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OBSERVATIONS ON THE SUBSTRATE SPECIFICITY OF AMINE OXIDASES

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A number of compounds have been tested as substrates of, first, the amine oxidases of rabbit and guinea-pig liver and of goat, pig, horse, and dog plasma and, second, the " diamine oxidase " of pig kidney. Of the three xylylenediamines [di(aminomethyl)benzenes] tested, *m*-xylylenediamine was found to be a substrate of the liver oxidase. All the plasma oxidases tested acted on both *m*- and *o*-xylylenediamine. Both 3- and 4-picolylamine (3- and 4-aminomethylpyridine) were readily oxidized by the liver enzymes; all the plasma oxidases tested oxidized 2-, 3-, and 4-picolylamine. 4-Picolylamine was more rapidly oxidized by horse and dog plasma than any other substrate hitherto examined.

Recent studies on the amine oxidases of mammalian plasma (Blaschko and Hawes, 1959; Blaschko, Friedman, Hawes, and Nilsson, 1959) have shown that the amine oxidases in general are a class of enzymes with a great diversity of substrate specificity. The inhibitor specificities of the plasma oxidases show that they are more closely related to the classical histaminase of pig kidney, although their substrate specificity is in many ways reminiscent of that of the intracellular amine oxidase.

In the following, experiments are reported on some new substrates of some of these oxidases. These substances were tested in the hope that they may be useful in the detection and characterization of these enzymes.

The enzymes tested in the present work were: the intracellular amine oxidase of mammalian liver, the histaminase (" diamine oxidase ") of pig kidney, the spermine oxidase of ruminant plasma (Hirsch, 1953; Tabor, Tabor, and Rosenthal, 1954), and the " benzylamine oxidase " of non-ruminant plasma (Bergeret, Blaschko, and Hawes, 1957).

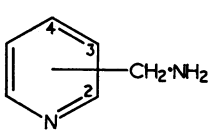
MATERIAL AND METHODS

Enzyme Preparations.—Dialysed extracts of guinea-pig and rabbit liver were prepared as described by Barlow, Blaschko, Himms, and Trendelenburg (1955). In a few preliminary experiments, carried out with Mrs. Jean M. Hagen (née Himms) in 1950, an acetone dried powder of rabbit liver was used as source of enzyme; this preparation, as well as the extract of pig kidney for the experiments on histaminase, has been described elsewhere (Blaschko and Hawkins, 1950). Sodium phosphate buffer of *p*H 7.4 was used throughout.

All the serum (or plasma) samples used, from the goat, pig, horse, or dog, were dialysed against 0.06 M-sodium phosphate buffer of *p*H 7.4. Serum was used in most experiments, but in a few instances we obtained fresh heparinized dog plasma from Professor I. de Burgh Daly and Dr. B. A. Waaler; the samples of dog serum were from animals used as blood donors in this department.

Substrates.—All compounds were used as the hydrochlorides. We are grateful to Dr. H. R. Ing, who in 1950 prepared for us the para and ortho isomers of xylylenediamine [di(aminomethyl)benzene, $C_6H_4(CH_2.NH_2)_2$]. The meta isomer has recently become available commercially from Messrs. Aldrich. We have also tested homo-*o*-xylylenediamine [*o*-(2-aminoethyl)benzylamine, $NH_2.CH_2.C_6H_4.CH_2.CH_2.NH_2$], kindly made for us by Dr. E. W. Gill, according to Helfer (1923).

The three isomeric picolylamines (2-, 3-, and 4-aminomethylpyridine) (I) were also obtained from Messrs. Aldrich.



(I)

Manometric Experiments—The rate of the enzymatic reaction was studied by measuring the initial uptake of oxygen after the addition of substrate at 37.5° in an atmosphere of O_2. Substrate concentrations after tipping were 10^{-2} M. In the experiments with liver amine oxidase, oxedrine [(±)-*p*-Sympatol] was used as the reference amine; with pig kidney enzyme, cadaverine; with goat plasma enzyme, spermine; with non-ruminant plasma enzymes (pig, horse, dog), benzylamine.

RESULTS

Xylylenediamines

Liver Oxidases.—Of the three isomers of xylylenediamine, only the meta isomer was oxidized at a significant rate. Its oxidation was rapid in rabbit liver, but relatively slow in guinea-pig liver. The results of these experiments are summarized in Table I in which it can be seen that homo-*o*-xylylenediamine was oxidized more rapidly than *o*-xylylenediamine itself; with the latter compound, the rate of oxidation was scarcely significant. However, *o*-xylylenediamine was not without affinity for the liver enzyme: it strongly depressed the rate of oxidation of oxedrine. With *p*-xylylenediamine in equimolecular amounts, the oxidation of oxedrine by rabbit liver was only about halved.

