CCLXVIII. THE OXIDATION OF ADRENALINE AND OTHER AMINES

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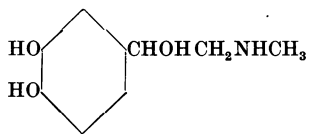
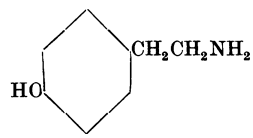
Mammalian liver and other organs contain an enzyme which catalyses the oxidation of adrenaline. By testing the ability of this enzyme to oxidize other amines of similar constitution it was found that certain groups in the adrenaline molecule, namely (*a*) the hydroxyl group in the side chain, (*b*) the phenolic hydroxyl groups and (*c*) the *N*-methyl group, were not essential for the oxidation to take place [Blaschko *et al.* 1937, 1, 2]. This suggested that the enzyme might be identical with the tyramine oxidase described by Hare [1928]. Their identity became still more probable when it was found that the reactions catalysed by these enzymes are chemically very similar, being in each case an oxidative



Adrenaline



Tyramine

deamination in the side chain [Richter, 1937]; but Hare's statement that adrenaline is not oxidized by tyramine oxidase made it appear that the adrenaline oxidase and tyramine oxidase must be two distinct enzymes.

Other enzymes which catalyse the oxidation of amino-compounds are also known and five such systems are described in the literature:

(1) Adrenaline oxidase [Blaschko *et al.* 1937, 1].
(2) Tyramine oxidase [Hare, 1928].
(3) Aliphatic amine oxidase [Pugh & Quastel, 1937].
(4) Histaminase [Best & McHenry, 1930].
(5) Amino-acid oxidase [Krebs, 1933].

The question of whether these systems are all distinct and separate from each other has not been fully investigated. The work of Best & McHenry [1930] and others leaves little doubt that histaminase is different from the systems (1), (2) and (5) as it is strongly inhibited by cyanide. Hare [1928] concluded that the tyramine oxidase was different from the amino-acid oxidase since her preparations did not oxidize tyrosine or phenylalanine. Pugh & Quastel [1937] showed that the aliphatic amine oxidase is distinct from the amino-acid oxidase since the former but not the latter enzyme is present in brain. The choline oxidase recently described by Mann & Quastel [1937] and Bernheim & Webster [1937] is clearly not in the same category since it is the alcohol group and not the amino-group of the choline molecule that is attacked.

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In the present paper it is shown that the first three systems, (1), (2) and (3), are similar in every property that has been investigated, and it is therefore suggested that they represent one enzyme which may be called "amine oxidase" which catalyses the oxidative deamination of a large number of different types of amines and which can be shown to be distinct from both histaminase and amino-acid oxidase.

These conclusions are based on the following series of experiments:

(1) Investigation of the distribution of the amine oxidases by testing preparations from different sources with representative substrates of each enzyme.

(2) Investigation of the action of inhibitors on the oxidation of different substrates.

(3) Competition experiments with mixed substrates.

Distribution of amine oxidases

The adrenaline oxidase was previously shown to occur in mammalian liver, intestine and kidney [Blaschko *et al.* 1937, 1]. The distribution of the tyramine oxidase and the aliphatic amine oxidase have not hitherto been systematically studied; the former was known to occur in liver and kidney, the latter in liver, kidney and brain.

In the present investigation a number of amine oxidase preparations from different sources have been examined by testing their ability to oxidize a series of amines and amino-compounds of different types. As substrates of the adrenaline oxidase *l*-adrenaline and *l*-*p*-sympatol were taken; for the tyramine oxidase tyramine and β-phenylethylamine; for the aliphatic amine oxidase heptylamine and *iso*amylamine; as substrates of the amino-acid oxidase *dl*-alanine and *dl*-methionine. The oxidations were carried out in the presence of 10^{-3} *M* cyanide which inhibits histaminase. The cyanide further protects the adrenaline from autoxidation or oxidation by the cytochrome oxidase system in the preparations [Green & Richter, 1937].

