The Role of Sulphur, Sulphide and Reducible Dyes in the Enzymic Oxidation of Cysteamine to Hypotaurine

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1. Cysteamine is oxidized to hypotaurine by an enzyme extracted from horse kidney, with sulphur or sulphide acting as a cofactor. It has been now found that, when the enzyme is omitted, sulphur and sulphide are able to catalyse the oxidation of cysteamine to cystamine by molecular oxygen. 2. Methylene blue may be used in catalytic amounts as a cofactor in the enzymic oxidation of cysteamine to hypotaurine in the place of sulphur or sulphide. The effect of methylene blue is not light-dependent and is not abolished by catalase. Other redox dyes with $E'_0$ higher than that of methylene blue are also used as cofactors. 3. A property common to all the cofactors is that they are necessary for the enzymic process in catalytic amounts, though they depress the final amount of hypotaurine produced when added over a critical concentration. All the cofactors share also the property of being catalysts for the non-enzymic oxidation of cysteamine to cystamine. 4. Methylene blue is reduced by cysteamine under anaerobic conditions, and is reoxidized in the presence of air. The rate of the reduction is not accelerated by the enzyme, indicating that the dye does not act in this reaction as a hydrogen carrier from the enzyme to oxygen. The possible mechanism of action of methylene blue and of the other cofactors is discussed.

An enzyme that catalyses the oxidation of cysteamine to hypotaurine in the presence of elemental sulphur or sulphide has been identified and purified (Cavallini, De Marco & Scandurra, 1962; Cavallini, Scandurra & De Marco, 1963). In this reaction sulphur is reduced to sulphide by the excess of cysteamine, and sulphide is oxidized to sulphur and polysulphides by molecular oxygen. As a consequence sulphur, sulphide and polysulphides are always present in the incubation mixtures if either sulphur or sulphide has been added (Cavallini et al. 1963). This finding suggested a possible role of sulphur as hydrogen carrier to oxygen. In the present paper it is shown that other reducible substances can function as cofactors.

MATERIALS AND METHODS

Enzyme preparation. The extraction and purification of the enzyme from horse kidney was reported by Cavallini et al. (1963). Enzyme purified to step 5, as described in that paper, has been used in the present work. When necessary, the final solution of the enzyme was concentrated by ultrafiltration by using the Ultrafilters of LKB Produkter A.B., Stockholm, Sweden.

Incubation mixtures. Unless stated otherwise each incubation mixture contained: 0.1 ml. of m-potassium phosphate buffer, pH 7.4; 1.2 mg. of enzyme (enzyme concentration was estimated from $E_{1%}^{1%} = 9.1$ at 280 m$\mu$); 50 mmoles of cysteamine hydrochloride; cofactor as indicated in the text; water to a final volume of 3 ml. Incubations were performed in stoppered flasks or Warburg vessels shaken at 38°, with air as the gas phase. The conventional Warburg apparatus was used for the determination of oxygen uptake. When sulphide was present, no NaOH solution was placed in the centre well, to prevent trapping of sulphide.

Determination of products. Quantitative determination of hypotaurine and determination of residual cysteamine or cystamine were performed with $^{35}$S cysteramine as substrate, by employing a radiochromatographic analysis of the final deproteinized solution (Cavallini et al. 1963). Determination of thiols and disulphides was performed by the Folin–Marenzi method as modified by Shinohara (1935). When sulphide was present in the solutions to be analysed by the Folin–Marenzi method, it was eliminated by acidification with 1.5 ml. of 2N-acetic acid followed by suction for 3 min. with a water pump. After the addition of 5 ml. of 2N-sodium acetate the procedure was continued as usual.

Reduction and reoxidation of methylene blue. This was followed by determining the extinction at 690 m$\mu$ in a Coleman photometer. A suitable amount of the incubation mixture was placed in the round-type cuvettes of the photometer and shaken at 38° in a Warburg bath. The cuvettes were provided with inlet and outlet tubes to allow bubbling of gas through the solution. At intervals the cuvettes were transferred to the photometer. Anaerobiosis was obtained by passing through the cuvettes nitrogen that had been purified over hot copper and saturated with water. Aero-
biosis was obtained by bubbling water-saturated air. The actual rate of reduction of methylene blue was determined in a Beckman model DU spectrophotometer by using a 1 cm. light-path cuvette in the form of a Thunberg tube. Air was removed by boiling at room temperature under vacuum, followed by refilling with pure argon. Evacuation and refilling were repeated three times, the last operation being a refilling with argon under slight pressure.