TABLE I

OXIDATION OF XYLYLENEDIAMINES BY RABBIT AND GUINEA-PIG LIVER

Substrate concentrations, 10^{-2} M. The oxygen uptakes are given in % of the uptake with oxedrine as substrate, calculated from the first 30 min. of incubation. The number of separate experiments contributing to the mean is given in parentheses.

Substrate:	*p*-Xylylenediamine	*m*-Xylylenediamine	*o*-Xylylenediamine	Homo-*o*-xylylenediamine
Source of enzyme:				
Rabbit liver ..	0 (1)	63 (2)	9 (1)	14 (3)
Guinea-pig liver	5 (1)	21 (3)	3 (2)	17 (3)

Pig Kidney Oxidase ("*Diamine Oxidase*").—No oxygen uptake occurred when the pig kidney preparation was incubated with either *p*- or *m*-xylylenediamine; with *o*-xylylenediamine there was a small uptake of oxygen. The oxygen uptake in the presence of both cadaverine and *o*-xylylenediamine was practically the same as in the presence of cadaverine alone, but when cadaverine and either *p*- or *m*-xylylenediamine were present in equal concentrations (10^{-2} M), oxygen uptakes were completely abolished. The action of these two isomers on the oxidation of cadaverine was therefore also tested in lower concentrations, and the oxygen uptakes in the first 30 min. of incubation are given in Table II.

TABLE II

INHIBITION OF DIAMINE OXIDASE OF PIG KIDNEY BY *m*- AND BY *p*-XYLYLENEDIAMINE

The oxygen uptakes in the first 30 min. of incubation are given in μl. O_2. Cadaverine concentration, 10^{-2} M.

Xylylenediamine Isomer Tested	Cadaverine Alone	With 10^{-3} M Diamine	With 10^{-4} M Diamine
Meta	52	2·5	16
Para	68·5	7·5	43

In an experiment with homo-*o*-xylylenediamine, there was no significant uptake of oxygen; in the presence of both cadaverine and homo-*o*-xylylenediamine (10^{-2} M), the rate of oxygen uptake was about one-half that with cadaverine alone.

Oxidases of Non-Ruminant Plasma (*Pig, Horse, and Dog*).—No significant oxidation of *p*-xylylenediamine was seen, but both the meta and the ortho isomers were readily oxidized. Homo-*o*-xylylenediamine was not oxidized.

Although *p*-xylylenediamine was not oxidized by the three non-ruminant sera, it was not without affinity for the enzyme: the experiment of Table III shows that the oxidation of benzylamine by pig serum was inhibited by *p*-xylylenediamine.

TABLE III

INHIBITION OF THE PLASMA OXIDASES BY *p*-XYLYLENEDIAMINE

Oxygen uptakes in the first 30 min. of incubation are given in μl. O_2.

Source of Enzyme	Substrate	Substrate Alone (μl.)	Substrate with *p*-Xylylenediamine		
			10^{-2} M	10^{-3} M	10^{-4} M
Pig serum	10^{-2} M-benzylamine	48	0	24·5	43
Goat ,,	10^{-2} M-spermine	117	0	20·5	93

Ruminant Plasma Oxidase (*Goat*).—As with the non-ruminant sera, there was no oxidation of *p*-xylylenediamine, although the oxidation of spermine was inhibited by this isomer (see Table III). Both the meta and the ortho isomers were oxidized by goat serum at similar rates, about 1/3 of the rate with spermine as substrate. Homo-*o*-xylylenediamine was not oxidized.

Picolylamines

Liver Oxidases.—The rate of oxidation of the three picolylamines depended upon the position of the side chain: 2-picolylamine was scarcely oxidized, but both 3- and 4-picolylamine were oxidized rather rapidly. With rabbit liver extract the rate of oxidation of 3-picolylamine, the isomer more rapidly oxidized, was more than 1/2 of the rate with oxedrine; with guinea-pig liver, the rate was less, about 1/5 of that of oxedrine.

Pig Kidney Diamine Oxidase.—None of the three compounds was oxidized significantly.

Non-Ruminant Plasma Oxidases.—Sera of all the three species tested oxidized all the three picolylamines. Pig serum acted on 3-picolylamine most readily. With horse and dog sera the rates of oxidation increased in the order: 2-, 3-, 4-picolylamine. This is shown in Table IV, where oxygen uptakes within the first 30 min. of incubation with the three sera have been listed.

TABLE IV

OXIDASES OF NON-RUMINANT PLASMA

Rates of oxidation of the three isomers of picolylamine, in comparison with the oxidation of benzylamine. The values are μl. O_2 consumed in the first 30 min. Substrate concentration, 10^{-2} M.