Amine oxidase in mammalian tissues

The enzyme preparations were made by grinding the tissue for 20 min. with sand, adding 1 vol. of *M*/15 phosphate buffer *p*H 7·3 and centrifuging. With brain, lung and intestine the supernatant solution was then made up to 2 vol. with buffer and used directly. With liver and kidney the extracts were further purified by dialysis. Sufficient *M* buffer was then added to make the solution *M*/15 with respect to phosphate before diluting to 2 vol. For the intestine preparations the intestine was first slit open down its length, washed with water, and allowed to stand for 10–20 min. in 0·01% hydroxyquinoline solution in order to destroy bacteria. The experiments were carried out with the Warburg apparatus. Each vessel contained 1·9 ml. enzyme solution, 0·1 ml. *M*/50 HCN and 0·2 ml. *M*/4 amine hydrochloride solution. This concentration of amine was sufficient to saturate the enzyme in the case of adrenaline and tyramine, and probably also for the other amines.

The results shown in Table I are representative of a number of experiments which were carried out with amine oxidase preparations obtained from different mammalian tissues. The figures give the increase in oxygen uptake in the course of 1 hr. in the presence of the added amine: the oxygen uptake of the enzyme preparation alone under the same conditions is also given. Histamine was oxidized slowly or not at all by these preparations in the presence of 10^{-3} *M*

Table I

Oxygen uptake (μl.) in 1 hr. at 37° in excess of that given by the enzyme preparation alone

	Guinea-pig				Rat			Pig	
Substrate	Liver	Intestine	Brain	Kidney	Liver	Kidney	Lung	Liver	Brain
Sympatol	202	131	14	45	63	6	16	57	7
Adrenaline	264	170	25	76	105	(65)	35	50	14
Tyramine	462	306	73	135	252	37	84	126	29
Phenylethyl-amine	66	29	1	15	24	8	8	20	2
Heptylamine	35	12	1	13	6	1	11	15	0
*iso*Amylamine	228	85	44	56	92	12	42	119	29
Histamine	0	0	—	0	2	0	7	—	2
Methionine	30	4	—	10	66	168	1	—	8
Alanine	26	4	—	13	83	246	6	—	2
Preparation alone	22	29	15	3	47	23	20	7	3

cyanide. The rate of oxidation of the amino-acids varied widely, showing the amino-acid oxidase to be present in some but not all of the preparations, and showing clearly that this enzyme is distinct from the amine oxidases. The three systems, adrenaline oxidase, tyramine oxidase and aliphatic amine oxidase, on the other hand, were found invariably to occur together. Some agreement can further be observed in the relative rates at which these three sets of amines were oxidized by the different preparations. The values for adrenaline were in general less reproducible than for the other amines; when the solution was strongly coloured at the end of the experiment, showing considerable autoxidation to have occurred, the figures obtained are given in brackets.

Amine oxidases in vertebrates

Amine oxidase preparations obtained from a number of other vertebrates were tested in a similar manner. The figures shown in Table II, which include

Table II

Oxygen uptake (μl.) in 1 hr. at 37° in excess of that taken up by the enzyme preparation alone. 10^{-3} *M* HCN. Conditions as previously described.

	Pigeon		Tortoise		Frog		Trout	
Substrate	Intestine	Liver	Intestine	Liver	Intestine	Liver	Intestine	Liver
Sympatol	36	33	172	83	7	31	11	12
Adrenaline	43	67	202	125	7	23	14	11
Tyramine	75	155	274	296	2	167	55	30
*iso*Amylamine	29	99	75	159	2	76	14	18
Preparation	7	13	22	24	3	15	3	0

representatives of the birds, reptiles, amphibians and fishes show that these enzymes are widely distributed throughout the vertebrates.

In this series of experiments some agreement can again be observed in the relative proportions in which the three amine oxidases are present.