Chemicals. [35S]Cystamine dihydrochloride (specific activity 23mc/m-mole) was obtained from The Radiochemical Centre, Amersham, Bucks. Labelled cysteamine was produced by thiol-disulphide exchange with an excess of unlabelled cysteamine (Cavallini et al. 1963). Colloidal sulphur was prepared and standardized according to the method of Scandurra, Mosti & Cavallini (1963).

RESULTS

Catalysis of the non-enzymic oxidation of cysteamine to cystamine by sulphur and by sulphide. The addition of sulphide to a solution of cysteamine buffered at pH 7.5, in the absence of the enzyme and of any other catalyst, produces a remarkable acceleration of the oxygen uptake (Cavallini et al. 1963). This observation suggested that sulphide might act as a catalyst for the non-enzymic oxidation of cysteamine to cystamine by a mechanism that may be of relevance for the enzymic process under study.

When a solution of cysteamine is shaken in the presence of air and of catalytic amounts of sulphur or sulphide it disappears rapidly. The effect is evident with 2μmoles of catalyst (Fig. 1) and increases with increasing concentrations of the catalyst (Table 1). At the end of the experiment, the cysteamine that disappeared was recovered as cystamine. No hypotaurine or other compounds were detected by paper chromatography of the final solution. The catalysis was almost abolished when the solution of sulphide used as catalyst was acidified to remove hydrogen sulphide and then neutralized. This indicates that the effect observed was actually due to sulphide and not to any metal impurity.

A possible explanation of the above findings is that sulphur may act as a hydrogen carrier between cysteamine and molecular oxygen. The ability of sulphur to be reduced to sulphide by thiols has been reported (Smythe, 1942; Meister, Fraser & Tice, 1954), and the ready oxidation of sulphide to sulphur and polysulphides is well known (Krebs, 1929; Haurowitz, 1941; Abel, 1956). Thus it may be assumed that sulphur and sulphide, which are always present in the reaction mixture, whichever compound was initially added, form a redox couple reduced by the thiol and oxidized by oxygen:

$$2RSH + S \rightarrow RSSR + H_2S$$

$$H_2S + \frac{1}{2}O_2 \rightarrow S + H_2O$$

This mechanism resembles that operative with more traditional catalysts for thiol oxidation (Young & Maw, 1958). However, an intermediate polysulphide of unknown nature may be the catalyst. This would avoid the large number of steps needed to reduce octatomic elementary sulphur to sulphide and to reverse this process. Compared with other catalysts like Cu²⁺ and Fe³⁺ the catalysis produced by sulphur and sulphide is, however, very poor.

Methylene blue as cofactor in the enzymic oxidation of cysteamine. Since the role of sulphur and sulphide

![Fig. 1. Catalysis of the non-enzymic oxidation of cysteamine to cystamine by sulphur and sulphide. Each vessel contained: 0.3ml of M-potassium phosphate buffer, pH 7.4; 50μmoles of cysteamine hydrochloride; water plus additions to a total volume of 3ml. Catalysts were added in the following amounts: ○, none; ▲, 2μmoles of Na₂S; Δ, 2μg-atoms of colloidal S; ●, 2μmoles of Na₂S acidified with excess of HCl to remove H₂S, then neutralized. Cysteamine was determined by the Folin–Marenzi reagent. At the end of oxidation the amount of cysteamine disappeared was recovered as cystamine. The gas phase was air, and the temperature was 38°C.](image)
in the enzymic oxidation of cysteamine to hypotaurine appears to be that of hydrogen carrier to oxygen, it was decided to investigate whether a traditional hydrogen carrier like methylene blue could replace sulphur and sulphide. Methylene blue oxidizes thiols to disulphides (Reid, 1931), and when it is in the reduced state is readily oxidized by molecular oxygen (Reid, 1930; Macrae, 1931). As illustrated in Fig. 2, methylene blue can function in catalytic amounts as a cofactor for the enzymic oxidation of cysteamine to hypotaurine. This is evident from the oxygen consumed in excess of the theoretical amount of 280 μl necessary for the oxidation of 50 μmoles of cysteamine to cystamine. Although catalytic amounts of methylene blue bring about the oxidation of cysteamine to a level of oxidation higher than cystamine, higher concentrations of methylene blue have a depressing effect. This behaviour bears a striking resemblance to that reported for sulphide, which, over a certain range of concentrations, is inhibitory (Cavallini et al. 1963). Results of some control experiments are collected in Fig. 3. These indicate that in the absence of enzyme, or with enzyme that has been heated in a boiling-water bath for 5 min., the oxygen uptake never exceeds the theoretical amount of 280 μl necessary for the oxidation to cystamine. One point that is apparent in Fig. 3 is that, when the enzyme has been boiled or omitted, the oxidation of cysteamine to cystamine is accelerated by methylene blue and no depression is observed with high concentrations of methylene blue.

