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THE OXIDATION OF CYSTAMINE AND HOMOCYSTAMINE BY MAMMALIAN ENZYMES

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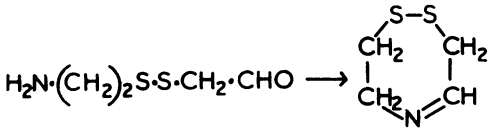
The oxidative deamination of cystamine and homocystamine by mammalian oxidases has been studied. The histaminase of pig kidney oxidizes homocystamine much more slowly than cystamine. The amine oxidase of mammalian liver (guinea-pig, rabbit) oxidizes homocystamine more rapidly than cystamine. Both amines are oxidized by plasma (or serum) of ruminants (ox, sheep, goat) and of the horse. In the enzymatic oxidation of homocystamine both amino-groups are removed; there is no evidence that a ring compound analogous to cystaldimine is accumulating.

The study of the substrate specificity of the mammalian amine oxidases is of interest because so many of the substrates of these enzymes have a high degree of biological activity on excitable tissues. These enzymes act also upon other amines, and there is good reason to believe that the oxidative deamination of these compounds is also a function of the mammalian oxidases.

Cavallini (1956) has recently reported that cystamine, $H_2N(CH_2)_2\ S.S(CH_2)_2\ NH_2$, is a substrate of the histaminase of pig kidney; he has also found that the amine is oxidized by the pea seedling oxidase of Kenten and Mann (1952). Evidence was obtained that, in the oxidation of cystamine, a ring compound was formed from the amino-aldehyde which is assumed to be the first product of the oxidation of cystamine:



This seven-membered ring compound was called cystaldimine (Cavallini, De Marco, and Mondovì, 1956, 1957).

Cystamine is of interest because cysteamine, its reduced form, is a constituent of co-enzyme A, and because it has a protective action against radiations (Bacq, Herve, Lecomte, Fischer, Blavier, Dechamps, Le Bihan, and Rayet, 1951). We have, therefore, studied the oxidation of cystamine by a number of mammalian oxidases. These oxidases included the amine oxidase of mammalian liver and also two other enzymes which have a substrate specificity similar to that of liver oxidase. These are the spermine oxidase of ruminant plasma (Hirsch, 1953; Tabor, Tabor and Rosenthal, 1954) and an enzyme recently found in horse serum and provisionally called benzylamine oxidase (Bergeret, Blaschko, and Hawes, 1957). Both spermine oxidase and benzylamine oxidase also act slowly on 5-hydroxytryptamine and on sympathomimetic amines.

Through the kindness of Dr. R. Cecil, we have recently obtained a small amount of homocystamine dihydrobromide, $H_2N(CH_2)_3\ S.S(CH_2)_3\ NH_2 \cdot 2\ HBr$, and we have studied the oxidation of this compound by the mammalian oxidases, in comparison with that of cystamine.

METHODS

The preparations of histaminase (an extract of an acetone-dried powder of pig kidney) and of liver amine oxidase did not differ from those already described (Barlow, Blaschko, Himms, and Trendelenburg, 1955). Horse serum and ruminant plasma (or serum) were dialysed against 0.067 M sodium phosphate buffer; usually the *p*H of the buffer was 7.4, but in a few experiments it was 6.5.

Uptake of oxygen was measured manometrically at a temperature of 37.5° and in an atmosphere of oxygen. In those experiments in which the initial uptake was measured, substrate concentration was 10^{-2} M; but when the reaction was followed to completion, substrate concentrations were lower.

Ammonia was determined using the microdiffusion method, followed by colorimetric determination with phenate-hypochlorite (Conway, 1947).

RESULTS

The observations of Cavallini *et al.* (1956) on the oxidation of cystamine by pig kidney extract were readily confirmed. The extracts oxidized homocystamine at a very much slower rate. In one of these experiments (at *p*H 7.4), the oxygen uptake of 1 ml. of extract incubated at 37.5° for 15 min. without added substrate was 8.5 μl. O_2. The additional amounts of oxygen consumed in the presence of 10^{-2} M cadaverine, cystamine, and homocystamine were 31.5, 37, and 4 μl. O_2 respectively.

Rabbit and guinea-pig liver preparations slowly oxidized both cystamine and homocystamine ; in every experiment the rate of oxygen uptake with homocystamine exceeded that with cystamine. Thus in an experiment with guinea-pig liver enzyme the amounts of oxygen consumed in the first 15 min. in the presence of 10^{-2} M tyramine, cystamine, and homocystamine were 156.5, 14.5, and 21 μl. O_2 respectively ; in a similar experiment with rabbit liver the amounts consumed were 106, 7.5, and 14.5 μl. O_2.