Species	Benzylamine	2-Picolyl-amine	3-Picolyl-amine	4-Picolyl-amine
Pig	47·5	21·5	30·5	24·5
Horse ..	12·5	12·5	19·5	28
Dog ..	32	20·5	53·5	63

In both horse and dog sera the rates of oxidation of 3- and 4-picolylamine exceeded the rates of oxidation of benzylamine, hitherto considered the most readily oxidized substrate of the non-ruminant plasma oxidase. This observation has been used to confirm the finding (Blaschko *et al.*, 1959) that in different samples of dog serum (or plasma) the enzymatic activity is very different. Table V is from 14 experiments, in each of which a different

TABLE V

OXIDATION OF BENZYLAMINE AND 4-PICOLYLAMINE BY DIFFERENT SAMPLES OF DOG SERUM OR PLASMA

Substrate concentrations: 10^{-2} M. The values are μl. of O_2 taken up between the 5 and 30 min. readings. S indicates serum, P heparinized plasma, M male, F female.

Sex	Sample	Benzylamine (Bz)	4-Picolylamine (Pic)	Ratio $\frac{Pic}{Bz}$
?	S	37	69	1·9
M	P	6	18·5	3·1
M	P	5·5	27	4·9
F	S	4	16·5	4·1
M	S	9·5	22·5	2·4
?	S	30·5	51	1·7
F	P	17	32·5	1·9
M	S	9	27	3·0
M	S	11·5	16	1·4
M	S	12·5	33·5	2·7
M	S	4	16·5	4·1
M	S	28·5	84·5	3·0
M	P	5·5	22·5	4·1
M	P	7	19·5	2·8

sample of dog serum (or plasma) has been tested with both benzylamine and 4-picolylamine. It can be seen that the value of the ratio, rate of oxidation of 4-picolylamine/rate of oxidation of benzylamine, varied between 1.4 and 4.9. It must be borne in mind that the rate of oxidation of benzylamine by dog serum was very small and therefore subject to a relatively large experimental error.

Ruminant Plasma Oxidase.—Goat serum readily oxidized the three isomers of picolylamine. In one experiment, the oxygen uptakes in the first 30 min. of incubation were, with 10^{-2} M concentrations of spermine, 126 μl. O_2; of 2-picolylamine, 48 μl. O_2; of 3-picolylamine, 48 μl. O_2; of 4-picolylamine, 58 μl. O_2. These rates are similar in order to those found with the sera from non-ruminants.

DISCUSSION

These results confirm earlier work from this laboratory which showed that the amine oxidase of non-ruminant plasma is an enzyme distinct from the intracellular amine oxidase, an important catalyst in the inactivation of amines with biological activity, in particular the catechol amines and 5-hydroxytryptamine. The substrate specificities of the plasma enzymes, benzylamine oxidase and spermine oxidase, are known to be closely similar (Blaschko and Hawes, 1959; Blaschko *et al.*, 1959), a relationship also brought out in the present study. The liver enzymes acted only on the meta isomer of xylylenediamine, whereas the two plasma enzymes oxidized both the meta and the ortho isomers. Also with the picolylamines, there were more similarities between the plasma enzymes than between them and the liver enzyme. The xylylenediamines must now be added to the group of diamines that are more readily attacked by amine oxidases than by " diamine " oxidases.

As the outcome of this work, benzylamine is no longer the amine most readily oxidized by the plasma oxidases from non-ruminants. In both dog and horse serum, 4-picolylamine was found to be oxidized at a much faster rate than benzylamine. This has proved a useful observation: in the dog and the horse, plasma oxidase activity is relatively low, and the use of a substrate more rapidly oxidized than benzylamine makes the study of the enzyme in these species easier and it may help in the detection of enzyme in species in which it has not hitherto been found.

In the dog, the varying amounts of the plasma oxidase remain puzzling. Since it has been shown that the dog enzyme acts on histamine (Blaschko *et al.*, 1959), the possibility has to be considered that the enzyme is identical with the histaminase studied by Carlsten, Kahlson, and Wicksell (1949). These Swedish authors found little histaminase activity in the blood plasma, but rather larger amounts of enzyme in the thoracic lymph. Histamine is a rather poor substrate of the plasma oxidase in comparison with benzylamine and 4-picolylamine. Since dog lymph has not yet been tested for enzymatic activity with substrates other than histamine, it cannot at present be decided whether the enzyme in the dog lymph is identical with the plasma enzyme here studied. Carlsten (1950) has shown that the enzymatic activity in the thoracic duct is dependent upon adrenocortical activity: adrenalectomy leads to a considerable increase in enzymatic activity in the thoracic lymph. It is tempting to consider the large differences between samples of dog serum

or plasma as a consequence of differing levels of adrenocortical activity. This could be settled by a study of the enzyme from the thoracic duct itself or by studying the effect of adrenalectomy on the plasma enzyme. However that may be, the high rate of oxidation of 4-picolylamine, when compared with that of histamine or benzylamine, may serve as a reminder that the natural substrate of the plasma oxidase in non-ruminants is still unknown.

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