Amine oxidases in invertebrates

A number of invertebrates have been tested in a similar manner for amine oxidases. Active preparations were obtained from the echinoderms *Asterias rubens* and *Echinus esculenti*, and from the mollusc *Patella vulgata*.

Table III

Oxygen uptake (μl.) in 1 hr. in excess of that taken up by the enzyme preparation alone. *Asterias* at 29°; *Echinus* at 37°; *Patella* at 25°. 10^{-3} *M* HCN. Conditions as previously described.

	Asterias		*Echinus*		*Patella*	
Substrate	Hepatic caecum	Stomach	Gonads	Alimentary tract	Muscle	Viscera
Sympatol	2	2	2	22	8	11
Adrenaline	(35)	(144)	2	24	10	41
Tyramine	20	21	12	48	36	85
*iso*Amylamine	19	15	44	145	7	17
Preparation	3	12	0	5	2	25

In several other organisms the amine oxidase could not be found. These observations may be summarized as follows:

Phylum	Species	Organs	Amines tested	Result
Vertebrata	All species tested	Liver etc.	T, I, S	+
Echinoderma	*Asterias rubens* L.	Hepatic caecum	,,	+
	,,	Stomach	,,	+
	Echinus esculentus L.	Gonads	,,	+
	,,	Alimentary tract	,,	+
Mollusca	*Patella vulgata* L.	Muscle	,,	+
	,,	Viscera	,,	+
	Helix aspersa	,,	T, I	−
Arthropoda	*Cancer pagurus* L.	Hepatopancreas	I	−
	,,	Gills	,,	−
Annelida	*Lumbricus terrestris*	Whole animal	,,	−
	Hirudo medicinalis	,,	,,	−
Coelenterata	*Tealia felina* (L.)	Muscle	T, I, S	−
	,,	Mesenterium	,,	−
	Actinea equina L.	Whole animal	,,	−
	Aurelia aurita (L.)	Denser parts	,,	−
Nematoda	*Ascaris lumbricoides* var. Suis	Whole animal	T, I	−
Fungi	*Agaricus campestris*	Basidium	,,	−
	Saccharomyces cerevisiae	Whole cells	,,	−
Phanerogams	*Solanum tuberosum*	Tuber	,,	−
	Ricinus communis	Seeds	,,	−
	Nasturtium officinale	Leaves	,,	−
	Cichorium intybus	,,	,,	−

With these organisms the oxygen uptake in 1 hr. at 26° under the conditions previously described was 0 ± 5 μl. With *Aurelia* the more solid parts of the animal were selected and the extract used undiluted. With *Saccharomyces* a 20% suspension was used. In describing the amines tested T = tyramine, I = *iso*amylamine and S = *l-p*-sympatol. Except where otherwise stated the amines were used as the hydrochlorides.

Action of inhibitors on amine oxidases

The amine oxidases are not inhibited by cyanide. This was known for the tyramine oxidase [Hare, 1928] and is true also for the aliphatic amine oxidase. On the other hand, all three systems are strongly inhibited by substances containing a hydrocarbon chain, such as the higher alcohols (e.g. octyl alcohol) or thymol.

Competitive inhibitors. *l*-Ephedrine and other *iso*propylamine derivatives are not oxidized by the amine oxidase preparations, but it can be shown that they have a marked affinity for the enzyme since in the presence of ephedrine the oxidation of adrenaline, *iso*amylamine or tyramine is inhibited. The inhibitory effect of ephedrine is of the competitive type since the degree of inhibition depends on the concentrations of both substrate and inhibitor. The following figures show this with *iso*amylamine as substrate:

*iso*Amylamine	0·0025 *M*	0·0025 *M*	0·02 *M*	0·02 *M*
l-Ephedrine	0	0·02 *M*	0	0·02 *M*
μl. O_2 uptake in 6 min.	20	10	28	21
% inhibition	—	50	—	25

A number of other amines which are not oxidized or which are oxidized only slowly show a similar inhibitory effect: in this group are to be included the tertiary amines triethylamine, tri*iso*amylamine and *dl*-1-hydroxy-2-hydrindamine.

