CLXIII. AMINE OXIDASE

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In a previous paper it was shown that animal tissues contain an enzyme, amine oxidase, which oxidizes a number of aliphatic and aromatic amines, including adrenaline [Blaschko *et al.* 1937]. This enzyme is distinct from the diamine oxidase, or histaminase, which oxidizes diamines [Zeller, 1938].

Amine oxidase was shown to occur in mammalian liver, intestine, kidney, brain and lung. A more detailed study has now been made of the distribution of the enzyme in mammalian tissues. In addition the previous observations on the specificity of the enzyme have been extended by testing a number of new amines as substrates.

I. *Distribution of amine oxidase in mammalian tissues*

Tissue extracts were prepared as described by Blaschko *et al.* [1937] by grinding with sand, centrifuging, dialysing and making up to 3 vols. with phosphate buffer of *p*H 7·3. The experiments were carried out with Warburg-Barcroft manometers: each vessel contained 1·9 ml. tissue extract, 0·1 ml. *M*/50 HCN and 0·2 ml. *M*/4 amine hydrochloride solution.

Table I. *Amine oxidase content of mammalian tissues*

Oxygen uptake (μl. O_2 0·63 g. fresh tissue/hr. at *p*H 7·3 and 37°).

Animal	Organ	Tissue extract alone	Increased uptake with Tyramine	Increased uptake with *iso*Amylamine
Pig	Liver	0	162	158
	Pancreas	2	47	—
	Heart	7	21	28
	Intestine	5	59	—
	Spleen	18	9	9
	Thyroid	2	4	—
	Kidney	37	285	—
Ox	Liver	8	501	315
	Heart	3	33	28
	Intestine	23	175	69
	Spleen	14	61	—
	Kidney	55	412	258
	Brain	13	16	31
Sheep	Liver	5	387	289
	Pancreas	2	11	—
	Heart	3	4	—
	Intestine	2	104	—
	Spleen	2	57	33
	Thyroid	10	27	—
	Kidney	7	349	266
	Brain	12	55	40

		Tissue alone	With tyramine	With *iso*amyl-amine	With hordenine	With *p*-sympatol
Guinea-pig	Testicle	0	24	17	1	8

The results of these experiments are given in Table I. In all the animals examined, liver, kidney and intestine show the highest activity; but most other organs examined were active to some extent. The relative activities of different organs in different species differ widely: for instance, the pig's heart is much more active than the sheep's heart, where the extra O_2 uptake in the presence of amines was found to be only just outside the limits of experimental error. In another experiment (not included in Table I) no enzymic activity was found in a preparation from dog's ventricle.

A number of similar experiments are not included in Table I as they were done under slightly different conditions. Among these are observations on the uterus, which contains considerable quantities of the enzyme.

One part of dog's uterus was ground with sand, 1 part phosphate buffer was added; the mixture was centrifuged for 5 min., the supernatant fluid being used for the experiment. 1·7 ml. of this preparation were incubated with 0·1 ml. $M/50$ HCN and 0·2 ml. $M/4$ amine hydrochloride. The O_2 uptakes in 45 min. were:

	µl.
Extract alone	7
Excess with tyramine	51
Excess with *iso*amylamine	38
Excess with *l-p*-sympatol	19

The activity of dog uterus, when compared with the other data given, is quite considerable. Sheep uterus was also tested; it contains appreciable amounts of the enzyme.

The adrenals also contain amine oxidase. In an experiment, in which to 1 part of sheep adrenals 1 part of phosphate buffer was added, 1·7 ml. of the extract (with 0·1 ml. $M/50$ HCN and 0·2 ml. $M/4$ amine hydrochloride) the subsequent O_2 uptakes in 55 min. were:

	µl.
Extract alone	106
Excess with tyramine	88
Excess with *iso*amylamine	43
Excess with *l-p*-sympatol	17

The relatively large O_2 uptake of the extract alone makes these figures somewhat less conclusive.

Guinea-pig skeletal muscle contains only small amounts of enzyme. 1·7 ml. of a preparation from guinea-pig muscle (1 part muscle + 2 parts phosphate buffer) showed in the presence of 0·1 ml. $M/50$ HCN and 0·2 ml. $M/4$ amine hydrochloride the following O_2 uptakes/hr.:

	µl.
Extract alone	14
Excess with tyramine	8
Excess with *iso*amylamine	7
Excess with tryptamine	12

The experiments reported in this and the preceding paper show that the enzyme has a much wider distribution than was previously believed. Kidney, liver and intestine contain the enzyme in highest concentration, but lungs, brain, uterus and—in some animals—spleen also give highly active preparations. The extent to which cortex and medulla of the adrenal contribute to the total activity of the organ remains to be examined. In view of the wide distribution the lack of enzymic activity of muscular tissue, especially skeletal muscle, is interesting. But here again species differences in the distribution of the enzyme may exist. The uterus is the only muscular organ that seems regularly to

contain amine oxidase in high concentration; this is of interest in view of the observation of Ewins & Laidlaw [1910] that tyramine disappears from the perfused cat's uterus. These authors found that tyramine is metabolized to *p*-hydroxyphenylacetic acid, which indicates that the disappearance of the amine is due to the amine oxidase.

