine oxidation are adaptive.

3. The oxidations take place most rapidly between pH 7.5 and 9.5.

4. The enzyme involved in the primary oxidation of tyramine is not formed in the absence of tyramine during growth and its formation is inhibited by anaerobic or acid growth conditions.

5. *Ps. pyocyanea* carries the oxidation of putrescine, cadaverine and agmatine to completion, the sole products being CO_2 , H_2O and NH_3 . The oxidation of tyramine and histamine is incomplete though the benzene and iminazole rings are destroyed. In the presence of semicarbazide a small yield of *p*-hydroxyphenylacetaldehyde can be obtained from the oxidation of tyramine.

6. The oxidation of histamine, putrescine, cadaverine and agmatine is inhibited by $M/100$ semicarbazide or $M/10,000$ cyanide.

7. Other amines, such as ethylamine, isoamylamine, aniline etc. produce an accelerated O_2 consumption when added to washed suspensions of freshly isolated *Ps. pyocyanea*, *Bact. coli*, etc.; this is probably not due to oxidation of the amine but to a catalytic effect on the 'blank respiration'.

The author wishes to express his thanks to Prof. Sir Frederick G. Hopkins for his interest and encouragement in this work, and to Prof. Friedmann for advice. The work has been carried out at intervals during the tenure of grants from the Royal Commission for the Exhibition of 1851, the Foundation of St John's College, Cambridge and the Beit Memorial Fellowship Trustees, to all of whom the author expresses his gratitude.

REFERENCES

- Arai, M. [1921]. *Biochem. Z.* **122**, 251.
Bamberger, E. [1899]. *Ber. dtsh. chem. Ges.* **32**, 1803.
Baumann, E. & Schmeller, M. [1930]. *Hoppe-Seyl. Z.* **188**, 251.
Berthelot, A. & Bertrand, D. M. [1912]. *C.R. Acad. Sci., Paris*, **154**, 1826.
Best, C. M. & McHenry, E. W. [1930]. *J. Physiol.* **70**, 349.
Clift, F. P. & Cook, R. P. [1932]. *Biochem. J.* **26**, 1800.
Clifton, C. E. [1937]. *Enzymologia*, **4**, 246.
den Dooren de Jong [1926]. Bijdrage tot de kennis van het mineralsatieproces.
Dissertation. Rotterdam
Edlbacher, S. & Zeller, A. [1937]. *Helv. chim. Acta*, **20**, 717.
Gale, E. F. [1940]. *Biochem. J.* **34**, 392, 846, 853.
—— [1941]. *Biochem. J.* **35**, 66.
Karström, H. [1938]. *Ergebn. Enzymforsch.* **7**, 350.
Koessler, K. K. & Hanke, M. T. [1919]. *J. biol. Chem.* **39**, 497.
—— [1922]. *J. biol. Chem.* **50**, 235.
Kohn, H. T. [1937]. *Biochem. J.* **31**, 1693.
Pucher, G. W. & Day, H. A. [1926]. *J. Amer. chem. Soc.* **48**, 672.
Pugh, C. E. M. & Quastel, J. H. [1937]. *Biochem. J.* **31**, 286.
Richter, D. [1937]. *Biochem. J.* **31**, 2022.
Van Slyke, D. D. [1929]. *J. biol. Chem.* **83**, 425.
Webster, M. D. & Bernheim, F. [1936]. *J. biol. Chem.* **114**, 265.
Willstätter, R., Kuhn, R., Lind, O. & Memmen, F. [1927]. *Hoppe-Seyl. Z.* **167**, 303.
Woods, D. D. & Clifton, C. E. [1937]. *Biochem. J.* **31**, 1774.
Zeller, E. A. [1938]. *Helv. chim. Acta*, **21**, 880, 1645.