A number of substances which are slowly oxidized show a relatively high affinity for the enzyme, and if added with another substrate decrease its rate of oxidation. Hordenine, the *N*-dimethyl derivative of tyramine, for example, is oxidized at about a fifth of the rate of *iso*amylamine, but the addition of an equimolecular amount of hordenine reduces the rate of oxidation of *iso*amylamine to about one-half:

*iso*Amylamine	0·02 *M*	0	0·02 *M*
Hordenine	0	0·02 *M*	0·02 *M*
μl. O_2 uptake in 15 min.	65	6	36

l-Ephedrine inhibits the three amine oxidases, but does not inhibit the amino-acid oxidase. This is shown for the oxidation of *dl*-alanine by an amino-acid oxidase preparation obtained from pig liver by the method of Krebs [1933]:

dl-Alanine	0·04 *M*	0	0·04 *M*
l-Ephedrine	0	0·04 *M*	0·04 *M*
μl. O_2 uptake in 20 min.	28	0	30

Competition experiments

If two substrates are oxidized by the same enzyme it is to be expected that the rate of oxidation of the two substrates together in saturation concentration will be less than the sum of the oxidation rates of each substrate alone. When tyramine was added to amine oxidase preparations in saturation concentration the further addition of either adrenaline or of *iso*amylamine did not increase the rate of oxidation.

(*a*) *Competition between tyramine and adrenaline for the amine oxidase of guinea-pig liver*

l-Adrenaline	0·0055 *M*	0·0055 *M*	0	0
Tyramine	0	0·0055 *M*	0·0055 *M*	0·011 *M*
μl. O_2 uptake in 7·5 min.	9	23·5	28	29

(*b*) *Competition between tyramine and* iso*amylamine*

*iso*Amylamine	0·04 *M*	0·04 *M*	0	0
Tyramine	0	0·04 *M*	0·04 *M*	0·08 *M*
μl. O_2 uptake in 6 min.	49	78	99	90

Control experiments were carried out to show that the rate of oxygen uptake was not limited by the rate of shaking or by the construction of the manometer vessels. That competition between adrenaline and tyramine occurs not only in "adrenaline oxidase" preparations but also in preparations of "tyramine oxidase" has been confirmed in an experiment made in collaboration with Dr H. I. Kohn [1937].

In a similar manner the competition between tyramine and tryptamine (β-indolethylamine), which is also rapidly oxidized by the amine oxidase, could be demonstrated:

(*c*) *Competition between tyramine and tryptamine*

Tyramine	0·02 *M*	0	0·02 *M*
Tryptamine	0	0·02 *M*	0·02 *M*
μl. O_2 uptake in 20 min.	59	49·5	52·5

In pig kidney preparations which will oxidize both amines and amino-acids no competition of these two substrates was found:

Tyramine	0	0·02 *M*	0·02 *M*	0·04 *M*
dl-Alanine	0·02 *M*	0·02 *M*	0	0
μl. O_2 uptake in 20 min.	9	52	40	40

It can be seen that the oxygen uptake in the presence of tyramine and alanine together is approximately equal to the sum of the uptakes of each substrate alone, although the tyramine is present in saturation concentration.

Specificity of amine oxidase

In order to obtain further information as to the different types of amines that are oxidized by the amine oxidase a number of different amines have been tested with the guinea-pig liver preparation.

The figures given for the relative rates of oxidation of the substrates represent percentages of the rate of oxidation of tyramine under the same conditions, i.e. in the course of 1 hr. at 37°. As before, the oxidations were carried out in the presence of 10^{-3} *M* cyanide to prevent autoxidation or oxidation of the more labile substrates by the cytochrome oxidase system. *M*/20 semicarbazide was also added as an aldehyde fixative so that the effects of the aldehyde oxidase and mutase could be eliminated. Each Warburg vessel contained 1·8 ml. enzyme preparation, 0·2 ml. *M*/4 amine hydrochloride, 0·1 ml. *M*/50 HCN and 0·1 ml. *M* semicarbazide. Tyramine was taken as the standard for comparison as it was the most rapidly oxidized substrate that had been found when this work was commenced. For most amines the figures given for the relative rates of oxidation are based on only two or three measurements and are intended to give only an approximate indication of the relative rates. When the rate of oxidation was less than about 10% of that of tyramine it became increasingly difficult to be certain whether the observed oxygen uptakes were real or due only to experimental error.