Methylene blue undergoes photo-oxidative reactions (Weil, Gordon & Buchert, 1951). However, we found that when light was excluded from the Warburg vessels the rate of enzymic oxidation of cysteamine in the presence of methylene blue was the same as in diffuse daylight.

In Table 2 are reported the amounts of hypotaurine and cystamine found after incubation of cysteamine under various conditions. Whenever hypotaurine was present a small radioactive ninhydrin-positive spot was also detected at Rf 0.40 in water-saturated phenol. This spot, which was identified as taurine, did not account for more than 5% of the hypotaurine produced. This small amount of taurine was presumably formed by the further oxidation of hypotaurine, either spontaneously or by some unknown enzymic side reaction. It was therefore added to the experimental values and reported as hypotaurine. Table 2

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**Fig. 2.** Enzymic oxidation of cysteamine to hypotaurine in the presence of different quantities of methylene blue. Each vessel contained: 1.2 mg. of enzyme; 50 μmoles of cysteamine; 0.1 ml. of m-potassium phosphate buffer, pH 7.4; water to a volume of 3 ml.; the centre well contained 0.2 ml. of 20% (w/v) KOH solution. Methylene blue was added in the following amounts (μmoles): ● (broken line), 0.0; ○, 0.01; ● (full line), 0.05; □ (full line), 0.1; ■, 1; △, 5; ▲, 10. The conditions for curve □ (broken line) were identical with those for curve ● (full line) except that 0.1 mg. of crystalline catalase was also added. The gas phase was air, and the temperature was 38°. The horizontal broken line indicates the theoretical O₂ uptake for the oxidation of 50 μmoles of cysteamine to cystamine.

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**Fig. 3.** Oxygen uptake during the oxidation of cysteamine; the conditions were as described in Fig. 1, except that in (a) the enzyme was omitted and in (b) the enzyme has been heated at 100° for 5 min. Methylene blue was added in the following amounts (μmoles): ● (broken line), 0.0; ○, 0.01; ● (full line), 0.05; □, 0.1; ■, 1; △, 5; ▲, 10.
Table 2. Products of the enzymic oxidation of cysteamine

<table>
<thead>
<tr>
<th>Enzyme added</th>
<th>Methylene blue added (µmoles)</th>
<th>Hypotaurine found (µmoles)</th>
<th>Cystamine oxidized (µmoles)</th>
<th>Residual cysteamine (µmoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active enzyme</td>
<td>—</td>
<td>1.5</td>
<td>48.5</td>
<td>0.0</td>
</tr>
<tr>
<td>0.01</td>
<td>5.9</td>
<td>44.1</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>32.1</td>
<td>17.9</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>35.4</td>
<td>14.6</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>7.0</td>
<td>43.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>5.0</td>
<td>0.0</td>
<td>50.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>10.0</td>
<td>0.0</td>
<td>50.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Enzyme boiled</td>
<td>—</td>
<td>0.0</td>
<td>50.0</td>
<td>0.0</td>
</tr>
<tr>
<td>0.1</td>
<td>0.0</td>
<td>50.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Enzyme omitted</td>
<td>—</td>
<td>0.0</td>
<td>6.0</td>
<td>44.0</td>
</tr>
<tr>
<td>0.1</td>
<td>0.0</td>
<td>15.6</td>
<td>34.4</td>
<td></td>
</tr>
<tr>
<td>Active enzyme:</td>
<td>—</td>
<td>0.0</td>
<td>50.0</td>
<td>—</td>
</tr>
<tr>
<td>Cysteamine added in the place of cysteamine</td>
<td>0.1</td>
<td>0.0</td>
<td>50.0</td>
<td>—</td>
</tr>
</tbody>
</table>