No significant uptake of oxygen occurred when human plasma was incubated with either cystamine or homocystamine. However, the amines were oxidized by horse serum. There is no spermine oxidase in horse serum, but we have already referred to the observation that it contains another oxidase which acts on many amines such as 5-hydroxytryptamine and benzylamine

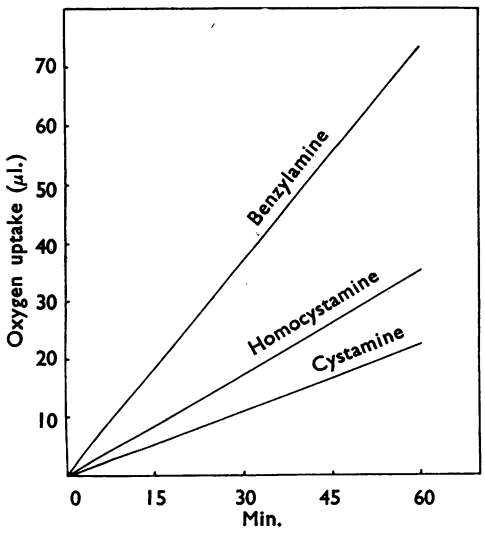


FIG. 1.—The oxidation of cystamine, homocystamine, and benzylamine by horse serum. The substrate concentration was 10^{-2} M and each flask contained 1.8 ml. dialysed serum.

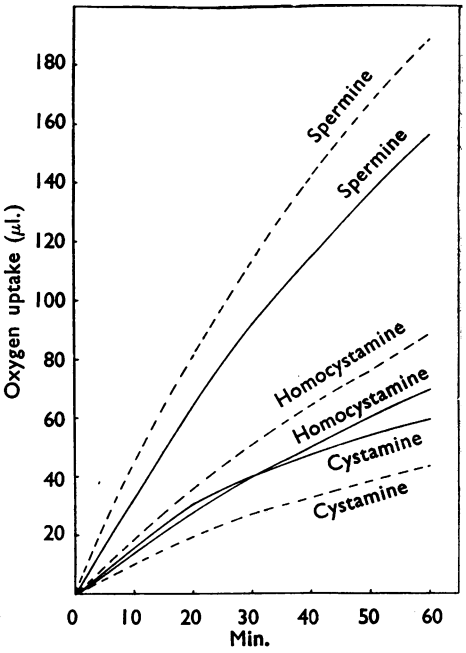


FIG. 2.—The oxidation of cystamine, homocystamine, and spermine by bovine serum. One sample of serum was dialysed against phosphate buffer of *p*H 7.4 (solid lines); another sample of the same serum against phosphate buffer of *p*H 6.5 (broken lines). The substrate concentration was 10^{-2} M. Each flask contained 1.0 ml. dialysed serum.

that are also oxidized by the amine oxidase of liver and other mammalian tissues (Bergeret *et al.*, 1957). Fig. 1 shows that homocystamine was oxidized more rapidly than cystamine.

With bovine serum, there was a difference in the rates of oxygen uptake at different *p*H. At *p*H 7.4, homocystamine and cystamine were oxidized at similar rates, but at *p*H 6.5 homocystamine was the more rapidly oxidized (Fig. 2). Sheep serum, which is known to contain spermine oxidase (Hirsch, 1953), also acted upon both cystamine and homocystamine. We have found that spermine oxidase is also present in goat plasma which oxidizes homocystamine more rapidly than cystamine (*p*H 7.4).

In all these experiments, a brown colour appeared in the flasks that were incubated with cystamine ; this is similar to the observation of Cavallini *et al.* (1957) with pig kidney extract. No colour appeared with homocystamine as substrate.

In their experiments with pig kidney extract, Cavallini *et al.* (1957) found that the reaction came to a standstill when one molecule of ammonia had been formed for every molecule of cystamine added. They found that in the

TABLE I

OXYGEN CONSUMPTION AND AMMONIA FORMATION IN THE OXIDATIVE DEAMINATION OF CYSTAMINE AND HOMOCYSTAMINE

Substrate concentration, $1{\cdot}25 \times 10^{-3}$ M; *o*-aminobenzaldehyde (*o*ABA) concentration, $1{\cdot}67 \times 10^{-3}$ M. The values are expressed in moles of O_2 consumed and NH_3 formed/mole of added substrate. * Indicates estimations at *p*H 6·5.