In the description of the tissues tested l = liver, i = intestine and k = kidney. All the amines were used as the hydrochlorides with the exception of hordenine sulphate and acetylcholine bromide. A warm supersaturated solution of di*iso*amylamine was used. The aniline, *p*-toluidine, ethylaniline and dimethylaniline were used in saturated solution. A small amount of the acetylcholine was hydrolysed during the experiment by choline esterase present in the preparations, but any considerable oxidation of acetylcholine should have been

Substrate	Tissues tested	Result	Relative rate
1. Methylamine	l, i	±	<2
2. Ethylamine	,,	+	<2
3. Propylamine	,,	+	7
4. Butylamine	,,	+	54
5. Amylamine	,,	+	19
6. Heptylamine	,,	+	5
7. *iso*Propylamine	,,	−	<2
8. *iso*Butylamine	,,	+	9
9. *iso*Amylamine	l, k, i	+	105
10. Allylamine	l, i	±	<2
11. Dimethylamine	,,	−	<2
12. Diethylamine	,,	−	<2
13. Dipropylamine	,,	−	<2
14. Dibutylamine	,,	±	<2
15. Di*iso*amylamine	,,	±	<2
16. Trimethylamine	,,	−	<2
17. Triethylamine	,,	−	<2
18. Tributylamine	,,	−	<2
19. Tri*iso*butylamine	,,	−	<2
20. Triamylamine	,,	−	<2
21. Tri*iso*amylamine	,,	−	<2
22. Glycine ester	,,	−	<2
23. Putrescine	,,	−	<2
24. Cadaverine	,,	−	<2
25. *cyclo*Hexylamine	,,	−	<2
26. Taurine	,,	−	<2
27. Colamine	,,	−	<2
28. Choline	,,	−	<2
29. Acetylcholine	,,	−	<2
30. $\gamma\gamma'\gamma''$-Trihydroxytripropylamine	,,	−	<2
31. *d*-Ornithine	,,	−	<2
32. *d*-Lysine	,,	−	<2
33. Aniline	l	−	<2
34. *p*-Toluidine	,,	−	<2
35. Ethylaniline	,,	−	<2
36. Dimethylaniline	,,	−	<2
37. Benzylamine	l, i	+	9
38. β-Phenylethylamine	,,	+	11
39. *l*-Phenylalanine	l	−	<2
40. *dl*-Alanine	i	−	<2
41. *l*-Methionine	,,	−	<2
42. Tyramine	l, i	+	100
43. *l*-Adrenaline	,,	+	65
44. *d*-Adrenaline	,,	+	45
45. *l*-*p*-Sympatol	,,	+	48
46. *d*-*p*-Sympatol	,,	+	—
47. *dl*-*m*-Sympatol	l	+	59
48. *dl*-Arterenol	l, i	+	51
49. Epinine	,,	+	125
50. Adrenalone	l	+	30
51. β-Phenyl-β-hydroxyethylamine	,,	+	46
52. *l*-Ephedrine	l, i	−	<2
53. *d*-Pseudoephedrine	l	−	<2
54. *dl*-Corbasil	,,	−	<2
55. Colephrin	,,	−	<2
56. *dl*-Hydroxyhydrindamine	,,	−	<2
57. Hydrastinine	,,	±	—
58. Mescaline	,,	+	5
59. Tryptamine	l, i	+	87
60. Histamine	l	−	<2
61. Hordenine	,,	+	12
62. Benzedrine	l, i	−	<2
63. Homorenon	l	+	16
64. *dl*-Alkamine	l, i	+	12
65. Dihydroxyphenylethylamine	,,	+	140
66. ω-Aminoacetocatechol	l	+	22

observed. In the above list are a few compounds previously tested by Hare [1928] and by Pugh & Quastel [1937].