Table 3. Stoichiometry of the oxidation

Incubation was for 4 hr. in Warburg vessels shaken at 38° in the presence of air. Each vessel contained: 1-2 mg. of enzyme; 0-3 ml. of potassium phosphate buffer, pH 7.4; 0-05 µmole of methylene blue; 45 µmoles of [35S]cysteamine; water to a volume of 3 ml.; the centre well contained 0-2 ml. of 20% (w/v) KOH. Hypotaurine and cysteamine were determined as described in the text. No cysteamine was present at the end of incubation. Values were calculated according to eqn. (5).

<table>
<thead>
<tr>
<th>Hypotaurine</th>
<th>Cysteamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>(µmoles)</td>
<td>Found</td>
</tr>
<tr>
<td>(µmoles)</td>
<td>Calc.</td>
</tr>
<tr>
<td>O2 uptake</td>
<td></td>
</tr>
</tbody>
</table>

Found: 768 32.6 12.4
Calc.: 30.6 14.4

The data confirms what is already apparent in Fig. 2, namely that methylene blue may act as a cofactor in the enzymic oxidation of cysteamine. It is also evident that methylene blue in excess of 0.1 µmole/3 ml. inhibits the enzymic formation of hypotaurine. No enzymic oxidation occurs when cysteamine is replaced by cysteamine (Table 2).

Stoichiometry of the reactions. Under the conditions described in Fig. 2, oxygen uptake ceases after 3-4 hr. After this time, cysteamine disappeared completely and hypotaurine and cysteamine are produced. This indicates that the reactions shown in eqns. (3) and (4) occur. Since

\[
\begin{align*}
\text{CH}_2\cdot\text{SH} + \text{O}_2 & \rightarrow \text{CH}_2\cdot\text{SO}_2\text{H} \\
\text{CH}_2\cdot\text{NH}_2 + \text{O}_2 & \rightarrow \text{CH}_2\cdot\text{S}\cdot\text{S}\cdot\text{CH}_3 + \text{H}_2\text{O} \\
\end{align*}
\]

...the addition of hydrogen peroxide, in an amount presumed to be produced by the reoxidation of reduced methylene blue, could replace methylene blue. To an incubation mixture identical with that described in the legend of Table 2, except that methylene blue was not added, were added 25 µmoles of hydrogen peroxide [Perhydrol (E. Merck A.-G., Darmstadt, Germany), suitably diluted]. After 4 hr. of incubation, a single substrate is oxidized to two final products, each requiring a different quantity of oxygen, the amount of each product formed can be calculated from:

Total O2 uptake (µl) = 22.4 (a - x) + 5.6x

where a represents µmoles of cysteamine added, and x and (a - x) represent µmoles of cysteamine oxidized respectively to cysteamine and to hypotaurine. Values calculated from eqn. (5) are in fairly good agreement with those determined experimentally (Table 3), which suggest that reactions (3) and (4) are the main reactions occurring during the enzymic oxidation of cysteamine in the presence of methylene blue.

Exclusion of hydrogen peroxide as an essential intermediate in the enzymic oxidation. The oxidation of reduced methylene blue by molecular oxygen is known to produce hydrogen peroxide (Reid, 1930; Macrae, 1931), and the oxidation of sulphide is also reported to yield hydrogen peroxide (Haurowitz, 1941). We therefore investigated whether the role of the cofactor in the enzymic process is that of hydrogen peroxide producer and whether hydrogen peroxide was involved in the oxidation of cysteamine to hypotaurine. For this purpose the enzyme was incubated with cysteamine and methylene blue in the presence of 100 µg. of catalase. However, Fig. 2 shows that the curves for the oxygen uptake in the presence and absence of catalase were identical. In a separate control it was established that cysteamine does not appreciably change the activity of catalase. It was also determined whether the addition of hydrogen peroxide, in an amount presumed to be produced by the reoxidation of reduced methylene blue, could replace methylene blue. To an incubation mixture identical with that described in the legend of Table 2, except that methylene blue was not added, were added 25 µmoles of hydrogen peroxide [Perhydrol (E. Merck A.-G., Darmstadt, Germany), suitably diluted]. After 4 hr. of incubation, a single substrate is oxidized to two final products, each requiring a different quantity of oxygen, the amount of each product formed can be calculated from:

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\[
\begin{align*}
\text{CH}_2\cdot\text{SH} + \text{O}_2 & \rightarrow \text{CH}_2\cdot\text{SO}_2\text{H} \\
\text{CH}_2\cdot\text{NH}_2 + \text{O}_2 & \rightarrow \text{CH}_2\cdot\text{S}\cdot\text{S}\cdot\text{CH}_3 + \text{H}_2\text{O} \\
\end{align*}
\]
tion no hypotaurine could be detected by the radiochromatographic procedure. The same negative result was obtained when the solution of 25 μmoles of hydrogen peroxide was added in portions every 30 min. for a 4 hr. period. These results therefore appear to exclude the possibility that hydrogen peroxide as such might play a role in the enzymic oxidation of cysteamine to hypotaurine.

Other substances active as a cofactor in the enzymic oxidation of cysteamine. The activity of different dyes was related to their redox potentials (Table 4). The values reported for enzymic activity in Table 4 are volumes of oxygen consumed in excess of the theoretical value of 280 μl. necessary for the oxidation of 50 μmoles of cysteamine to cystamine. In no experiment was cysteamine present in the reduced form at the end of the incubation. Thus the reported values are measures of the amount of hypotaurine produced. Since the effect of methylene blue varies with its concentration, the other dyes were tested at three different concentrations. With 2,6-dichlorophenol-indophenol and thionine it was ascertained, by using labelled cysteamine, that hypotaurine was actually produced. With 1 μmole of the dye, under the conditions described in Table 4, 7-2 μmoles of hypotaurine were obtained in the presence of dichlorophenol-indophenol, and 19.5 μmoles in the presence of thionine, after incubation for 4 hr. Of the dyes that were tested only those with $E'_0$ equal or higher than +0.011 were active as cofactors, although their activities differed. It is noteworthy that all the active dyes are inhibitory above a given concentration, as was observed with methylene blue and with sulphide.

The concentration of dye that gives maximum activity in the enzymic oxidation depends on the value of $E'_0$ and tends to rise as $E'_0$ increases. Like methylene blue all the active dyes are able to catalyse the oxidation of cysteamine to cystamine in the absence of the enzyme when tested in the range of concentration indicated in Table 4.

The following compounds showed no cofactor activity: NAD, NADP, FMN, ubiquinone, mena-dione, ascorbic acid, folic acid, cytochrome c, haematin, 2,4-dinitrophenol, Fe$^{2+}$, Cu$^{2+}$, Zn$^{2+}$. Elementary selenium and selenite were active, whereas tellurium and tellurite were not.

Reversible non-enzymic reduction of methylene blue by cysteamine. The most likely role for methylene blue in the non-enzymic oxidation of cysteamine would appear to be that of hydrogen carrier from thiol to oxygen, as was assumed for sulphur. Indication that this mechanism is operative has been obtained by following changes of extinction at 690 μm under anaerobic conditions in mixtures containing cysteamine and methylene blue. The effect of the enzyme on this process has also been investigated. Cysteamine causes the reduction of methylene blue under anaerobic conditions (Fig. 4). Under aerobic conditions the reoxidation of the reduced dye by molecular oxygen is so fast that the dye is kept in the oxidized form, even when the thiol is in large excess with respect to the dye. Reoxidation depends strongly on the vigour of oxygenation. If air is not bubbled or the vessel is not shaken the solution remains colourless, diffusion of oxygen being the limiting factor. The presence of enzyme does not appreciably change the reduction rate of methylene blue by cysteamine, as shown by the similarity of the curves illustrating anaerobic experiments in the presence and in the absence of the enzyme. However, the reoxidation rate seems lower in the presence of the enzyme.