How do the observations on the distribution of the enzyme contribute to the understanding of its function in the animal body? It has already been pointed out that the presence of the enzyme in the intestine may serve to protect the body from the effects of amines formed by bacterial activity in the intestinal lumen. This is supported by the fact that the diamine oxidase also occurs in high concentration in the intestine and may therefore serve to prevent the diamines (histamine, putrescine, cadaverine) from reaching the general circulation. It appears unlikely that an enzyme system which shows such a widespread distribution should have only a localized detoxicating function, and the amine oxidase may well have a more general significance in cell metabolism.

Another function has been suggested for this enzyme in the work of Gaddum & Kwiatkowski [1938], namely that it may serve to inactivate the adrenergic transmitter substance of the post-ganglionic sympathetic neurones released on stimulation of the sympathetic nerve. This theory will be dealt with more fully elsewhere, but it must be said that the experiments reported here do not support the conception. A number of organs with a good sympathetic nerve supply, it is true, are rich in amine oxidase (e.g. the intestine, liver and uterus); but on the other hand heart muscle is poor in enzyme and the brain contains relatively large amounts. The sympathetic supply to the rabbit's ear on which Gaddum & Kwiatkowski's experiments were done, is probably mostly to the blood vessels of the skin; attempts to demonstrate the presence of the enzyme in the skin from rabbit's ears failed, although it must be admitted, in view of the toughness of the tissue, that it is possible that the enzyme may not have been successfully extracted.

II. *Specificity of amine oxidase*

Blaschko *et al.* studied the specificity of the guinea-pig oxidase by testing the oxidation of 66 amines of different types. A number of new amines have now been tested. The conditions in these experiments were as previously described. The results shown in Table II agree with and confirm the conclusions previously

Table II

Substrate	Tissue tested (l = liver, i = intestine)	Relative rate
67. *l-sec*-Butylamine	l, i	<2
68. Novocain	l, i	<2
69. Glucosamine	l, i	<2
70. Camphylamine	l, i	3
71. Piperidine	l, i	<2
72. 3:4-Methylenedioxy-δ-phenyl-β-aminobutane	l, i	<2
73. α-Phenylethylamine	l, i	<2
74. Hordenine methylchloride	l, i	<2
75. β-Phenylethylmethylamine	l	29
76. β-(3-Methoxyphenyl)-ethylamine	l	24
77. β-(3:4-Dimethophenyl)-ethylamine	l	9
78. Ethoxy-6-methylaminomethyl-2-cumarane (887 F)	l	<2
79. Methylaminomethyl-2-cumarane (879 F)	l	<2
80. Piperidinomethyl-3-benzodioxane (933 F)	l	<2
81. ε-Aminocaproic acid	l	<2
82. Agmatine	l	<2

arrived at as to the specificity of the amine oxidase. The absence of any oxidation with *l-sec*-butylamine shows that the inability of the enzyme to oxidize compounds of the type $R(CH_3)CHNH_2$ extends to the aliphatic as well as to the aromatic series of amines.

This result had previously been established only for aromatic compounds such as ephedrine. A number of ephedrine derivatives which have since been tested all conform to this general rule. A further point of interest is in the behaviour of the methoxy-phenyl derivatives: the monomethoxy compound No. 76 shows a relative rate 24, the dimethoxy compound, No. 77 gives 9, and the trimethoxy compound mescaline (No. 58 of our preceding paper) has a relative rate of 5. This shows that the relative rate decreases with increasing substitution by methoxyl groups.

III. *Effect of urea on the enzyme*

That urea has a denaturing action on certain proteins is well known. Guinea-pig liver preparations are inactivated by treatment with concentrated urea solutions.

Equal amounts of guinea-pig liver extract and 6*M* urea solution were mixed and the enzymic activity towards tyramine was tested immediately. The activity of the extract+urea was found to be only 24% of that of the extract alone. Another sample of the mixture was tested after keeping in the refrigerator for 24 hr.; it showed no activity. The remainder of the extract-urea mixture was then dialysed against distilled water for 24 hr. to remove the urea; no activity had reappeared when tested. This makes it likely that urea irreversibly inactivates the enzyme. In this respect the amine oxidase differs from succinic dehydrogenase, which has recently been shown to retain its activity after treatment with concentrated urea solutions [Hopkins *et al.* 1939].

Summary

1. The enzyme amine oxidase is shown to have a very wide distribution in the mammalian body.
2. The list of substances tested as possible substrates has been extended; the results confirm the conclusions previously arrived at.
3. Urea (3*M*) irreversibly inactivates the enzyme.

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