From the 66 compounds tested certain conclusions as to the specificity of the amine oxidase system can be drawn. Only compounds containing an amino-group at the end of a hydrocarbon chain were oxidized. Compounds with a substituent on the α-carbon atom, such as *iso*propylamine, benzedrine, ephedrine and the α-amino-acids, were not attacked. In the case of ephedrine this cannot be due to lack of affinity for the enzyme since it has been shown that ephedrine can act as a competitive inhibitor.

Compounds such as methylamine and ethylamine with no pronounced hydrocarbon chain were oxidized only slowly or not at all, while compounds such as cadaverine and histamine in which the hydrocarbon chain is interrupted by a second polar group were also not oxidized. This suggests that the free hydrocarbon chain is an essential part of the substrates and may be concerned in determining their affinity for the enzyme.

β-Phenylethylamine and many of its derivatives were oxidized. This group includes adrenaline, tyramine and many substances with sympathicomimetic activity. Among these is arterenol (noradrenaline) which has a pressor effect of the same order of magnitude as that of adrenaline [Barger & Dale, 1910]. Mescaline was oxidized only very slowly: this may possibly be due to the presence of the three large methoxyl groups in the molecule.

The aliphatic tertiary amines such as triethylamine and tri*iso*amylamine were not oxidized at a measurable rate, but hordenine which contains a tertiary dimethylamino-group showed a slow but definite oxidation.

The relative rates of oxidation were found to be reproducible for different preparations from guinea-pig liver, but they differed for the amine oxidases of different animals. The rate of oxidation of di*iso*amylamine for example was too slow to measure with the guinea-pig preparations, but with pig liver it gave a relative rate of 27 %. Similarly the relative rate of oxidation of hordenine was much faster with the pig liver than with the guinea-pig liver preparation.

Variation of rate of oxidation with substrate concentration

The relation between the substrate concentration and the initial rate of oxidation was measured for adrenaline and tyramine. In order to minimize the possibility of errors arising from the delay in adding the substrate in the different manometer vessels the calculation of the Michaelis constant was based on the rates of oxidation between the third and ninth minutes after the beginning of the reaction. Except with the lowest substrate concentrations the rate of the reaction was sufficiently nearly linear for this purpose.

With the adrenaline 1 mg. reduced glutathione per ml. enzyme solution was added in order to ensure that no autoxidation occurred. The results of each series of experiments were plotted and the $p(S)$ values determined from the graphs. The $p(S)$ gives the negative logarithm of the molar substrate concentration at which the initial reaction rate is half the rate at saturation concentration. In three experiments with tyramine the figures obtained for the $p(S)$ with guinea-pig liver preparations were 2·6, 2·8 and 2·9; mean value 2·75. In three experiments with adrenaline the values obtained were 2·2, 2·0 and 2·25; mean value 2·15. This means that the affinity of the enzyme for tyramine is greater than for adrenaline, but of the same order of magnitude.

Discussion

Identity of amine oxidases. In view of the evidence obtained in the present work the independent existence of the three amine oxidases, adrenaline oxidase, tyramine oxidase and the aliphatic amine oxidase must be considered improbable.

There is no known method by which the identity of any two enzymes can be conclusively established; but it can be shown that they are so similar in their properties that their identity becomes increasingly probable. In the case of the three amine oxidases it has been shown that they are similar

(*a*) in the chemical reactions they catalyse,
(*b*) in their distribution in the animal kingdom,
(*c*) in their behaviour towards inhibitors, and
(*d*) in that their substrates compete with one another.