Substrate	Cystamine				Homocystamine			
Source of Enzyme	Oxygen Uptake		Ammonia Formation		Oxygen Uptake		Ammonia Formation	
	Without *o*ABA	With *o*ABA	Without *o*ABA	With *o*ABA	Without *o*ABA	With *o*ABA	Without *o*ABA	With *o*ABA
Pig kidney ..	0·68	0·54	1·37	0·83	—	—	—	—
Ox serum ..	1·10	—	—	—	1·98*	—	—	—
,, ,, ..	0·93	—	1·60	—	1·71*	—	1·94*	—
,, ,, ..	1·11	0·57	1·72	0·89	—	—	—	—
,, ,, ..	1·44	0·68	1·53	0·76	1·40	1·56	1·91	1·74
,, ,, ..	—	—	—	—	1·95	1·82	1·74	2·00
Goat plasma ..	1·02	0·80	1·74	1·40	1·84	1·81	1·80	1·97

presence of *o*-aminobenzaldehyde a yellow pigment was formed; this was analogous to the observations of Tabor (1951) on putrescine and cadaverine.

In Table I we have summarized our observations on the relation of oxygen uptake and ammonia formation in the absence and in the presence of *o*-aminobenzaldehyde. In these experiments, the manometer flasks contained 2 ml. of fluid, the concentration of amine (cystamine or homocystamine) was $1{\cdot}25 \times 10^{-3}$ M; the *o*-aminobenzaldehyde concentration was $1{\cdot}67 \times 10^{-3}$ M.

It can be seen that the oxygen uptake with ox serum and with cystamine as substrate was between 0.93 and 1.44 moles of O_2/mole of substrate; ammonia formation was between 1.53 and 1.72/mole of substrate. In other words, there was evidence for the removal of more than one of the two amino groups. In the presence of *o*-aminobenzaldehyde, both oxygen uptake and ammonia formation were reduced: O_2 uptake was 0.57 and 0.68 moles in two experiments; ammonia formation was 0.89 and 0.76 moles.

These results are consistent with the interpretation that, in the presence of *o*-aminobenzaldehyde, cystaldimine was accumulating. In the absence of the reagent, where ammonia formation was near two moles/mole of cystamine, oxygen consumption was close to one mole. This suggested that an aldehyde was accumulating as an end-product of the oxidation. In two experiments with ox serum as source of enzyme and cystamine as substrate, at the end of the incubation and after deproteinization of the incubation mixture with trichloroacetic acid, we added a saturated solution of 2:4-dinitrophenylhydrazine in 2 N HCl. A slight colour change to orange occurred and a very slight precipitate formed, indicating that some carbonyl compound was accumulating in the mixture.

Table I also gives our results with homocystamine. There was no evidence of cyclization. In the presence as well as in the absence of *o*-aminobenzaldehyde, ammonia formation and oxygen consumption were higher than with cystamine as substrate. Ammonia formation was between 1.74 and 2.00 moles/mole of homocystamine, indicating that the second amino-group was also attacked. In the experiments with homocystamine, there was no evidence of the formation of a hydrazone when 2:4-dinitrophenylhydrazine was added after the incubation.

In some of the experiments with cystamine, oxygen consumption had not entirely stopped at the end of incubation.

In the presence of *o*-aminobenzaldehyde, a yellow colour developed in the flasks incubated with cystamine as well as in those incubated with homocystamine.

DISCUSSION

The experiments described show that both cystamine and homocystamine are oxidized by mammalian enzymes. The oxidation of cystamine by the histaminase of pig liver, described by Cavallini (1956), was confirmed. In addition, we have found that cystamine is a substrate of two other oxidases, the spermine oxidase of ruminant plasma and the benzylamine oxidase of horse serum. In most species examined, the plasma enzymes acted more rapidly on homocystamine than on cystamine. The oxidation of cystamine by preparations of mammalian liver was slow; homocystamine was attacked at a slightly faster rate.

Our knowledge of the metabolism of cystamine has recently been reviewed (Eldjarn and Pihl, 1956; Cavallini, 1956). It is not certain whether or not cystaldimine can appear as an end-product of metabolism, but in our experiments the total

formation of ammonia, in the absence of *o*-aminobenzaldehyde, always exceeded one mole for each mole of cystamine added. It is likely that a considerable amount of amine was oxidized beyond the cystaldimine stage.

With homocystamine as substrate, a yellow colour appeared in the presence of *o*-aminobenzaldehyde, but otherwise there was no indication that a ring compound was accumulating.

Homocystamine was less rapidly oxidized than cystamine by the pig kidney enzyme. This is in agreement with what is known of the action of histaminase on polymethylene diamines. The slightly higher rate of oxidation of homocystamine by mammalian liver can also be interpreted on the basis of similar observations with the polymethylene diamines (Blaschko and Hawkins, 1950). The two plasma enzymes, spermine oxidase and benzylamine oxidase, acted upon both cystamine and homocystamine. Their specificity requirements are not so well known, but it appears that their position is intermediate between that of liver amine oxidase and histaminase.

To what extent histaminase takes part in the breakdown of cystamine is not yet known; the present experiments show that for some mammalian species the action of the plasma oxidases must also be considered.

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