The observation that the enzyme does not affect the rate of reduction of methylene blue by cysteamine suggests that the function of methylene blue in the enzymic reaction is not that of a hydrogen acceptor from the enzyme, as for many typical dehydrogenases or oxidases. In this case the addition of the enzyme in anaerobic conditions should have produced a reduction of methylene blue faster than that observed with cysteamine alone. The inability of the enzyme to accelerate the reduction rate of methylene blue has been established by determining the half-reduction time of the dye in

<table>
<thead>
<tr>
<th>Redox dye (in parenthesis)</th>
<th>Amount of dye (μmoles) required to oxidize cysteamine to cystamine (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-1</td>
</tr>
<tr>
<td>Neutral red (−0-325)</td>
<td>−</td>
</tr>
<tr>
<td>Indigo disulphonate (−0-125)</td>
<td>−</td>
</tr>
<tr>
<td>Triphenyltetrazolium chloride (−0-08)</td>
<td>−</td>
</tr>
<tr>
<td>Indigo tetrasulphonate (−0-046)</td>
<td>−</td>
</tr>
<tr>
<td>Methylene blue (+0-011)</td>
<td>545</td>
</tr>
<tr>
<td>Thionine (+0-083)</td>
<td>359</td>
</tr>
<tr>
<td>Phenazine methosulphate (+0-08)</td>
<td>210</td>
</tr>
<tr>
<td>Tolypylene blue (+0-115)</td>
<td>361</td>
</tr>
<tr>
<td>2,6-Dichlorophenol-indophenol (+0-217)</td>
<td>149</td>
</tr>
</tbody>
</table>

Table 4. Redox dyes as cofactors for the enzymic oxidation of cysteamine to hypotaurine

Experimental conditions were as described in Fig. 2. Values are reported as μl. of O$_2$ consumed over the theoretical value of 280 μl. necessary for the oxidation of 50 μmoles of cysteamine to cystamine. Incubation was continued to cessation of O$_2$ uptake (4 hr.). At the end of incubation the Folin–Marenzi test for reduced thiols was negative in every incubation mixture. Under the same conditions, but with boiled enzyme, the uptake of O$_2$ stopped at 280 μl. $E'_0$ values are taken from Long (1961) except those for triphenyltetrazolium chloride and phenazine methosulphate, which were taken respectively from Glock & Clifford (1953) and from Singer & Kearney (1957).
cuvettes metric by 4. as described. The Each incubation was carried out under aerobic conditions. The half-reduction time of a solution identical with that described in Fig. 2, but scaled up threefold (i.e. to 9 ml). The addition of 1, 2 and 4 mg. of enzyme caused slight variations that were not statistically significant.

**DISCUSSION**

The present results indicate that certain reducible dyes may be used as cofactors for the enzymic oxidation of cysteamine to hypotaurine in place of sulphur or sulphide. All the compounds found so far that can act as cofactors for the enzymic oxidation to hypotaurine can also catalyse the non-enzymic oxidation to cystamine. The depression of the final amount of hypotaurine produced, observed when the cofactors are raised above a critical concentration, could be explained by assuming that during the enzymic process two different catalytic reactions are running side by side: one, catalysed by the cofactor, leading to the non-enzymic production of cysteamine (reaction 4); the other catalysed by the enzyme, leading to the production of hypotaurine (reaction 3). The increase in concentration of cofactor, when the enzyme is limiting, is likely to favour the non-enzymic oxidation to cystamine.

The inability of the enzyme to accelerate the reduction rate of methylene blue under anaerobic conditions suggests that the role of methylene blue as cofactor in the enzymic reaction is not the traditional one of linking the enzyme with oxygen. The inability of cystamine to act as substrate shows, on the other hand, that the function of methylene blue and other cofactors in the enzymic process is not that of producing cystamine. Two reasonable explanations for the role of methylene blue, and possibly for the other cofactors, may then be advanced. The first is that the cofactor could act as an activator for the enzyme molecule; the second is that the catalytic oxidation of cysteamine performed by the cofactors is necessary to produce transient intermediate compounds (thiyl and oxygen radicals), which are used by the enzyme to yield hypotaurine. In the first case the ability of the cofactors to catalyse the oxidation to cystamine is only accidental, whereas in the second case it represents an indispensable preliminary step for the production of the actual substrates for the enzyme.

It is difficult to decide between these two alternatives, although the ability of all the cofactors to catalyse the non-enzymic oxidation of cysteamine to cystamine seems unlikely to be fortuitous.

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**REFERENCES**


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