The relative rates at which the different substrates were oxidized by enzyme preparations from different sources also showed considerable agreement, though in a few cases distinct differences were observed. To take an extreme example the relative rate of oxidation of hordenine was 12 % with the guinea-pig but over 50 % with the pig liver preparations [Kohn, 1937]. These differences may be partly due to the presence of varying amounts of other enzymes such as aldehyde oxidase in the preparations. Another possible source of error is that inhibitors present in the preparations might affect the rates for different substrates to

different extents, depending on their different affinities for the enzyme. Yet these possible sources of error can hardly account for the differences observed for example in comparing the figures for the vertebrate preparations with those for the invertebrates. These differences might be interpreted as meaning that there exist different amine oxidases with closely similar properties; the more probable explanation is that the differences are due to slight variations in the specificity of the enzymes obtained from different sources.

Physiological significance of amine oxidase. A number of amines are known to disappear when given to the intact animal or added to the perfusion fluid of intact organs. Ewins & Laidlaw [1910] showed that tyramine is converted into the corresponding *p*-hydroxyphenylacetic acid. They assumed that the amino-group was first removed by hydrolysis to give the corresponding alcohol. This assumption appeared to receive some support from the work of Guggenheim & Löffler [1916] who also isolated the alcohol tyrosol from tyramine, and obtained the alcohols as well as the acids from a number of other amines such as *iso*-amylamine and tryptamine.

Hare [1928] and Bernheim [1931] concluded from her experiments with tissue extracts that in the case of tyramine an oxidation is involved, but as she did not isolate the intermediate aldehyde it was not clear whether this was a primary or secondary reaction.

In view of the evidence now available it can be concluded that the primary attack on the amines is brought about by the amine oxidase here described. That the corresponding aldehydes which are the primary oxidation products have not been isolated under the conditions of feeding or perfusion experiments is not surprising as the aldehydes would be expected to be further metabolized by other enzymes such as the aldehyde oxidase and mutase. The products which have been isolated are in fact those which would be expected to be formed if these enzymes are responsible for the removal of the aldehyde.

Whether amines other than adrenaline are formed as products of animal metabolism is not known; but it is known that many bacteria that constitute the normal and pathological flora of the intestinal lumen are capable of forming amines. If these substances, many of which are toxic, are formed in the intestine, it is not surprising to find a system present which destroys them. From this point of view it may be noted that the enzyme occurs in highest concentration in intestine and liver.

As far as adrenaline is concerned it is now shown that it is oxidized by the amine oxidase in the same manner as many other amines. The affinity of adrenaline for the enzyme is less than that of tyramine but of the same order. Whether the amine oxidase can be held responsible for the removal of adrenaline in the minute concentrations in which it occurs in the body can be decided only by further physiological experiments. The observations on the inhibition of the amine oxidase reported in this paper suggest a possible method of approach to this question.

When the concentration of adrenaline in the body is high, as under experimental conditions, there is no reason to doubt that the amine oxidase may be active in removing adrenaline. Weinstein & Manning [1937] have recently reported that a substance with the properties of protocatechuic acid appeared in the urine of rabbits to which adrenaline had been administered. This shows that under the conditions of their experiments the adrenaline molecule is oxidized in the side chain and suggests that the amine oxidase may be concerned.

SUMMARY

1. The enzymes adrenaline oxidase, tyramine oxidase and aliphatic amine oxidase are similar (*a*) in their distribution in the animal kingdom, (*b*) in their behaviour with inhibitors, and (*c*) in that their mixed substrates show competition. It is therefore concluded that these three systems are identical.

2. The amine oxidase was found to be present in every vertebrate tested and in a number of invertebrates.

3. Competition was observed to occur between the substrates (*a*) tyramine and adrenaline, (*b*) tyramine and *iso*amylamine, and (*c*) tyramine and tryptamine.

4. The affinities of the substrate for the enzyme were found to be $p(S) = 2{\cdot}75$ for tyramine and 2·15 for adrenaline.

5. The specificity of the amine oxidase has been examined by testing the oxidation of 66 amines of different types. For those amines which were oxidized the relative rates of oxidation have been measured.

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